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STUDY OF THE IMMOBILIZATION OF THE PROBIOTIC STRAIN *LACTOBACILLUS PARACASEI-010-K* ON A NATURAL SORBENT FOR USE IN POULTRY FARMING

Annotation: *This article presents the results of a study on the immobilization of cells of the probiotic strain Lactobacillus paracasei-010-K on natural adsorbents.*

*Methods of cell immobilization can increase their stability, reduce the lethal effect of microorganisms on cells during their passage through the gastrointestinal tract, improve visceral parameters and clinical outcomes compared with free cells. The local mineral vermiculite was used to immobilize lactic acid bacteria. The sample is characterized by a high content of macro and microelements compared to samples of vermiculites from other deposits in the country and it has high adsorption activity (*20-50% by volume*). To immobilize the cells of the strains, the mineral adsorbent vermiculite was added in amounts of 0.25, 0.5, 0.75 and 1.0 (%) to daily cultures of lactic acid bacteria with titers of $1*10^7$ CFU/ml in the MRS (de Man, Rogosa u Sharpe agar) nutrient medium. The adsorption-immobilization process was carried out at 37°C with stirring for 24 hours. Liquid preparations containing lactobacillus cells and adsorbent were stored at a temperature of 4-6°C for 30, 60 and 90 days to assess the stabilizing effect of the adsorbent and determine its optimal amount. In the course of studies on the immobilization of microorganisms on enterosorbents, it was found that vermiculite does not adversely affect the bioactivity and viability of lactic acid bacteria cells. Moreover, when vermiculite was introduced into the MRS medium at a concentration of 0.5%, the titer of lactic acid bacteria increased to $1*10^9$ CFU/ml. The resulting preparations containing cultures of lactic acid bacteria are further used to introduce them into the composition of feed additives for poultry farming.*

Key words: *probiotic, prebiotic, immobilization, sorbent, biological product, strain, lactic acid bacteria.*

Introduction. The development of new bio-products to produce high-quality poultry products is an important task for the agro-industrial complex and opens up new opportunities to ensure national food security. To solve the problem of healthy nutrition, it is necessary to improve the quality characteristics of poultry feed by creating and using biological products based on natural agrominerals and lactic acid bacteria. They not only have nutritional and immunological properties, but also optimize mineral nutrition, correct metabolism, increase poultry productivity and improve product quality [1].

The development of highly effective biological preparations based on natural aluminosilicates makes it possible to replace expensive similar products with cheaper ones made from local raw materials [2]. The mechanism of action of biologics based on probiotic cultures stimulates the microflora of the gastrointestinal tract, helps to establish an optimal microbial balance, increases the body's resistance, improves growth and development.

Products based on probiotics should contain a much number of microorganism cells (not least 10^6 -10^7 CFU/g) [3]. The use of such a large number of microbial cells is accompanied by the adverse effects of adverse factors of various origins. The main factors of adverse effects include processing conditions (e.g. temperature, oxidation), storage conditions (packaging materials and environment: humidity, oxygen, temperature) and deterioration in the gastrointestinal tract (low pH in the stomach, bile salts in the small intestine). However, the survival rate of probiotics during storage and passage through the gastrointestinal tract is usually low [4].

One possible solution to this problem is the immobilization of bacterial cells [5]. Cell immobilization techniques can increase cell stability, reduce the lethal effects of microorganisms on cells, and improve histological parameters and clinical outcomes compared to free cells. Immobilization methods improve the survival of probiotics in food and during gastrointestinal transit.

Mineral enterosorbents serves as a reliable protection for bacteria, attracting aggressive substances to its surface. Probiotics obtained by immobilization enter the large intestine, preserving

most of the microorganisms. The presence of enterosorbent in the composition of drugs affects not only the bioavailability, but also the therapeutic activity of drugs, i.e. probiotic bacteria attaching to the inner wall of the intestine accelerates the growth and reproduction of beneficial microorganisms, the population of bacteria belonging to conditionally pathogenic and pathogenic microflora rapidly decreases [6].

The presence and magnitude of the charge play a dominant role in the interaction of the cell and the adsorbent, and the nature of the adsorption force in such an interaction is mainly determined by the chemical composition of the bacterial cell wall and the functional groups of the adsorbent itself. It is known that gram-positive bacteria, including lactic acid bacteria, have negatively charged surfaces [7].

N.V. Potekhina's research has shown that the negative charge on the cell surface is caused by anionic polymers in the cell wall. This primarily applies to the macropolymer peptidoglycan. The negative charge of peptidoglycan is formed due to the carboxyl groups of γ-glutamic and meso-diaminopimeric acids and the terminal residue of the D-Ala peptide subunit.In addition to teichoic and lipoteichoic acid, anionic compounds such as teichuronic acid and sugar-1-phosphate polymers make a significant contribution to the formation of the polyelectrolyte gel structure of the cell wall [8].

Adsorption immobilization of microbial cultures is currently used in various fields of research, and in some cases has already found technical application [9]. The popularity of this approach is due to the fact that the formulations obtained by this method are significantly superior in their beneficial properties to formulations using conventional excipients.

Highly effective adsorbents can be used directly to change the bacterial flora in local areas of the body. An example of this approach is the use of natural mineral adsorbents containing colloidal silicon dioxide, which has been proposed for the treatment of foodborne toxic infections and non-infectious inflammatory diseases.

When developing immobilized forms of biological preparations, the most important point is the choice of a sorbent carrier. According to the classification of sorbents by chemical structure, there are: carbon adsorbents; silica gels; zeolites; aluminogels; aluminosilicates and other inorganic sorbents [10]. Mineral sorbents can be isolated as zeolites and aluminosilicate – vermiculite.

Vermiculite is one of the most promising natural aluminosilicates suitable for use in agriculture [11]. It is a natural mineral that is a product of hydrothermal decomposition of biotite, phlogopite, some types of chlorite and other magnesium-rich silicates [12]. Vermiculite is a silicate of magnesium, aluminum and iron, consisting of SiO_2 (%) (about 35-45), MgO (about 20-40), Al_2O_3 (about 7-15) and Fe_2O_3 (about 10) [13]. Vermiculite improves the digestive process by increasing the area of biochemical reactions in the intestine and sorbing low molecular weight metabolites [14]. In the production of vermiculite, high heat treatment increases volume, increases permeability and reduces weight. The resulting product is very light and sterile [15]. The material has a relatively high moisture retention capacity (200-325% by weight and 20-50% by volume), thermal conductivity (0.065-0.062 W) and a golden, accordion-like appearance. An important property determining the industrial value of vermiculite is its ability to increase in volume (swell) by 6-8 times when heated above 300°C [16].

The purpose of this work is to study the effect of different concentrations of vermiculite on cell immobilization of the probiotic strain Lactobacillus paracasei-010-K.

Research methods. The object of this study is the probiotic strain Lactobacillus paracasei-010-K. The high biological potential of the Lactobacillus paracasei-010-K strain isolated by us from koumiss suggests that it has probiotic properties and can be used as a probiotic formulation for poultry farming. Based on this study, a strain passport was compiled and deposited in the Collection of the Republic of Microbiology of the Republic of Kazakhstan [17].

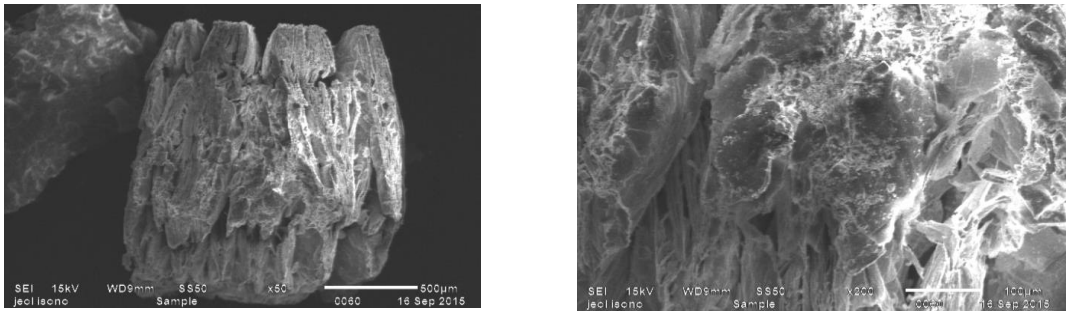
A mineral adsorbent, vermiculite, was used to immobilize lactic acid bacteria. Vermiculite is a local product of the Kulantau deposit (Turkestan region, Kazakhstan), with a grain size of 0.5-3.0 mm and was obtained from the processing company AVENUE LLP (Kulantau deposit).

The immobilization of strain cells was carried out according to the authors' method described in [18]: the mineral adsorbent vermiculite was added in amounts of (%) 0.25, 0.5, 0.75 and 1.0 during daily cultivation of probiotic strains of lactic acid bacteria in the MRS (de Man, Rogosa и Sharpe agar) nutrient medium in titers of $1*10^7$ CFU/ml. The process of adsorption immobilization was carried out by mixing, which consists in the fact that the contact of the adsorbent with a suspension

of cells in a culture medium occurs with continuous stirring for day at the temperature of 37°C. Mixing was carried out on orbital shakers (OS-20, BioSan, USA) at a speed of 128 rpm. Next, the supernatant was separated from the sorbent with a pipette and seeded by the Koch method on a dense medium of MRS to confirm a decrease in the number of free cells in the culture fluid. Further, synbiotic liquid preparations containing lactobacillus cells and sorbent were stored in a household refrigerator (DS 325000, Beko, Turkey) at a temperature of 4-6°C for 30, 60 and 90 days to assess the stabilizing effect of the sorbent and determine its optimal amount. The growth dynamics of immobilized crops was determined by sequentially diluting the immobilizer in a liquid medium of MRS and seeding dilutions on a dense medium of MRS according to GOST ISO 11133-2016.

The experiment was performed in one repeat. Statistical processing of the results was carried out using standard methods.

Results and discussions. First of all, the electron microscopic structure of vermiculite was investigated. Figure 1 shows electronic photographs and their surface, visualized at different magnifications.



(a) (b)

Figure 1 – Electronic image of vermiculite crystals. Increased volume ×50(a) and ×200(b)

Electron microscopic studies have revealed that vermiculite has a complex microsurface relief formed by microcrystals and aggregates, represented in most cases by a finely dispersed mass. Microcrystal aggregates are concentrated in microgeodes and microcracks located relatively evenly in the rock.

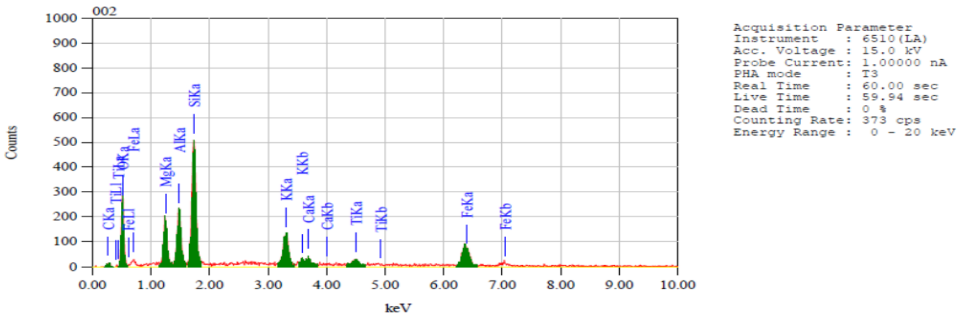
According to the study of the physico-chemical properties of vermiculite, the color was brown, greenish-brown, yellow-brown, grayish with a greenish and silvery tint, odorless, the appearance was scaly, loose masses, worm-like. The volume weight is 123.25g/l, humidity is 0.90%, pH is 7.11. The peculiarity of this type of vermiculite is that it does not contain asbestos impurities, which is typical for some vermiculite deposits, and does not contain carcinogenic and harmful to human and animal health impurities. It also has a high adsorption activity.

A sufficiently high content of macro- and microelements in the composition of vermiculite distinguishes it from other natural minerals. The sample is characterized by a high content of SiO_2 and Fe_2O_3 - about 17 and 20%, respectively (Figure 2). In addition, organic compounds are present on the surface of vermiculite. Therefore, during the immobilization of lactic acid bacteria on vermiculite, the carbon groups of the hydrophobic part of the adsorbent come into contact with the cell surface.

Therefore, studies have shown that vermiculite retains the original compositional proportions of mineral elements, and the prospects for using this product as a feed additive for poultry farming and as a basis for the preparation of biological products.

To assess the stabilizing effect of the adsorbent, immobilizates containing a consortium of lactic acid bacteria and vermiculite were stored at a temperature of 6°C for 90 days. The results showed that the greatest stabilizing effect was achieved at a concentration of 0,5% vermiculite.

As can be seen from the data shown in Table 1, vermiculite is able to increase the concentration of bacterial cells by 55%. The presented data provide confidence that positively charged vermiculite adsorbents are an effective means for the immobilization and concentration of lactic acid bacteria and their metabolites.



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.3036

Element	(keV)	Mass%	Error%	Atom%	Compound	Mass%	Cation	K
C K	0.277	4.49	0.19	8.97				1.0717
O K	0.525	31.28	0.18	46.96				32.0550
Mg K*	1.253	6.40	0.10	6.32				5.0774
Al K*	1.486	7.22	0.10	6.43				6.3000
Si K*	1.739	17.80	0.11	15.23				17.3864
K K*	3.312	8.18	0.18	5.02				10.2950
Ca K*	3.690	1.79	0.22	1.07				2.3291
Ti K*	4.508	2.27	0.29	1.14				2.5391
Fe K*	6.398	20.59	0.64	8.86				22.9463
Total		100.00		100.00				

Figure 2 – Mineral composition of experimental vermiculite

Table 1 – Titer of cells of strain *Lactobacillus paracasei-010K* when growing on an MRS medium with the addition of different consentration of vermiculite

Shelf life, CFU/ml titer		Concentrations of vermiculite on the nutrient medium			
	the control group	experienced groups			
		0,25%	0,5%	0,75%	1,0%
at the beginning	10^7	10^7	10^8	10^8	10^7
30 days	10^7	10^7	10^8	10^8	10^7
60 days	10^6	10^8	10^9	10^8	10^6
90 days	10^4	10^7	10^9	10^8	10^6

However, since these data are essentially the results of static experiments, they do not provide information about the degree of reversibility of sorption, which is important for the cultivation process, or about the impact of long-term survival of the culture. To determine these properties, experiments were conducted on control crops obtained from the combined preparation after different storage periods – after 30, 60 and 90 days. The table shows that the data on assessing the viability of a culture at a concentration of 0.5% sorbent, for short shelf life (30 days), differ significantly by more than 2 orders of magnitude for longer periods (90 and 60 days, respectively).

Thus, based on the model of the *Lactobacillus paracasei-010K* strain, the sorbent vermiculite is an effective carrier for obtaining composite immobilized forms of biological products, has a high sorption capacity both with respect to whole cells.

Conclusion

In the course of our research, we selected concentrations of a stabilizing sorbent – vermiculite, which, when added to the liquid medium of lactic acid bacteria culture in an amount of 0.5%, contributes to both intensification and prolongation of the vital activity of lactobacillus culture. The resulting synbiotic drug can be used as a feed additive to normalize the intestinal microflora of farm birds and fish. The presence of vermiculite and an active strain of lactic acid bacteria in the composition of the drug affects not only the bioavailability, but also the therapeutic activity of the drug.

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ҚҰС ШАРУАШЫЛЫҒЫНДА ҚОЛДАНУ ҮШІН *LASTOBASILLUS PAGACASEI-010К* ПРОБИОТИКАЛЫҚ ШТАММЫНЫҢ ТАБИҒИ СОРБЕНТТЕ ИММОБИЛИЗАЦИЯЛАНУЫН ЗЕРТТЕУ

Мақалада табиғи сорбентте Lastobasillus pagacasei-010К пробиотикалық штаммының жасушаларының иммобилизациясын зерттеу нәтижелері келтірілген.

*Жасушаларды иммобилизациялау технологиясы бос жасушалармен салыстырғанда тұрақтылықты арттыруға және асқазан-ішек жолдары арқылы өту кезінде микроорганизмдер жасушаларының қырылуын азайтуға, органолептикалық көрсеткіштер мен клиникалық нәтижені жақсартуға мүмкіндік береді. Лактобактерияларды иммобилизациялау үшін жергілікті минерал – вермикулит қолданылды. Бұл үлгі басқа да кенорындарының вермикулит үлгілерімен салыстырғанда құрамындағы макро және микроэлементтердің көп мөлшерімен сипатталады, сонымен қатар ол жоғары адсорбциялық белсенділікке ие (көлемі бойынша 20-50%). Штаммның жасушаларын иммобилизациялау үшін MRS қоректік ортасындағы сүт қышқылы бактерияларының 1*10^{7} КТБ/мл титрі бар тәуліктік культурасына 0,25%, 0,5%, 0,75% және 1,0% мөлшерінде минерал-сорбент вермикулит қосылды. Адсорбциялық иммобилизация процесін 37°С температурада 24 сағат бойы араластыру әдісі арқылы жүргіздік. Одан кейін сорбенттің тұрақтандырушы әсерін бағалау және оның оңтайлы мөлшерін анықтау үшін лактобактериялар жасушалары мен сорбент бар сұйық препараттар 4-6°С температурада 30, 60 және 90 тәулікке сақтауға қойылды. Энтеросорбентте микроорганизмдердің иммобилизациялануын зерттеу барысында вермикулиттің сүт қышқылы бактерияларының жасушаларының физиологиялық белсенділігі мен өміршеңдігіне кері әсер етпейтінін, сонымен қатар вермикулиттің 0,5% концентрациясын MRS ортасына енгізілуі сүт қышқылы бактерияларының титрінің 1*10^{9} КТБ/мл дейін жоғарылауына ықпал ететіні анықталды. Сүт қышқылы бактериялары бар препарат бұдан әрі құс шаруашылығына арналған азықтық қоспалардың құрамына енгізу үшін пайдаланылатын болады.*

Түйін сөздер: *пробиотик, пребиотик, иммобилизация, сорбент, биопрепарат, штамм, сүтқышқылды бактериялар.*

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ИЗУЧЕНИЕ ИММОБИЛИЗАЦИИ ПРОБИОТИЧЕСКОГО ШТАММА *LACTOBACILLUS PARACASEI-010-К* НА ПРИРОДНОМ СОРБЕНТЕ ДЛЯ ПРИМЕНЕНИЯ В ПТИЦЕВОДСТВЕ

*В статье представлены результаты по исследованию иммобилизации клеток пробиотического штамма Lactobacillus paracasei-010-К на природном сорбенте. Методы иммобилизации клеток позволяют повысить их стабильность, снизить летальное воздействие микроорганизмов на клетки во время их прохождения через желудочно-кишечный тракт, улучшить висцеральные показатели и клинические исходы по сравнению со свободными клетками. Для иммобилизации лактобацилл использовали минерал – вермикулит, который является продуктом местного производства. Для образца характерно высокое содержание макро и микроэлементов по сравнению с образцами вермикулитов других месторождении страны, так же он обладает высокой адсорбционной активностью (20-50% по объему). Для иммобилизации клеток штаммов минеральный адсорбент вермикулит добавляли в количестве 0,25%, 0,5%, 0,75% и 1,0% к молочнокислым бактериям, ежедневно культивируемым в питательной среде MRS с титрами от 1*10^{7} КОЕ/мл. Процесс адсорбции-иммобилизации проводили при 37°С с перемешиванием в течение 24 часов. Жидкие препараты, содержащие клетки лактобацилл и адсорбент, хранились при температуре 4-6°С в течение 30, 60 и 90 дней для оценки стабилизирующего эффекта адсорбента и определения его оптимального количества. В ходе исследований по иммобилизации микроорганизмов на энтеросорбентах было установлено, что вермикулит не оказывает негативного влияния на биоактивность и жизнеспособность клеток молочнокислых бактерий. Более того, при внесении вермикулита в среду MRS в концентрации 0,5% титр молочнокислых бактерий увеличился до 1*10^{9} КОЕ/мл. Полученный препарат, содержащий культуру молочнокислых бактерии, далее будет использован для введения в состав кормовых добавок для птицеводства.*

Ключевые слова: *пробиотик, пребиотик, иммобилизация, сорбент, биопрепарат, штамм, молочнокислые бактерии.*

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ИССЛЕДОВАНИЕ НОВЫХ РУБЛЕННЫХ ПОЛУФАБРИКАТОВ ИЗ СУБПРОДУКТОВ

Аннотация: *Удовлетворение растущего мирового спроса на белок приводит к возникновению проблем с точки зрения предложения. Расширение использования животных белков за счет более широкого использования мясных субпродуктов и могло бы стать частью решения, при условии одобрения потребителями. В этом исследовании изучалась перспективность использования субпродуктов из туши говядины такие как почки, сердце, язык, рубец, а также местное растительное сырье порошок тары в получении рубленных полуфабрикатов(котлет). Кроме того, увеличение асортимента мясных продуктов позволит предприятиям быть более гибкими и адаптивными к изменяющимся рыночным условиям. Новые продукты могут привлечь внимание потребителей и стать ключевым конкурентным преимуществом перерабатывающих предприятий. Расширение линейки продукции также способствует увеличению доходов и улучшению финансового положения предприятия за счет увеличения объемов продаж.*

Целью исследования является рациональное использования вторичного мясного сырья для производства новых продуктов лечебно-профилактического питания с повышенной биологической ценностью. В данной статье были исследованы сенсорные и органолептические показатели, физико-химические показатели (белка, жиры, углеводы, влаги, золы,), витаминный состав, а также текстурный профильный анализ новых рубленных полуфабрикатов из субпродуктов с добавлением местного растительного порошка тары.

Ключевые слова: *субпродукты, рубленые полуфабрикаты, тары, текстурный профиль.*