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PHYSIOLOGIC AND BIOCHEMICAL PROPERTIES OF PROBIOTICALLY VALUABLE LACTIC ACID BACTERIA ALLOCATED FROM GASTROINTESTINAL TRACT OF CHICKENS

On the basis of the conducted researches physiological and biochemical properties of probiotically valuable lactic acid bacteria allocated from a gastrointestinal tract (GIT) of chickens are defined and analysed. The allocated bacteria are checked on compliance of their properties to the requirements imposed to probiotic substances. According to the results of the carried-out analysis lactic acid bacteria strains with the greatest probiotic potential are selected.

Introduction. Nowadays dangerous infectious diseases are widespread on many poultry farms of Belarus and the former Soviet Union, and the antibiotic therapy (having a number of serious shortcomings) is greatly applied to fight against them [1].

The alternative to antibiotic therapy in animal husbandry is the use of the probiotics the basis of which is bacteria allocated from healthy animals with a complex of valuable properties. Application of probiotics provides not only treatment of serious infectious diseases of poultry, but also serves as a preventive measure.

Thus, one of the most perspective directions of biotechnology is the development and production of probiotics – therapeutic and preventive preparations and functional food having positive impact on human and animal health.

A number of requirements is imposed to probiotic preparations: non-pathogenicity and non-toxicity; existence of antagonistic properties in relation to the main opportunistic strains of GIT microbiota bacteria that can be connected as with the formation of lactic acid during lactic-acid fermentation of lactose, and with allocation of bacteriocins as well; existence of lytic activity, the antibiotic resistance caused by chromosomal determinants, localized not as a part of mobile elements; ability to fixing, survival and reproduction in GIT [2].

Lactic acid bacteria even since I. I. Mechnikov's works deserved the reputation of the most demanded probiotics having complex of useful properties: good adhesion to the cells of intestine villi, ability to produce antimicrobial agents (various bacteriocins, lactic acid), stimulation of the immune answer of the owner. Now probiotic lactic bacteria of different types are used, mainly representatives of *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Enterococcus*. They find application both in prevention and treatment of dysbacteriosis and gastroenteritis of people and animals. At the same time researches on allocation and improvement of new probiotic bacteria don't stop [3, 4]. It, on the one hand, is caused by the increased popularity of probiotics against the problems caused by large-scale use of antibiotics in agriculture and prompt

accumulation of polyresistant to antibiotics bacteria forms: unlike antibiotics, probiotics provide replacement therapy and prevention of a disease, competing in gastrointestinal tract with a pathogenic microbiota. On the other hand, it is known that the specificity in adhesion of some bacteria to surface of cells of intestine villi of a certain owner takes place, this fact determines the need of looking for the bacteria most effectively colonizing GIT mucous of this or that owner.

Main part. As the purpose of the presented research was allocation of lactic-acid bacteria (LAB) from the dung of chickens and the subsequent selection of the most perspective probiotic strains on the basis of compliance of their physiologic and biochemical properties to requirements imposed to probiotic preparations, so the allocated bacteria were checked on the existence of a number of signs such as antagonistic properties to sanitary-indicative bacteria, lytic activity, antibiotic resistance and ability to develop in the conditions close to the conditions of hens GIT.

The lactic bacteria allocated from dung of chickens of Smolevichsky poultry farm acted as an object of the research.

For allocation of probiotically valuable lactic acid bacteria incubation the samples of dung in various elective conditions were incubated (Fig. 1.)

Among 134 allocated strains 50 strains belong to lactic bacteria. They are checked on compliance of their physiologic and biochemical properties to the requirements imposed to probiotic bacteria.

Existence of lytic activity will allow probiotic bacteria to take part in splitting of macromolecules on monomers, thereby increasing availability of these substances to the organism of the owner.

Determination of lytic activity was carried out by means of all-usable methods [5]. According to the results of determination of lytic activity it is established that 28 of 50 LAB possess proteolytic activity (56%), 5 – amilolitic (10%) and only 1 strain of LAB possesses both proteolytic and amilolitic activities. The lactic bacteria possessing cellulolytic activity was not revealed.

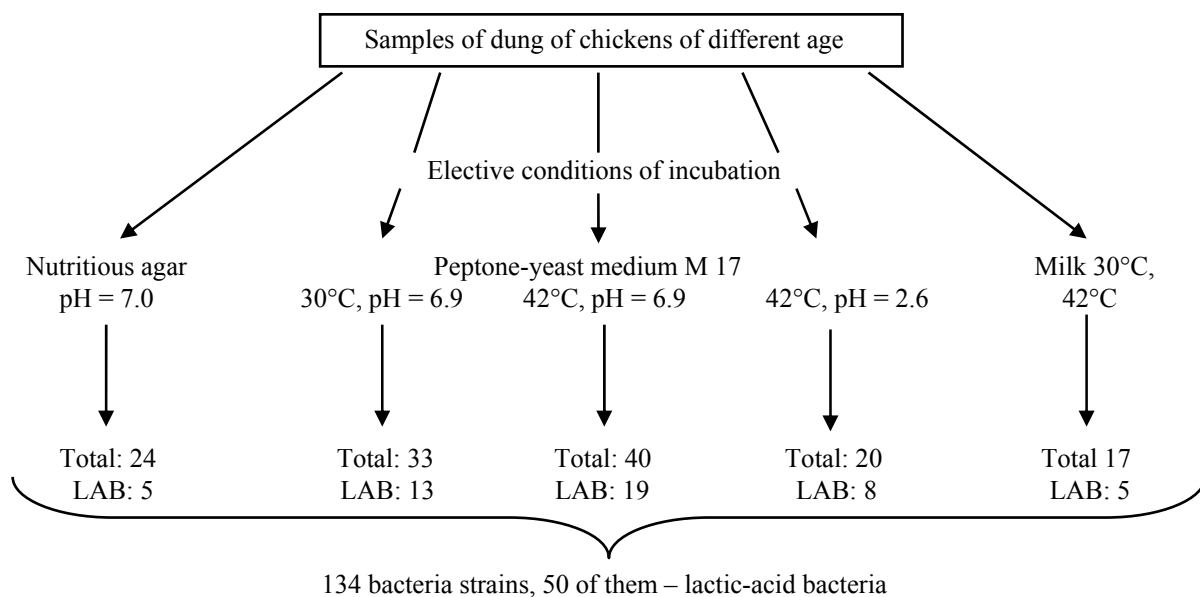


Fig. 1. The elective conditions of incubation used for allocation of various types of bacteria from dung of chickens

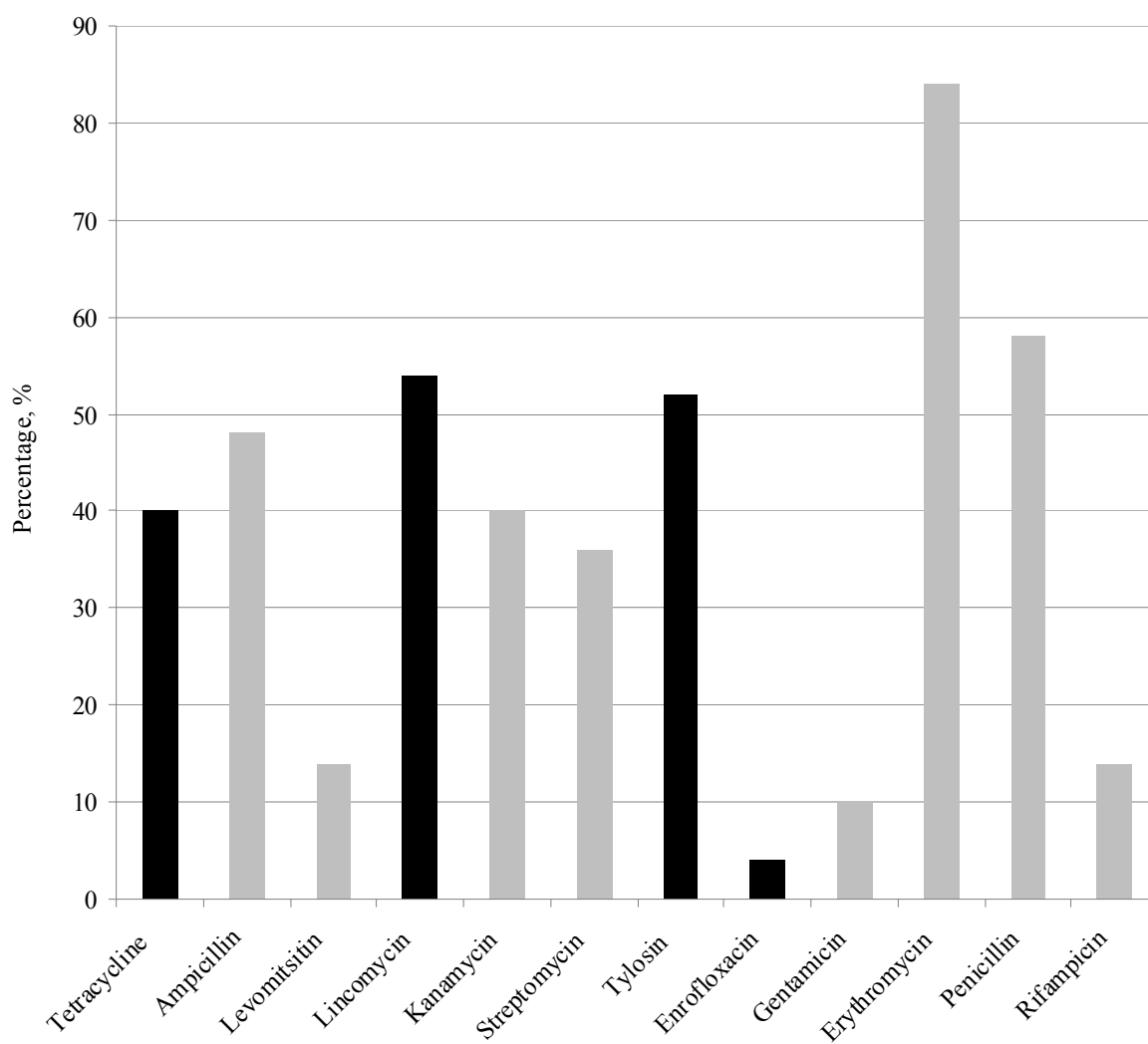


Fig. 2. Antibiotic resistance of allocated LAB

The important property of the analyzed lactic bacteria is the antibiotic resistance. Today certain schemes of an antibiotic therapy including the application of such preparations as lincomycin, enrofloxacin, doxycycline, colistin, tetracycline, oxytetracycline, tilosine and etc. are used on poultry farms for prevention and treatment of bacterial infections. Thus the probiotic LAB having the resistance to the mentioned antibiotics can develop in the course of antibiotic therapy in GIT of hens.

Antibiotic resistance was defined by the disk diffusion method [6]. In Fig. 2 the received results are presented. The percentage of LAB resistant to those antibiotics that are used today on poultry farm is marked with the black colour, in other words, these bacteria will keep viability in the process of poultry treatment by the mentioned substances.

On the other hand, the considerable relative content of LAB antibiotically resistant to applied antibiotics testifies to a wide circulation of a resistance to these preparations and, therefore, to the initial inefficiency of application of such kind of antibiotic therapy scheme. The exception is made only by enrofloxacin (only 4% of resistant bacteria) that have been applied on poultry farm recently. It must be kept in mind that LAB are more sensitive to various chemical agents, including antibiotics, than other representatives of GIT microbiota.

In addition, as it is seen from the chart, allocated LAB have resistance at least to 5 antibiotics among them - erythromycin and penicillin. But these two antibiotics have not already been applied for a long time on poultry farms.

The explanation of this may be the fact that chickens after hatching quickly settle the initially sterile GIT by pecking of dung of adult poultry which contains the microbiota resistant to antibiotics applied earlier serves. Thus, resistance to antibiotics remains in populations of an intestinal microbiota of chickens very long, even after the exception of an antibiotic from the therapy program.

One of the most important properties of probiotic bacteria is their ability to develop in the conditions of GIT of the owner: resistance to the low values of pH, to the contents of phenol in the environment and high concentration of NaCl.

Lactic acid bacteria, being acidophila, easily transfer high acidity of poultry GIT. Data of ability of bacteria to develop in the presence of phenol and NaCl are presented in Table 1.

Table 1

Phenol and NaCl resistance of bacteria

Total number of strains fermenting milk	Resistant to 0,2% phenol	Resistant to 4% NaCl
50	42 (84%)	23 (46%)

It is seen the considerable part of lactic acid bacteria shows resistance to the content of phenol and NaCl in the environment, this fact testifies that they can stand dwelling conditions of chickens and hens GIT well.

Antagonistic properties of investigated bacteria were defined in relation to sanitary-indicative bacteria *Salmonella typhimurium* TA 100 and *Staphylococcus aureus* 25922.

During determination of antagonistic properties of investigated strains we faced the problem of weak expressiveness of these properties. For registration even insignificant antagonistic activity the new method of determination of antagonistic properties was developed. It allowed additionally to reveal antagonism of 10 LAB strains (20% of total number of the analysed strains) which antagonistic properties could not be registered by the traditional methods. The method was based on the application of sterile microporous membranes.

The suspensions of bacteria-antagonists were brought on membranes surface and crops were incubated. After the removal of membranes on the lawn of test-bacteria zones of the growth inhibition were found (Fig. 3).

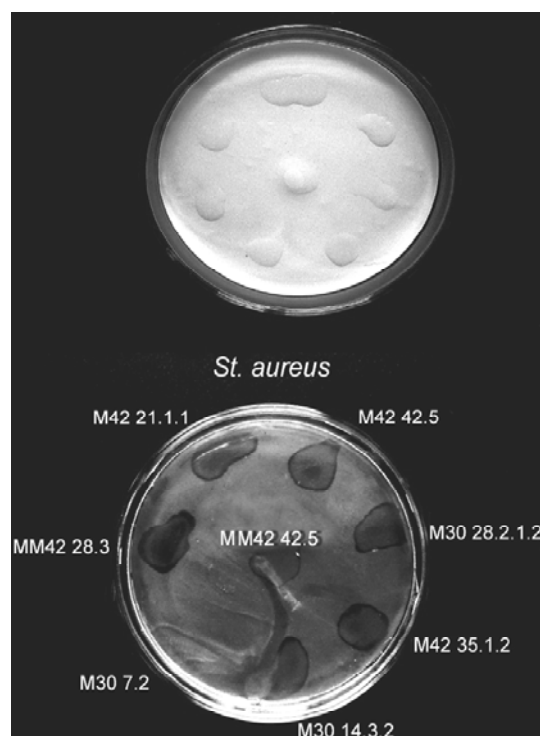


Fig. 3. Antagonistic properties of lactic bacteria in relation to test culture of *St. aureus* 25922 in the developed method: above – aliquots of culture broth of antagonists on bacterial filter surface; below – zones of the growth inhibition of test bacteria

Results of determination of antagonistic properties of investigated lactic bacteria are presented in Table 2.

Table 2
Antagonistic properties of LAB

Total number of strains fermenting milk	Express antagonism in relation to test-bacteria		
	<i>S. typhimurium</i> TA 100	<i>St. aureus</i> 25922	<i>S. typhimurium</i> TA 100 + <i>St. aureus</i> 25922
50	33 (66%)	33 (66%)	29 (58%)

As it is seen from Table 2, a significant amount of lactic bacteria shown antagonistic properties in relation to sanitar-indicative test bacteria and therefore can be a basis for creation of a probiotic preparation.

Antagonistic properties of bacteria are considered to be the most important property of probiotic strains and therefore while selecting probiotically valuable strains mainly they were taken into account.

Conclusion. By the results of the conducted researches and the analysis of physiologic and biochemical properties of the allocated lactic acid bacteria two of 50 strains were selected and they were carried to the sort of *Enterococcus* on the basis of the analysis of a gene sequences 16S of RNA. Both of the strains possess antagonistic activity in relation to sanitary-indicative bacteria *Salmonella typhimurium* TA 100 and *Staphylococcus aureus* 25922, proteolytic activity, are resistant to antibiotics applied on poultry farms. Besides, *E. faecalis* is

capable to develop in the presence of phenol and in considerable concentration of NaCl as well.

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