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CORRELATION BETWEEN THE LEVEL OF ANTAGONISTIC ACTIVITY AND THE COMPOSITION OF NUTRIENT MEDIA

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Introduction

Antagonistic properties of lactic acid bacteria associated with the production of organic acids, thermolabile and thermostable high and low molecular weight antibacterial substances and antibiotics. Moreover, some substances produced by lactic acid bacteria are characterized by high antagonistic activity even at low concentrations in the medium. This category includes antibiotic substances (lactocil, lactobrevin, nisin, lactobacillin, etc.) [Стоянова, 2012].

Due to the widespread occurrence in nature and the ability to form a wide range of metabolites with valuable properties, according to many microbiologists and biotechnologists, lactic acid bacteria can become a real alternative to substances harmful to the health and life of macroorganisms, such as antibiotics, pesticides, fungicides, preservatives, etc. [Стоянова,2012; Асташкина, 2010].

The research aim was determining the effect of medium composition on the level of antagonistic activity.

Materials and methods

In our study, we used strain *L. vaccinostercus* ONU 2 which was isolated from sea sponges. Determination of antagonistic properties was carried out *in vitro* by a hole-diffuse method [Presti et al., 2015; Servin, 2004; Schillinger, 1989] in relation to opportunistic microorganisms.The following indicator strains were used in the work *Staphylococcus aureus* ATCC 25923*, Salmonella enteric* NCTC 6017*, Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6896*, Klebsiella pneumonia* ATCC 10031*, Pseudomonas aeruginosa* ATCC 27853*, Bacillus subtilis* ATCC 6633.

To order to determine the components of culture media that enhance the antagonistic activity of strains of lactic acid bacteria, we used a number of culture media that were different in composition in quantitative composition (Table 1).

Results

Order to determine the components of culture media that enhance the antagonistic activity of strains of lactic acid bacteria, we used a number of culture media that were different in composition in quantitative composition (Table 2).

When we analyzed the obtained data on the indicators of antagonistic activity, it was shown that only when using medium No. 1, medium No. 4 and medium No. 8, the indicators of antagonistic activity against most opportunistic microorganisms were significant (Tab.2).

Table 1

The composition of the nutrient media used in the design of the experiment (g/l)

	peptone	trypton	MPB	glucose	maltose	lactose	saccharose	Yeast extract	Sodium acetate ($C_2H_3O_2Na$)	Potassium dihydroorthophosphate KH_2PO_4	Potassium hydroorthophosphate K_2HPO_4	Ammonium citrate	Tomato juice (ml)	casein	gelatin	NaCl	Ascorbic acid	cysteine	$(NH4)_2SO4$	tween 80 (ml)	Etanol (ml)
Medium № 1	15,0			20,0				5,0	5,0	5,0		2,0	100,0							1,0	
Medium № 2				5,0		5,0	5,0	5,0	1,5					20,0	2,5	4,0	0,5				
Medium № 3		5,0	5,0	5,0				2,5	3,0					5,0			0,5				
Medium № 4	10,0		2,0	20,0				5,0	5,0		2,0	2,0								1,0	
Medium № 5			5,0	20,0				5,0					100,0	10,0		5,0		0,3			
Medium № 6							7.0	3.0		13.6				3.0					4.0	1,0	
Medium № 7			2,0	20,0				5,0	20,0	0,5	0,5			10,0			0,3			1,0	40,0
Medium № 8		10,0	3,0	4,0	4,0			5,0			2,6	2,0						0,5		1,0	
Medium № 9			5,0	10,0				5,0			2,0			10,0		5,0		0,5		1,0	

Table 2

Indicators of antagonistic activity of the strain *L. vaccinostercus* ONU 2 against culture-pathogenic strains when using different composition media

Indicator strain	Diameter of the zone of lack of growth (mm)								
	Medium № 1	Medium № 2	Medium № 3	Medium № 4	Medium № 5	Medium № 6	Medium № 7	Medium № 8	Medium № 9
S. enterica NCTC 6017	9	0	0	9	0	0	0	12	1
St.aureus ATCC 25923	1,5	1	1	2	2	0	0	3	1
P. vulgaris ATCC 6896	10	0	0	9	0	0	0	13	1,5
K. pneumoniae ATCC 10031	11	0	1	12	0	1,5	0	15	1,5
E. coli ATCC 25922	11	1	0	11	1	1	1,5	12	10
B. subtilis ATCC 6633	10	0	1	9	0	0	0	9	1,5
P. aeruginosa ATCC 27853	13	1	0	11	0	0	0	15	1,5

That is, we can draw a preliminary conclusion that the indicators of antagonistic activity depended on the composition of the medium.

The one-way analysis of variance according to Kruskal-Wallis confirmed that the composition of the medium affects the level of antagonistic activity. The Kruskal-Wallis H-test is a nonparametric analogue of one-way analysis of variance for comparing independent groups [Медик и др., 2007; Унгуряну идр., 2016]. The obtained calculated values of the Kruskal-Wallis criterion are 31.63 at a significance level of p-value = 0.0001, which indicates the adoption of an alternative hypothesis (Fig. 1).

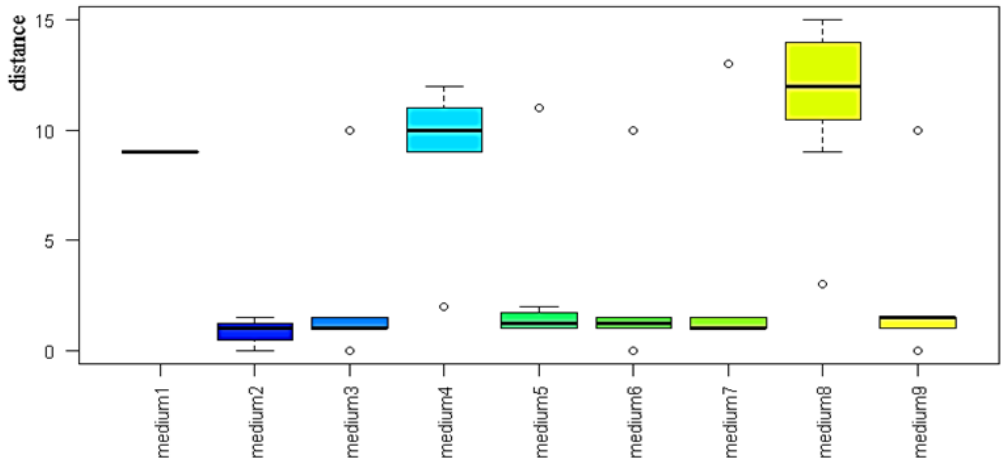

Fig. 1. The results of one-way analysis of variance in terms of antagonistic activity (Kruskal-Wallischi-squared = 31,63, df = 8, p-value = 0,0001)

After analyzing table 1, we excluded from it those components that were encountered in isolated cases and conducted a correlation analysis in order to determine the presence and level of connection between the signs of "antagonistic activity" and "environment component". As a result, the values of the Pearson correlation coefficients (r) between the parameters were calculated, and the concentrations of the components of the nutrient media were also studied. The results are presented in graphical form (Fig. 2).

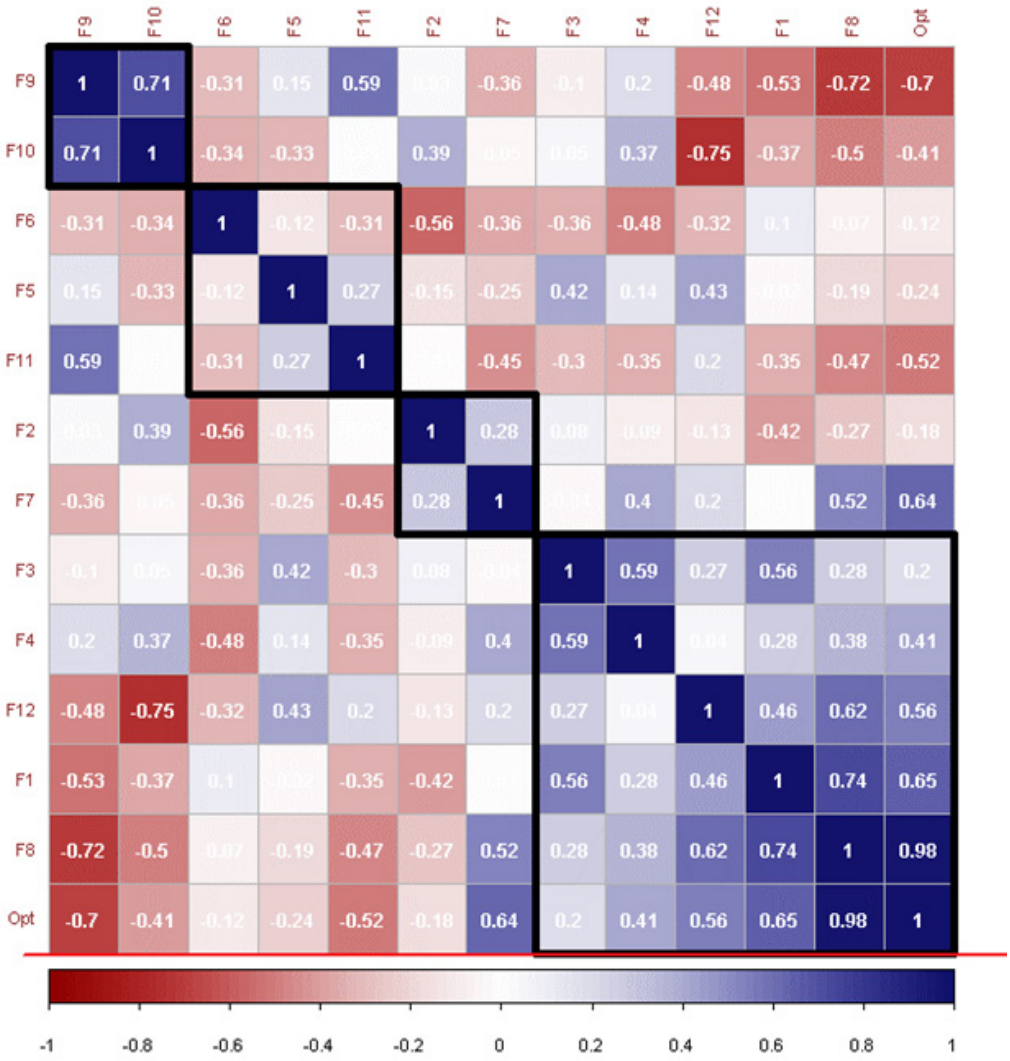

Fig. 2. Pearson correlation coefficients (r) between the antagonistic activity indicators of the strain *L. vaccinostercus ONU* 2 and the composition of the nutrient medium

Designations: F1 - peptone, F2 - MPB, F3 - glucose, F4 – yeast extract, F5 – sodium acetate, F6 – potassium dihydroorthophosphate, F7 – potassium phosphate, F8 – ammonium citrate, F9 - casein, F10 - NaCl, F11 – ascorbic acid, F12 – ethanol and Opt – an indicator of antagonistic activity

Based on the results, we determined that the following factors positively affect the level of antagonistic activity: peptone (r = 0.65), ammonium citrate (r = 0.98), potassium phosphate (r = 0.64), ethanol (r = 0.56), yeast extract (r = 0.41), glucose (r = 0.21), ascorbic acid (r = -0.52) and NaCl (r = -0.41). The obtained Pearson correlation coefficients indicate that in order to increase the level of antagonistic activity, the necessary organic factors that contribute to the rapid increase in cell concentration in the medium, and inorganic components are more necessary for maintaining the achieved concentration, are a source of nitrogen and phosphorus.

Conclusion

As is known, most lactic acid bacteria are known for their antagonistic properties [Бабич и др., 2015; Sharmaet al., 2017]. Therefore, it is very important to fully reveal the potential of the strains that can be used as producers or as the basis of a bacterial preparation against opportunistic bacteria.

During the work, we used the method of one-way analysis of variance (ANOVA) and correlation analysis, which allowed us to determine the components of the media that provided the maximum effect of the antagonistic activity of the strain *L. vaccinostercus* ONU 2. So among those that were "most influential" were peptone, glucose, yeast extract, ammonium citrate, KH_2PO_4 and ethanol.

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