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THE EFFECT OF AEROSIL A-300 ON THE GROWTH OF BACTERIA *LACTOBACILLUS PLANTARUM*, *BIFIDOBACTERIUM ADOLESCENTIS* AND *STREPTOCOCCUS LACTIS*

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Summary. The article presents data on the effect of Aerosil A-300 on the growth of bacteria *Lactobacillus plantarum* No. 7-317, *Bifidobacterium adolescentis* No. 17-316, *Streptococcus lactis* No. 5. The results of studies have shown that the most optimal for the growth of bacteria content of Aerosil A-300 in the environment is 2.0%. It has been found that when Aerosil was added to nutrient media, bacteria grew and actively accumulated a significant number of viable cells: $3.8–4.5\times10^7$ CFU/cm^3 (control $1.1–2.5\times10^7$ CFU/cm^3) according to the average technological parameters pH 7.0 and the temperature of 37 °C. Studies have shown that the relative increase in the number of cells, by which we assessed the effect of Aerosil on growth, was in *Lactobacillus plantarum* No. 7-317 (87.5 ± 12.0), which is 23% higher than control, in *Bifidobacterium adolescentis* No. 17-316 — (79.2 ± 11.9), which exceeded the control data by 14%

Keywords: fumed silica, nutrient medium, probiotic strains, cultivation

Introduction. Analysis of the technology of production of bacterial drugs showed that an important and promising direction is to improve the initial — basic stages of production, namely the creation of the most productive conditions for biomass accumulation — optimization of nutrient medium (Gujvinska and Paliy, 2018a, 2018b, Raskoshnaya et al., 2016).

Standardization of production media in terms of nutritional value is one of the main factors determining the quality of the finished product. According to the literature (Gujvinska et al., 2018; Kovalenko et al., 2010; Kozlovska et al., 2012), complex nutrient media are regulated for the cultivation of probiotic strains: hydrolysates and extracts of milk, casein, liver, baker's yeast and muscle. The results of the literature data indicate that these media contain a complete set of 19 amino acids (AMA). According to the literature (Danylenko et al., 2013; Kigel et al., 2003; Poltavska, 2006; Ravliuk and Dekhtiarenko; 2016), the main components of the nutrient medium for growing bifidobacteria are sources of amino acid nitrogen, trace elements (sulfur), vitamins, growth factors, peptones, sodium chloride, components to increase the density of the medium and others.

Yeast is a source of amino acids, growth factors, vitamins; the source of peptones and minerals is casein hydrolyzate; the source of sulfur — sulfur-containing amino acid — L-cystine; because bifidobacteria are strict anaerobes, to increase the density of the nutrient medium, in order to complicate the diffusion of air into the medium, agar is used; sodium chloride is used to maintain the appropriate osmotic pressure of the nutrient medium; Lactose, which is metabolized by bifidobacteria and lactobacilli, is used as a source of sugars.

Lactic acid bacteria are very common in nature (Poltavska, 2006; Ravliuk and Dekhtiarenko, 2016). The study of the biological properties of lactobacilli and bifidobacteria, as well as other microorganisms, requires the ability to long-term preservation of cultures (Kovalenko, 2014; Timchenko, 2010; Yankovs'kiy, 2005). This is necessary both to maintain the collections of lactic acid bacteria in a highly active state, and for the manufacture and storage of probiotics (Kovalenko, 2002; Khyzhniak, 2014).

These circumstances required the search for a new composition of the nutrient medium based on the components from domestic raw materials, which would reduce its cost and increase growth properties, in particular to accelerate the growth of lactobacilli, bifidobacteria and the accumulation of their bacterial mass.

One of the substances that has good adsorbing and stabilizing properties is Aerosil — amorphous anhydrous silicon dioxide, belongs to the group of synthetic active highly dispersed mineral fillers. In pharmacy and bioindustry it is used as an excipient, stabilizer, gelling agent, adsorbent, adjuvant and a substance that improves the fluidity of tablets, ointments, gels and other mixtures.

The aim of our research was to study the effect of Aerosil A-300 in the composition of growth substrates on the growth of bacteria *Lactobacillus plantarum* No. 7-317, *Bifidobacterium adolescentis* No. 17-316, *Streptococcus lactis* No. 5 in their cultivation.

Materials and methods. The study was performed using strains *L. plantarum* No. 7-317, *B. adolescentis* No. 17-316, *St. lactis* No. 5.

Perfection the technology of cultivation of production strains *L. plantarum* No. 7-317, *B. adolescentis* No. 17-316, *St. lactis* No. 5 on different growth substrates was performed with the addition of Aerosil A-300. For this purpose, samples of media for the cultivation of lactic acid bacteria were prepared. Aerosil A-300 was added to them in final concentrations of 1.0%, 2.0%, and 3.0%. After that, lactic acid bacteria at a concentration of 10^7 CFU/cm^3 were added to the medium with Aerosil and cultured at a temperature of 37 °C.

Aerosil 300 (fumed silica, CAS number 112945-52-5) white powder, amorphous, non-porous. Insoluble in water, acids and dilute alkalis. It has good sorption properties. Specific surface area 200 ± 25 m^2/g; the average particle size is 12 μm; the pH of 4% aqueous dispersion is from 3.6 to 4.3. Numerous studies have confirmed that Aerosil has therapeutic effect in diseases of the gastrointestinal tract.

The effect of Aerosil on the growth activity of lactic acid cultures was assessed using the indicator of relative growth of biomass of microorganisms during the daily period of their growth (X):

$$X = \frac{N_{24}}{N_0},$$

where: N_0 — the initial concentration of the cells of microorganisms; N_{24} — the final concentration of the cells of microorganisms.

and the stimulation indicator — relative growth of bacteria in experiment and control (SI):

$$SI = \frac{X_{exp}}{X_{control}},$$

where: SI in the control medium was taken as a unit; X_{exp} — experimental medium; $X_{control}$ — control medium without Aerosil.

The activity of the strains was evaluated by the results of skim milk fermentation for 72 hours and the ability to form acid according to the method of Bannikova (1987).

The number of living microbial cells was determined by serial dilutions of the resulting suspension in saline, followed by seeding of bacterial cultures per 0.1 cm^3 from dilution 10^6 to MRS-4 medium.

All studies were performed in triplicate. Statistical processing of the results was performed according to traditional methods of variation statistics using the program MS Excel and Statistica 10.

Results. It should be noted that modern biotechnology for the production of complex probiotics is based on the separate cultivation of different strains and their subsequent combination in certain proportions.

Given the symbiosis of beneficial bacterial flora in the human body, the peculiarities of the growth of cultures used (oxygen bonding, vitamin production, the need for certain nutrients, etc.), the need for nutrients in the recovery phase in the enteric environment, it is important and expedient to combine deep cultivation of different strains and the introduction of a prebiotic component, which will have a significant clinical effect. In addition, not only living cells are useful, but also the products of their metabolism (organic acids, bacteriocins, vitamins, etc.), which also have a positive effect on biochemical reactions in the body, confirming the need to preserve the culture medium in the combined preparation before drying.

We started the system of maintaining the biological properties of production strains of lactic acid bacteria at a high level and improving the system of their preservation by improving the medium for their cultivation.

The work was carried out to improve the technology of cultivation of production strains of *L. plantarum* No. 7-317, *B. adolescentis* No. 17-316, *St. lactis* No. 5 on growth substrates with the addition of Aerosil A-300, which can significantly stimulate growth activity and production of biologically active substances of some species of bacteria.

During the experiment, samples of media for the cultivation of lactobacilli and bifidobacteria were prepared, to which Aerosil A-300 was added in various concentrations from 1.0% to 3.0%. Then lactobacilli and bifidobacteria were added to the medium with Aerosil at a concentration of 10^7 CFU/cm^3. The cultures were incubated at a temperature of 37 ± 0.5 °C (Table 1).

Table 1 — Study of the growth of bacteria *B. adolescentis* No. 17-316 and *L. plantarum* No. 7–317 in the medium with Aerosil (M ± m)

Bacteria	Indicators	The amount of Aerosil A-300 in the test medium,%			
		1.0	2.0	3.0	Control
L. plantarum No. 7–317	SI	0.36 ± 0.04	0.76 ± 0.15	0.26 ± 0.16	1.0
	pH	6.7	7.1	6.8	7.2
B. adolescentis No. 17-316	SI	0.39 ± 0.08	0.91 ± 0.72	0.26 ± 0.12	1.0
	pH	7.1	6.9	7.2	7.2

Note: the stimulation index (SI) is the relative increase in the number of bacteria in the experiment and control.

The results of studies have shown that the most optimal for the growth of bacteria content of Aerosil A-300 in the medium is 2.0%. Thus, the stimulation index was 0.76 ± 0.15 for lactobacilli and 0.91 ± 0.72 for bifidobacteria.

The next stage of work was the preparation of experimental medium with Aerosil A-300 (2%) for the cultivation of lactobacilli and bifidobacteria.

Studies have shown that the relative increase in the number of cells, by which we assessed the effect of Aerosil on growth, was 87.5 ± 12 in *L. plantarum* No. 7-317, which is 23% higher than control; and 79.2 ± 11.9 in *B. adolescentis* No. 17-316, which exceeded the control data by 14% (Table 2).

Thus, if we take the index of stimulation of bacterial growth in the medium without Aerosil A-300 per unit, then at the optimal concentration of Aerosil administered into the medium (2.0%), it was maximum and amounted to 0.76 ± 0.15 for *L. plantarum* No. 7-317 and 0.91 ± 0.72 — for *B. adolescentis* No. 17-316.

It is known that when lyophilized probiotics are introduced into the intestine, only 10% of bacterial cells attach to its surface, colonizing the mucous membrane, and the rest of the cells are excreted. Therefore, one of the main tasks that arises in the development of effective probiotics is to achieve the maximum number of living cells in 1 dose of the drug. There is a direct relationship between the number of microbial cells of lactic acid bacteria that enter the body and the degree of their adhesion to the intestinal mucosa. The results obtained by us allowed us to conclude about the biocompatibility of probiotic strains of lactic acid bacteria and Aerosil A-300. Therefore, this sorbent is promising for the creation of effective complex drugs. In the combined drug, which will include both a probiotic and a sorbent, a significant proportion of bacterial cells can be adsorbed on the sorbent, forming a larger area of contact with the mucous membrane, and thus enhance the colonization resistance of the latter.

Table 2 — The effect of Aerosil A-300 on the growth of bacteria *L. plantarum* No. 7-317 and *B. adolescentis* No. 17-316 (M ± m)

Medium	Number of microbial cells, $\times 10^7$ CFU/cm^3		Relative cell growth		pH of the medium	
	L. plantarum No. 7-317	*B. adolescentis No. 17-316*	*L. plantarum No. 7-317*	*B. adolescentis No. 17-316*	*L. plantarum No. 7-317*	*B. adolescentis No. 17-316*
Experimental	4.2 ± 0.29	4.5 ± 0.17	87.5 ± 12.0	79.2 ± 11.9	7.1 ± 0.3	6.9 ± 0.2
Control	1.1 ± 0.56	2.5 ± 0.29	67.3 ± 13.0	68.2 ± 12.0	7.3 ± 0.4	7.1 ± 0.3

Note: relative cell growth — initial and final (after 24 h of growth) concentration of cells of the microorganism.

The next step was to study how Aerosil A-300 affects the growth and activity of lactobacilli and bifidobacteria. Studies have shown that the number of microbial cells grown on media with Aerosil increased — *L. plantarum* No. 7-317 ($3.8 \pm 0.29 \times 10^7$ CFU/cm^3) compared to control ($1.1 \pm 0.56 \times 10^7$ CFU/cm^3), *B. adolescentis* No. 17-316 ($4.5 \pm 0.17 \times 10^7$ CFU/cm^3) compared to control ($1.3 \pm 0.46 \times 10^7$ CFU/cm^3), *St. lactis* No. 5 ($3.8 \pm 0.37 \times 10^7$ CFU/cm^3) compared to control ($2.5 \pm 0.29 \times 10^7$ CFU/cm^3), and correlated with the rate of acid production (Table 3). It was found that the rate of acid formation in *L. plantarum* No. 7-317 was 145 ± 20 °T, and in the control group this figure was 115 ± 17 °T. It should also be noted that the rate of acid formation in *B. adolescentis* No. 17-316 was higher on the experimental medium and was 153 ± 30 °T, while in the control — 105 ± 20 °T. Good acid formation was on the experimental medium in *St. lactis* No. 5 — 141 ± 21 °T, and in the control this figure was much lower — 104 ± 17 °T. During the experiment, it was found that the most optimal concentration of Aerosil in the medium is 2.0%. Thus, during 24 hours of growth, the number of lactic acid and bifidobacteria increased and ranged from 3.8×10^7 CFU/cm^3 to 4.5×10^7 CFU/cm^3.

Table 3 — Indicators of activity of lactobacilli and bifidobacteria grown on medium with Aerosil A-300 (M ± m)

Medium	Number of microbial cells, $\times 10^7$ CFU/cm^3			Acid formation, °T		
	L. plantarum No. 7-317	*B. adolescentis No. 17-316*	*St. lactis No. 5*	*L. plantarum No. 7-317*	*B. adolescentis No. 17-316*	*St. lactis No. 5*
Experimental	4.2 ± 0.29	4.5 ± 0.17	3.8 ± 0.37	145 ± 20	153 ± 30	141 ± 21
Control	1.1 ± 0.56	1.3 ± 0.46	2.5 ± 0.29	115 ± 17	105 ± 20	104 ± 17

Conclusions. The possibility of culturing bacteria *Lactobacillus plantarum* No. 7-317, *Bifidobacterium adolescentis* No. 17-316, *Streptococcus lactis* No. 5 on the proposed nutrient medium with the addition of Aerosil A-300 was established.

It was found that the most optimal concentration of Aerosil in the medium for bacterial growth is 2.0%. Growth rates are different: the average activity of acid formation 146 ± 10 °T, the average number of live bacteria — $4.16 \pm 0.27 \times 10^7$ CFU/cm^3; cell morphology is characteristic.

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