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DEVELOPMENT OF *LACTOBACILLUS* STRAINS COMPOSITION FOR ENHANCING OF ANTAGONISTIC ACTIVITY AGAINST *SALMONELLA ENTERICA*

Currently, the use of antibiotics for the treatment of most diseases leads to the emergence of the so-called "multiple resistance" in pathogens, which significantly reduces the effectiveness of treatment.

Therefore, controlling infections through a nonantibiotic approach is urgently needed. The potential use of *Lactobacillus* to control typhoid fever represents a promising approach, as it may exert protective actions through various mechanisms.

Lactobacilli have a long history of safe use, especially in the dairy industry [3]. Different *Lactobacillus* strains can function as microbial barriers against gastrointestinal pathogens through competitive exclusion of pathogen binding, modulation of the host's immune system, and production of inhibitory compounds, such as organic acid (e.g., lactic acid and acetic acid), oxygen catabolites (e.g., hydrogen peroxide), proteinaceous compounds (e.g., bacteriocins) and etc [4; 5; 7; 10]. Herewith, the combination of different strains among themselves in different proportions allows to achieve the best result.

The research aim was to investigate change of the antagonistic activity of lactobacilli strains when they are co-cultivated in different combinations.

Materials and Methods

In our study, we used strains of *Lactobaciilus* which were isolated from sea sponges. Determination of antagonistic properties was carried out *in vitro* by a hole-diffuse method [6; 7; 8] in relation to the *Salmonella enterica* NCTC 6017.

At the first stage of optimization, was checked the change of the antagonistic activity provided they were introduced in different combinations, but in equal volume (Table 1).

Table 1

The combination of the investigated strains of lactobacilli

№ experience	Option (combination that was used)	Strains that used
1	2	3
1	[1+2+3+4]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8+ *Lactobacillus vaccinostercus* ONU2
2	[1+2+3]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8
3	[1+2]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU19.2b

Table continued

1	2	3
4	[1+3]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU8
5	[1+4]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus vaccinostercus* ONU2
6	[4+2]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU19.2b
7	[4+3]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU8
8	[4+2+3]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8
9	[4+2+1]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus bifermentans* ONU55.1a
10	[4+3+1]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU8 + *Lactobacillus bifermentans* ONU55.1a
11	[2+3]	*Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8

At the second stage, were used the optimization matrices based on the analysis of variance adapted for Greek-Latin squares (Table 2).

Table 2

Matrix for a three-factor experiment for three levels according to the principle of Greek-Latin squares

A	**B**		
	b1	**b2**	**b3**
a1	c1	c2	c3
a2	c2	c3	c1
a3	c3	c1	c2

Calculations to identify the most effective combination, according to the results of an experiment based on analysis of variance, allow us to estimate the influence of existing factors and separate their effects from variability, which is provided by a random [1; 2].

Results

According to the results of an antagonistic study of the investigated strains in previous experiments, we selected four strains that were different in the investigated index relative to the indicator strain *Salmonella enterica* NCTC 6017:

Lactobacillus vaccinostercus ONU2 (low antagonistic activity);

Lactobacillus bifermentans ONU55.1a (high antagonistic activity);

Lactobacillus parabuchneri ONU8 (average antagonistic activity);
Lactobacillus parabuchneri ONU19.2b (average antagonistic activity).

In order to check the change in the level of antagonistic activity of the investigated strains of lactobacilli when co-cultivated, we used the scheme given in Table 1. In this case, the number of nocturnal culture per strain was the same (25.0 μl).

The obtained results showed that there is a change in the antagonistic activity index compared to control in the joint cultivation of strains in relation to *Salmonella enterica*.

The maximum response, with a control over 23.0%, was recorded at pairwise joint cultivation of the strong and second medium strains (variant [1 + 3]), strong and weak strains (option [1 + 4]), weak and first medium strains (option [4 + 4]) and both middle strains (variant [2 + 3]) (Fig. 1).

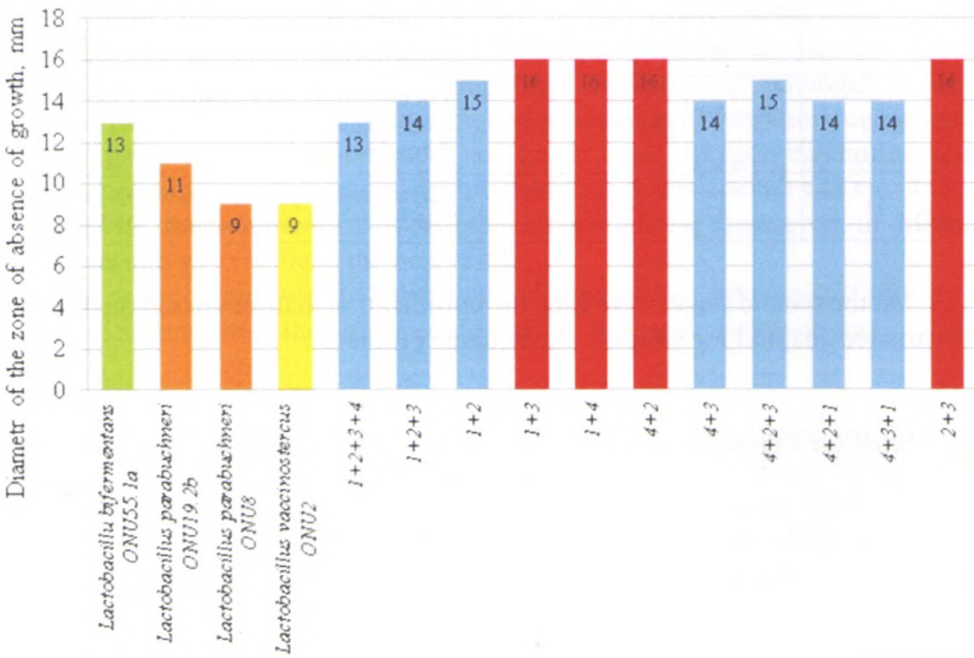

Fig. 1. Antagonistic activity of the investigated strains of lactobacilli in relation to *Salmonella enterica* NCTC 6017 in the joint cultivation of strains

That is, according to the results of the study, it was shown that co-cultivation of lactobacilli strains may increase their antagonistic activity, but for the indicator strain it is necessary to select their combination of strains.

Therefore, in the next series of experiments, we used a mathematical optimization method based on a one-factor dispersion analysis of the Greco-Latin squares matrix adapted to determine the optimal combination of lactobacilli strains. which would increase their aggregate antagonistic activity.

As we can see from the optimization results presented in Figure 2, the maximum effect on the increase of the antagonistic activity of the lactobacillus complex in relation to *Salmonella enterica* NCTC 6017 showed an over-control of over 50.0% in (Fig. 2).

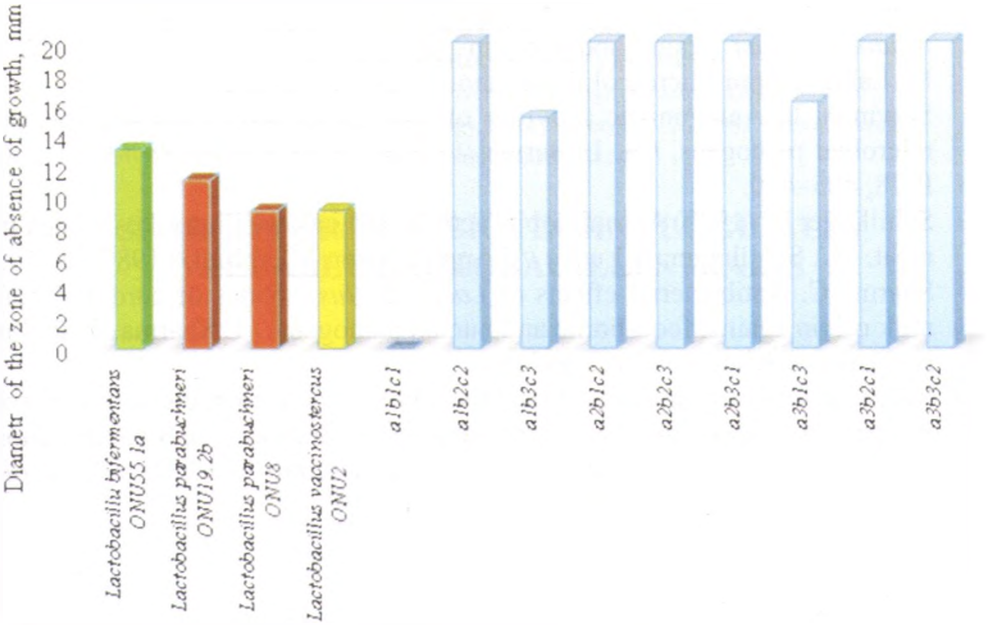

Fig. 2. Antagonistic activity of combinations of investigated strains of lactobacilli in relation to *Salmonella enterica* NCTC 6017 using an optimization matrix

The combination of the strains *Lactobacillus bifermentans* ONU55.1a (25.0 µl), *Lactobacillus parabuchneri* ONU19.2b (25.0 µl) and *Lactobacillus parabuchneri* ONU8 (50.0 µl) in the final volume appeared to be the most effective against the indicator strain *Salmonella enterica* NCTC 6017. 5.0 ml of MRS medium.

That is, losing all the results we can say that the use of compositions lactobacilli significantly increases the level of antagonistic activity against opportunistic bacteria, and mathematical planning helps to find the optimal combination.

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