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Сравнительный анализ традиционных и модернизированных питательных сред для культивирования лактобактерий

The comparative analysis of traditional and modernized nutrient media for the cultivation of lactobacilli

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Аннотация. *Питательные среды для культивирования лактобактерий используются для их роста и размножения в лабораторных условиях, а также при производстве молочных продуктов. В состав разработанной питательной среде входят: обезжиренное молоко, дрожжевой экстракт, лактоза, лактулоза, агар-агар, дистиллированная вода. Данный комплекс компонентов обеспечивает лактобактерии необходимыми питательными веществами, включая углеводы, белки, витамины и минералы. Составные компоненты питательной среды являются доступными и дешевыми для приобретения. В работе проводилось культивирование различных видов лактобактерий. (Lactobacillus acidophilus,, Lactococcus lactis, Lactobacillus bulgaricus; Lactobacillus casei) на разных питательных средах (MRS, Молочный агар с пребиотиками). Из результатов опытов следует, что интенсивность роста лактобактерий на питательной среде «Молочный агар с пребиотиками» выше, в сравнении с аналогами промышленного производства. Наибольшая интенсивность роста пробиотиков наблюдается в образцах, выращенных на закваске для йогурта, в состав которой входят пробиотики видов: Lactococcus lactis; Lactobacillus bulgaricus; Lactobacillus casei, выросшей на питательной среде «Молочный агар с пробиотиками», она составляет 3,7 * 10^{-9} КОЕ/гр. Подтверждена высокая экономическая эффективность разработанной питательной среды по сравнению с зарубежными аналогами. Разработана рецептура производственной питательной среды, необходимой для культивирования пробиотических микроорганизмов. Установлено, что интенсивность роста различных лактобактерий на данной среде выше в сравнении с аналогами промышленного производства. Подтверждена высокая экономическая эффективность разработанной питательной среды по сравнению с зарубежными аналогами.*

Ключевые слова: питательная среда, лактобактерии, пробиотики, культивирование, интенсивность роста, себестоимость

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Abstract. *Culture media for lactobacilli cultivation are used for their growth and reproduction in laboratory conditions, as well as in the production of dairy products. The composition of the developed nutrient*

*medium includes: skimmed milk, yeast extract, lactose, lactulose, agar-agar, distilled water. This complex of components provides lactobacilli with essential nutrients, including carbohydrates, proteins, vitamins and minerals. The constituent components of the nutrient medium are affordable and cheap to purchase. The work involved the cultivation of various types of lactobacilli. (Lactobacillus acidophilus, Lactococcus lactis, Lactobacillus bulgaricus; Lactobacillus casei) on various nutrient media (MRS, Milk agar with prebiotics). It follows from the results of the experiments that the growth rate of lactobacilli on the nutrient medium "Milk agar with prebiotics" is higher in comparison with analogues of industrial production. The greatest intensity of probiotic growth is observed in yogurt starter culture, which includes probiotics of the following species: Lactococcus lactis; Lactobacillus bulgaricus; Lactobacillus casei, grown on the nutrient medium "Milk agar with probiotics", it is 3.7 * 10^9 CFU/ gr. The high economic efficiency of the developed nutrient medium in comparison with foreign analogues has been confirmedA formulation of a production nutrient medium necessary for the cultivation of probiotic microorganisms has been developed. It has been established that the growth rate of various lactobacilli on this medium is higher in comparison with analogues of industrial production. The high economic efficiency of the developed nutrient medium has been confirmed in comparison with foreign analogues.*

Keywords: nutrient medium, lactobacilli, probiotics, cultivation, growth rate, cos

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Introduction. Healthy microflora plays an important role in the normal functioning of the entire body. Recently, the study of the composition and principles of operation of the gastrointestinal tract has attracted great interest. At the beginning of the twentieth century, studies were carried out that established the presence of various types of probiotic microorganisms inside the intestines of the human body [5, 7, 12].

Probiotics are live microorganisms that provide benefits to the host when administered to the body in adequate quantities. The human microflora is constantly thinning, probiotic microorganisms die, this is due to poor nutrition, unhealthy lifestyle, poor sleep, and constant stress. Problems with intestinal microflora affect the overall health of a person, have a detrimental effect on the condition of the skin, hair, nails, and worsen the condition of the immune, respiratory, digestive, cardiovascular, and nervous systems of the body. These harmful factors led to the creation of a new form of medicines, later called probiotic drugs. Later, special nutrient media were developed that were used to cultivate certain types of bacteria, which became a medicine for humans that helped restore their own microflora. Their quality, viability, and functions depend on the quality and composition of the nutrient medium for cultivating various types of microorganisms [1, 2, 6].

One of the types of probiotic microorganisms are lactobacilli, which have the ability to metabolize lactose and milk sugar. They play an important role in maintaining the health of humans and animal ecosystems. This type of probiotic microorganisms is characterized by increased requirements for the nutrient media used for their cultivation. To stimulate their growth, various additives are added to such media to enhance the growth of microorganisms. In recent years, a large number of nutrient media have been created, differing in composition, characteristics, production methods, growth intensity and pricing policy [3, 4, 13].

The market for these products in Russia is not well developed. Previously, nutrient media were purchased from foreign companies and there was no need for their domestic production, because foreign nutrient media were of good quality and relatively low cost. However, in modern conditions of social-market relations, a massive outflow of foreign products has arisen on the part of the European Union and the United States. The decrease in imports of nutrient media and starter cultures had a strong impact on the Russian market of medicines and the market of functional food products. A sharp shortage of products enriched with probiotic components has created conditions for the development of the Russian market of nutrient media and probiotic starters [8, 11].

For many types of research work, laboratory and clinical tests, probiotic microorganisms are used, so formulations of nutrient media for their cultivation can help these researchers

continue their work, regardless of imported supplies of nutrient media. Enterprises producing products enriched with probiotics will be able to use this nutrient medium, the cost of which is several times lower than average market prices, and thereby reduce the cost of the finished product [9].

Cultivation of probiotic microorganisms in laboratory conditions is of great importance, because probiotics are used in medicine, in the food industry, and in cosmetology.

The purpose of the study was to modernize the "Milk Agar" nutrient medium and compare its growth qualities with traditional media for cultivating microorganisms of the genera *Lactococcus, Lactobacillus.*

Materials and research methods. The work compared the growth intensity of 4 different probiotic starter cultures on two nutrient media: MRS and "Milk agar with prebiotics".

In the experiment, the control was MRS medium (control) g/l: bacteriological agar, bacteriological peptone, dextrose, K_2HPO_4, magnesium sulfate, manganese sulfate, meat extract, sodium acetate, Tween 80, yeast extract, ammonium citrate.

As part of the experiment, a nutrient medium was developed based on the Milk Agar medium, the main component of which is skim milk. In the experimental nutrient medium, prebiotic components and yeast extract were added to the main components, since it was experimentally established that they affect the growth rate of probiotic microorganisms.

The composition of the experimental liquid nutrient medium "Milk agar with prebiotics" included the following components, g/l: skim milk, yeast extract, agar-agar, lactose, lactulose, distilled water.

Liquid nutrient media were prepared as follows:

- Control:

The finished mixture of dry components (m =62 g) was mixed with 1 liter of distilled water. The solution was thoroughly mixed and heated to dissolve all components. The culture medium was then sterilized by autoclaving at 121 $^{\circ}$C for 12 minutes.

- Experimental:

A dry mixture of components was prepared, for this purpose yeast extract (m = 3 g), agar-agar (m = 2 g), lactose (m = 20 g), lactulose (m = 10 g) were mixed, skim milk (V = 400 ml) and distilled water (V = 575 ml). The resulting solution was thoroughly mixed and heated to dissolve all components. The nutrient medium is then sterilized by autoclaving at 110 $^{\circ}$C for 15 minutes. The solid nutrient medium differs in composition from the liquid nutrient medium in the amount of agar-agar (equal to 15 g/l).

The following types of probiotic cultures were selected for laboratory testing:

– *Lactococcus lactis subsp lactis* – Manufacturer: FGBNU, "Experimental Biofactory", Russia.

– *Lactococcus mixture lactis; Lactobacillus bulgaricus; Lactobacillus with asei* (yogurt starter) – VIVO. Manufacturer: Vivo LLC Industry", Russia.

– *Lactobacillus acidophilus – Manufacturer:* CJSC Pharmaceutical Company LECCO, Russia.

– *Lactococcus mixture lactis subsp lactis, Lactococcus lactis subsp cremoris, Lactococcus lactis subsp dracetilactis* (cheese starter) – Manufacturer: Federal State Budgetary Institution "Experimental Biofactory", Russia.

Description of the experiment:

Inoculation of microorganisms was carried out simultaneously on control and experimental media. Each flask was filled with 15 ml of nutrient medium and 0.1 g of probiotic starter. All samples were cultivated under anaerobic conditions for 48 hours at 37 ^{o}C.

6, 24, 36 hours after the start of the fermentation process, 1 ml of solution was taken from each test tube to determine the growth rate. For this purpose, the tenfold measurement method was used. Microorganisms were reseeded onto solid nutrient media, which were then cultivated in an anaerostat at 37 ^{o} C for 48 hours. CFU/g was calculated for each sample using the following formula:

$$N_f = \frac{Ni \times Vf}{Vi} \times D$$

Where:

Nf - final number $_{of}$ bacteria (after dilution)

N_i - initial number of bacteria (taken for dilution)

V_f - volume of the final culture (after dilution)

V_i - volume of initial culture (taken for breeding)

D - tenfold dilution factor (the number of times the culture was diluted)

The experiment was carried out in 10 speeds. The results obtained were statistically processed using STATISTICA 10.0 software; the nonparametric Mann–Whitney test was used to compare data from independent groups. Below are the results of the experiments, each value is an arithmetic mean.

Research results and their discussion. A comparison of the growth intensity of the same strain on the MRS nutrient medium and the Milk Agar with Probiotics nutrient medium is presented in Figures 1, 2.

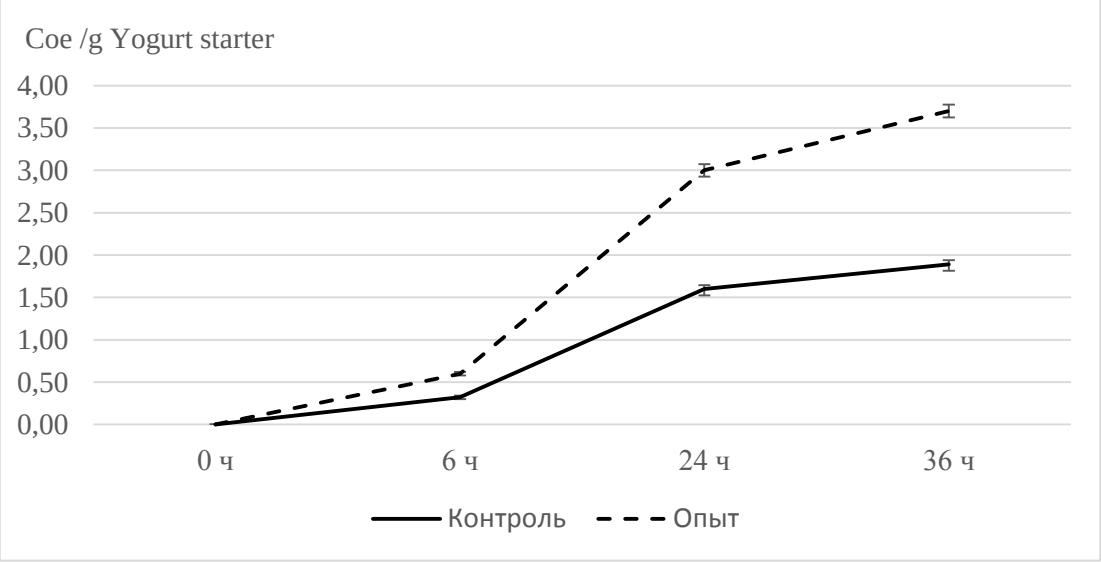

Figure 1 – Dependence of the growth intensity of probiotics (*Lactococcus lactis; Lactobacillus bulgaricus; Lactobacillus with asei*) on various nutrient media depending on the cultivation time

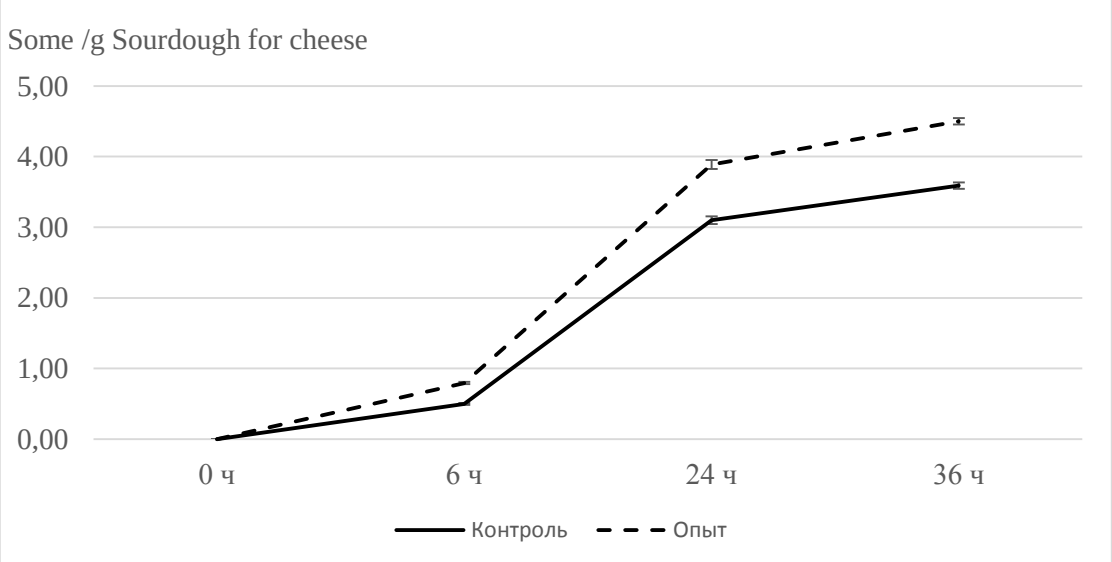

Figure 2 – Dependence of the growth intensity of probiotics (*Lactococcus lactis subsp lactis, Lactococcus lactis subsp cremoris, Lactococcus lactis subsp dracetilactis*) on various nutrient media depending on the cultivation time

The graphs show the dependence of the growth rate of probiotic microorganisms. The highest growth rate of microorganisms is observed in the period from 6 to 24 hours, which is characteristic of the exponential growth phase.

After completing the fermentation process, using two different nutrient media, it was found that the number of CFU/g was higher in the Milk Agar with Probiotics medium. This pattern is observed in both figures.

The highest intensity of probiotic growth is observed in yogurt starter, which contains probiotics of the following species: Lactococcus lactis ; Lactobacillus bulgaricus ; Lactobacillus with asei grown on the nutrient medium "Milk agar with probiotics", it is $3.7 * 10^9$ CFU/g.

The content of CFU/g in the samples at the end of the fermentation process, after 36 hours, is presented in Table 1.

Table 1 – Growth rate (in CFU/g) of probiotic microorganisms on various nutrient media

Name of strains	CFU on MRS	CFU on "Milk agar with prebiotics"
L. lactis; Lactobacillus bulgaricus; Lactobacillus with asei	$1.9 * 10^9$	$3.7 * 10^9$
L. lactis subsp lactis, L. lactis subsp cremoris, L. lactis subsp dracetilactis	$3.6 * 10^9$	$4.5 * 10^9$
L. _ lactis	$2.7 * 10^9$	$3.7 * 10^9$
L. _ acidophilus	$3.5 * 10^9$	$4.4 * 10^9$

The results of the study show that the presented probiotic microorganisms grow more actively on the nutrient medium "Milk agar with prebiotics". The number of cells cultured on this nutrient medium is greater than their number on MRS medium in:

– 1.97 times for strains *L. _ lactis; Lactobacillus bulgaricus; Lactobacillus with asei*.

– 1.25 times for strains *L. _ lactis subsp* lactis, *L. lactis subsp cremoris*, *L. lactis subsp dracetilactis*.

– 1.4 times for *strain L. lactis.*

– 1.26 times for *strain L. acidophilus*

The cost of 1 liter of the nutrient medium "Milk agar with prebiotics" is calculated in detail in Table 2.

Table 2 – Calculation of the cost of the nutrient medium "Milk agar with prebiotics"

Component	Weight in nutrient medium	Cost per mass used in the environment, rub.
Skimmed milk	400 ml	17.6
Yeast extract	3 g	7.9
Lactose	20 g	8
Lactulose	10 g	7
Agar-agar	2 g	6.4
Distilled water	575 ml	6.4
Total	58.2 r / l	

The total cost of the nutrient medium "Milk agar with prebiotics" was 68.2 rubles/l, and the MRS medium was 535.5 rubles/l.

Conclusion. The study showed the effectiveness of the experimental nutrient medium "Milk agar with prebiotics". The low cost of the resulting medium makes it possible to produce it on an industrial scale. The efficiency of this nutrient medium is sufficient for it to be competitive. The article presents a recipe for the production of liquid and solid variations of nutrient media. All components can be purchased from domestic manufacturers. The conducted studies can serve as a model for studying the use of other types of raw materials for the preparation of nutrient media.

The obtained result can be considered quite optimal as an import-substituting raw material. The development will allow Russian manufacturers of probiotic products to obtain a

more profitable solution regarding the purchase of high-quality raw materials and production of their own.

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