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BIOTECHNOLOGY CULTIVATING THE PHYSIOLOGICALLY ADAPTED LACTOBACILLI TO CREATE MICROBIAL BIO-ADDITIVE FOR POULTRY

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Abstract:

This article presents the results of research on the selection of the most suitable nutrient medium, ensuring maximum biomass growth of lactic acid microorganisms. The objects of research were pure cultures of microflora of the gastrointestinal tract of quails - Lactobacillus agilis, Lactobacillus intermedius and Lactobacillus salivarius. For the cultivation of microorganisms used 4 nutrient medium: medium for lactic acid microorganisms (Uglich), molasses autolysate medium (MAM), glucose-peptone medium (GPM) and medium MRS. In the cultivation of bacteria, the time and temperature of growth served as variable parameters. In the process of cultivation, the analysis of the dynamics of consumption of reducing substances and the titer of microorganisms was carried out. According to the results of the cultivation of microbial cultures, an active consumption of the carbon substrate was found in the nutrient media variants used, it was found that by 24 h of cultivation a maximum of cells was observed. Based on the experiments performed, it was established that with different parameters, the highest cell titer was achieved by 24 h at a temperature of 38.0 ° C on molasses autolysate medium. Thus, molasses autolysate medium can be recommended under production conditions as the cheapest substrate for the further development of probiotic biologics for the poultry industry.

Key words: *lactobacilli, nutrient medium, cultivation, titer, dynamics, temperature, time, reducing substances, microscopy, morphology.*

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INTRODUCTION:

The widespread use of antibiotics in feed composition has now led to a decrease in the natural resistance of the organism of poultry. Long-term antibiotic therapy provokes the suppression of the bird's own microflora, disruption of the body's metabolic processes, and has a negative effect on the reproductive system [1, 2].

An alternative to the use of antibiotics is the correction of bird endomicroecology with the help of live microorganisms, which, when administered in physiological amounts, benefit the health of the host organism [3, 4]. The most preferable for these purposes are the strains that are included in the natural for this species and evolutionarily fixed microbial associations. They should have an increased functional adaptation to the physiological characteristics of the farmed birds [5; 6]. Thus, the selection of nutrient media for newly isolated beneficial microorganisms from the gastrointestinal tract of birds is a relevant study, and the development of new domestic microbial preparations of microbial origin with complex action is a promising and economically sound direction in the poultry industry [7; 8].

MATERIAL AND METHODS:

The work was carried out in the research laboratory of the Department of Biotechnology, Biochemistry and Biophysics FSBEI HE Kuban GAU, the purpose of which was to determine the nutritional needs and optimal conditions for the cultivation of lactobacilli used to intensify the processes of obtaining their bioMAMs. The objects of research were lactobacilli - *Lactobacillus agilis, Lactobacillus intermedius* and *Lactobacillus salivarius*, which were isolated from the blind processes of the gastrointestinal tract by an independent microbiological method, real-time quantitative polymerase chain reaction and metagenomic methods [9].

The first stage of research included the cultivation of bacteria on standard nutrient media when grown on rocker flasks in a laboratory rotary shaker.
The cultivation time for all bacterial cultures was 48 h, with an optimal temperature of 38 ° C and rocking speed of 50 rpm.

To optimize the nutritional needs of lactic acid microorganisms used environment of the following composition:
1. Environment for lactic acid bacteria: yeast extract - 0.2%; corn extract - 0.3%; glucose syrup - 2%; ascorbic acid (sodium citrate) - 4 g / l; KH_2PO_4 - 2 g / l.
2. The composition of the medium MRS, g / l: peptone - 10.0; yeast extract - 20.0; glucose - 20.0; dipotassium phosphate - 2.0; sodium acetate - 5,0; triammonium citrate - 2.0; magnesium sulfate - 0.2; manganese sulfate 4-water - 0.05.
3. The composition of the medium GPM, g / l: Na_2HPO_4 12-water - 3.2; KH_2PO_4 - 0.3; $MgSO_4$ - 0.5; NaCl - 0.5; peptone - 2.0; yeast extract - 0.05; glucose - 25.
4. The composition of the medium MAM: molasses fodder - 45 g / l; K_2HPO_4 - 2 g / l; yeast autolysate - 10 ml / l.
The second stage of the research was to determine the optimal cultivation temperature for each strain of the microorganism.

Culture *Lb. salivarius, Lb. intermedius* and *Lb. agilis* are thermophilic, i.e., growing at sufficiently high temperatures, due to their habitat, namely, the gastrointestinal tract of the bird, where the normal temperature varies, depending on the type of bird, within 38–41 °C.

To determine the microflora titer, 1.0 ml of each culture was taken and placed in a flask with a volume of 100 cm^3 and 99.0 ml were poured with sterile saline, left for 1 hour. A 1: 100 dilution was obtained. After that they prepared a series of consecutive tenfold dilutions up to 10^{-9}. Separate sterile tips were used for each dilution. Sowing in Petri dishes was carried out according to (GOST 10444.11-89 (clause 4.2.2) on Laktobakagar from dilutions 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}. From dilutions 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} with a sterile tip of the automatic dispenser, 1 ml of the suspension was transferred into 4 petri dishes, into which sterile, molten nutrient medium was poured, cooled to 38–40 °C. In a circular motion, the petri dishes were mixed in the medium and left to agar with inoculated media were placed in a thermostat and kept at (38 ± 1) °C for 72 hours. By the number of grown colonies, according to (GOST 9225-84 (4.5.3) determined total titer of microorganisms.

The number of viable cells in 1 ml of the drug (X), was calculated by the formula:

$$X = N \times P \quad (1)$$

where: N – average of the number of colonies in Petri dishes;
P – serial number of the tenfold dilution in which the growth of bacteria is noted.

In the process of cultivation on rocker flasks, the analysis of the dynamics of consumption of reducing substances (RS) with an initial concentration of 4% was carried out. The method for the determination of reducing substances is based on the reducing ability of the monoforms of sugars - glucose and fructose.

RESULTS AND DISCUSSION:

According to the results of growing microbial cultures in rocking flasks, active consumption of the carbon substrate was found in the nutrient media used, it was found that by 24 h of cultivation a maximum of cells was observed in the exponential phase and a transition to a stationary phase was observed.
As a result of the study, the most effective was MAM

(*Lb. agilis* - 2.0 × 10^{10} CFU / ml; *Lb. intermedius* - 1.1 × 10^{9} CFU / ml; *Lb. salivarius* - 5.0 × 10^{10} CFU / ml) and medium for lactic acid bacteria, Uglich (*Lb. agilis* - 3.5 × 10^{10} CFU / ml; *Lb. intermedius* - 7.0 × 10^{9} CFU / ml; *Lb. salivarius* - 3.1 × 10^{10} CFU / ml). When growing lactobacilli on GPM medium, the following results were obtained: *Lb. agilis* - 4.3 × 10^{9} CFU / ml; *Lb. intermedius* - 1.0 × 10^{9} CFU / ml; *Lb. salivarius* - 5.2 × 10^{9} CFU / ml, and on MRS: *Lb. agilis* - 6.2 × 10^{9} CFU / ml; *Lb. intermedius* - 1.5 × 10^{9} CFU / ml; *Lb. salivarius* - 5,2 × 10^{9} CFU / ml.

Also in the process of cultivation, the dynamics of consumption of reducing substances (RS) on MAM were recorded, the results of which are shown in Figure 1.

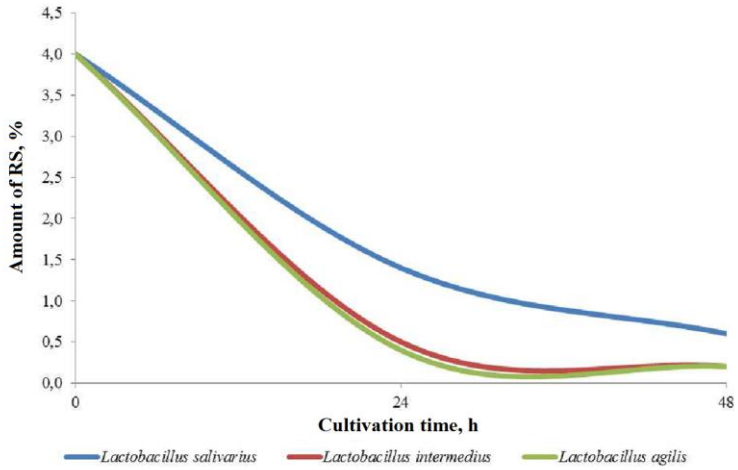

Figure 1: Dynamics of consumption of reducing substances by cultures of lactobacilli at MAM

According to the results of monitoring the consumption of the carbon substrate of the studied microorganisms, it can be concluded that it is already depleted by 24 hours from the start of the cultivation of lactobacilli.

Further, in the experiment, it was interesting to determine the optimal cultivation temperature of the tested microorganisms in order to increase the cell biomass as soon as possible in the shortest growing time. It should be noted that the results of the cultivation of microorganisms presented above were obtained at a growth temperature of 38 ° C.

Then, a series of experiments was laid out in the research work to determine the maximum thermotolerance of these cultures when grown on a molasses autolysate medium for 48 hours. The results of the dependence of the number of lactic acid bacilli cells on temperature are presented in Figures 2 and 3.

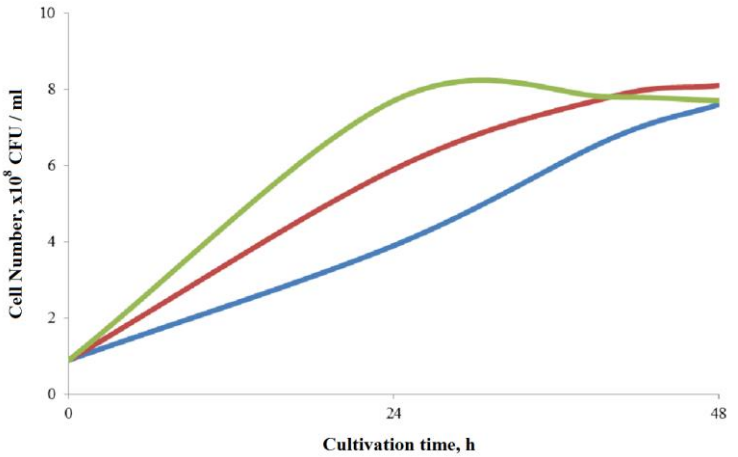

Figure 2: Dynamics of change in the number of lactic acid bacilli cells at 39 °C.

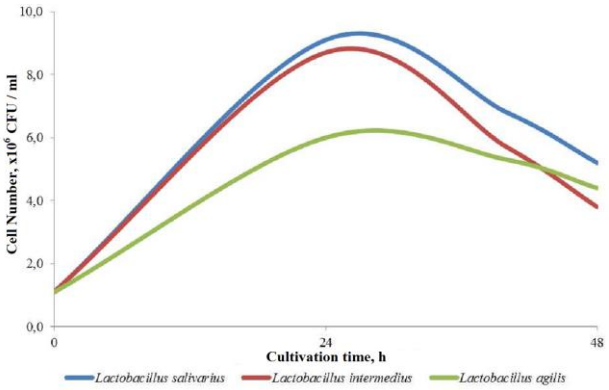

Figure 3: Dynamics of change in the number of cells of lactic acid bacilli at 40 ° C.

The result of cultivation at 41 °C is not represented, since at this temperature no culture showed growth relative to the seed titer. However, after the cultivation temperature was lowered to 38 ° C, the growth was restored in the same volume, which indicates that the culture did not die, but entered the phase of growth inhibition, which continued until the temperature decreased by 2–3 °C.

Based on the results of cultivation on various media and at different temperatures, it was established that the highest cell titer was achieved by 24 hours from the start of the cultivation, regardless of the composition of the media. Longer growth and increase in temperature leads to a decrease in titer and, as a rule, an increase in the number of non-viable cells.

Separately, during cultivation, microscopic monitoring of the state of the cells, changes in their morphology and the presence of extraneous microflora were performed.

For microscopic control, Carl Zeiss Axio Imager research microscopes were used in both light-field and phase-contrast modes. The preparations were fixed in Carnoy's fixative and stained by Gram. Microscopy was performed with oil immersion with a 100 × objective lens and a 10 × eyepiece (Figure 4-6).

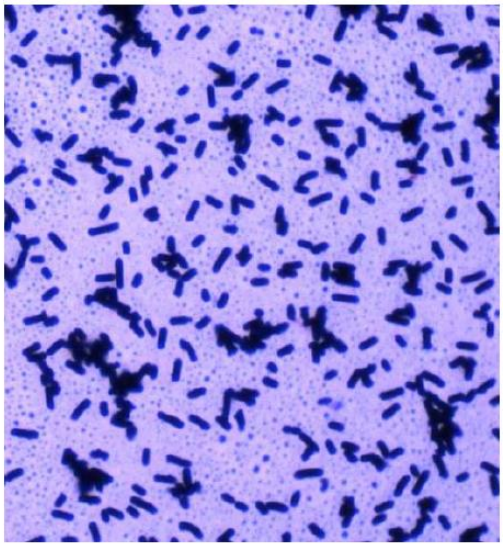

Figure 4: *Lactobacillus salivarius*, 24 h growth, Gram stain, 1200-fold increase

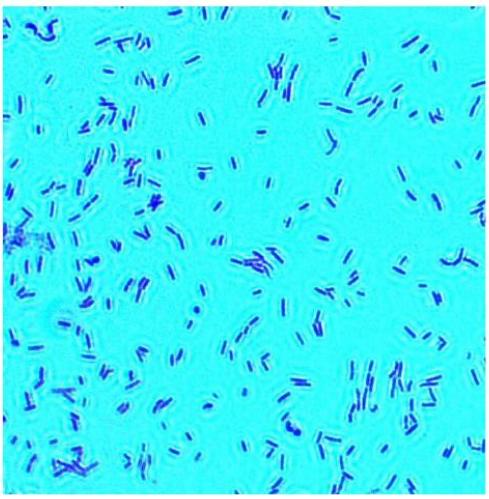

Figure 5: *Lactobacillus agilis*, 24 h growth, gram stain, 1200-fold increase

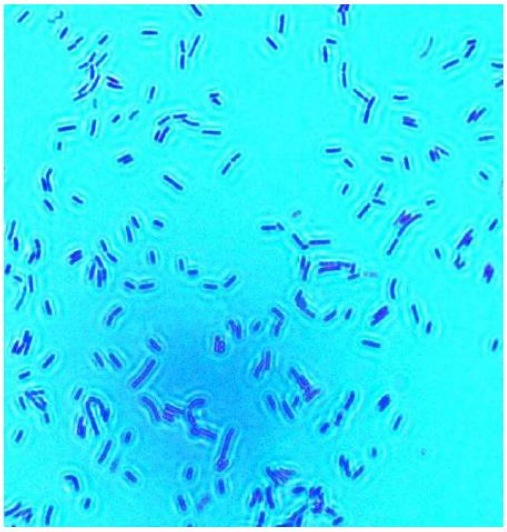

Figure 6: *Lactobacillus intermedius*, 24 h growth, gram stain, 1200-fold increase

The morphology of the cells has a constant size in almost all cultures used - these are short rods from 3-4 μm and thickness to 0.5 μm. Polymorphism inherent in some types of lactic acid bacteria is absent. As the culture ages, all cells tend to be somewhat shortened and crushed.

CONCLUSION:

The results of the studies showed that molasses autolysate is the most cost-effective nutrient medium, while the optimum temperature and time regimes are 38 ° C and 24 hours, respectively. molasses autolysate medium can be used in production conditions with the further development of biological products for the poultry industry.

REFERENCES:

1 Rodionova AS. et al. The autochthonous microflora of wild birds is the basis of therapeutic and prophylactic drugs for industrial birds.XXVIII winter youth scientific school "Perspective directions of physical and chemical biology and biotechnology". Collection of theses. M.: FANO of Russia, 2016.

2 Donnik IM, Lebedeva IA. The condition of the stomach and intestines of broiler chickens when using the probiotic preparation monosporin. Kuban veterinary medicine. 2011; 3: 15-16.

3 Koshchaev AG, Koschaeva OV, Kalyuzhny SA. Probiotic trilactobact in quail feeding. Polythematic network electronic scientific journal of the Kuban State Agrarian University. 2014; 95: 633-647.

4 Kirillova ER. et. al. Metagenomic profiling of bacteriocenoses of the digestive tract of various lines of quails. Proceedings of the All-Russian International Conference on the 40th anniversary of the Department of Genetics of the Kazan Institute of Fundamental Medicine and Biology university. Kazan. 2016: 55-56.

5 Migina EI, Lysenko YuA, Koshchaev AG. Study of the toxicological and irritating action

of the probiotic feed additive Trilacososorb for use in the quailing. Veterinary Medicine of Kuban. 2014; 4:13-16.

6 Semenov VV, Kononenko SI. Method of Improving Feed Conversion. Collection of Scientific Works of the All-Russian Research Institute for Sheep and Goat Breeding. 2012; 1(5): 114-117.

7 Koshchaev AG. et. al. Chlorella and trichoderma as functional feed additives for quails. Agrarian Science. 2012; 7:28-29.

8 Chikov A. et. al. The effectiveness of probiotics with increased fiber content in the diet of pigs. Combined feeds. 2012; 7:95-96.

9 Chernykh OU. et. al. Experience Of Diagnostics And Containment Of Foot And Mouth Disease Of Cattle In Krasnodar Region, Russia. Journal Of Experimental Biology And Agricultural Sciences. 2017; 6(5): 786-792.