

# Antagonistic Activity of Lactic Acid Bacteria

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## Abstract

**Objective:** Studying the antagonistic activity of cultures of lactic acid bacteria strains bred at the Research Institute of Biotechnology of the Gorsky State Agrarian University compared to some representatives of pathogenic and conditionally pathogenic microflora: *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris*.

**Materials and Methods:** In studying the antagonistic activity of lactic acid bacteria, studies were performed with the use of the following methods of analysis generally accepted in microbiology: The method of serial dilutions in liquid nutrient medium and the method of diffusion in agar with the use of agar nutrient medium (1, 5). The material for the study was pure cultures of microorganisms' breeding strains bred at the Research Institute of Biotechnology of the Gorsky State Agricultural University: *Streptococcus salivarius* RNCIM V-11174, *Lactobacillus helveticus* RNCIM V-11175, *L. helveticus* RNCIM V-11176, and *S. salivarius* RNCIM V-11177.

**Results:** The article shows the data obtained in the study of antagonistic properties of lactic acid bacteria strains bred by the Research Institute of Biotechnology of the Gorsky State Agricultural University: *S. salivarius* RNCIM V-11174, *L. helveticus* RNCIM V-11175, *L. helveticus* RNCIM V-11176, and *S. salivarius* RNCIM V-11177 deposited in the All-Russian collection of industrial microorganisms of the FSUE GosNIIGenetika for *S. aureus*, *E. coli*, and *P. vulgaris*. It has been determined that the studied strains of lactic acid bacteria included into the starter cultures for obtaining probiotic food products contain high number of living cells of lactic acid bacteria - up to  $10^{10}$  CFU/cm<sup>3</sup> and are antagonistically active to pathogenic and conditionally pathogenic microflora. The results of the research confirm the feasibility of using pure cultures of lactic acid microorganisms bred at the Research Institute of Biotechnology of the Gorsky State Agricultural University for the preparation of bacterial starter cultures of lactic acid microorganisms due to their physiological, biochemical, and antagonistic activity.

**Conclusion:** The results prove the feasibility of using the studied strains of lactic acid bacteria in preparing starter cultures for the production of functional probiotic products based on their high physiological, biochemical, and antagonistic activity to pathogenic and conditionally pathogenic bacteria. The studied cultures of lactic acid bacteria strains have various abilities to suppress the growth of pathogenic and conditionally pathogenic microorganisms, which should be considered in choosing lactic bacteria for preparing starter cultures and producing probiotic dairy products and functional food products.

**Key words:** Antagonistic activity, lactic acid microorganisms, microflora, probiotic, strain

## INTRODUCTION

The antagonism between various groups of microorganisms is widely spread in the nature. The most widely known is the antagonism of lactic bacteria to pathogens of intestinal infections.

Studies of isolation and selection of new strains of lactic bacteria are important.<sup>[1]</sup>

The promising and important field of biotechnology is searching for new strains of lactic bacteria for creating new probiotic

products.<sup>[2]</sup> To improve the functional properties of dietary products, lactic acid bacteria are widely used.<sup>[3]</sup>

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An important biotechnological aspect in medicine is the use of preparations based on probiotic microorganism's bacteria and gradual replacement of traditional medicines in certain cases.<sup>[4,5]</sup>

Antagonistic properties of lactic bacteria are based on their ability to synthesize<sup>[6]</sup> organic acids, carbon dioxide, and specific antibiotic substances.<sup>[6]</sup>

It has been established that strains of the same species of a microorganism may be widely different from each other by their antagonistic ability.<sup>[7]</sup> Therefore, correctly chosen symbiosis of various strains of the same species of the microorganism contributes to improving the antagonistic properties of a certain combination of cultures of various strains.<sup>[8]</sup>

In this regard, searching for and choosing new strains of lactic acid microorganisms with high antimicrobial properties are relevant.

The research was aimed at studying the antagonistic activity of lactic acid bacteria breeding bred at the Research Institute of Biotechnology of the Gorsky State Agrarian University toward intestinal infections pathogens.

## OBJECTS AND METHODS OF RESEARCH

Objects of research were pure cultures of lactic acid bacteria bred by the Research Institute of Biotechnology of the Gorsky State Agricultural University - *Streptococcus salivarius* RNCIM V-11174, *Lactobacillus helveticus* RNCIM V-11175, *L. helveticus* RNCIM V-11176, and *S. salivarius* RNCIM V-11177 extracted from raw self-souring cow milk, as well as field strains of the representatives of pathogenic and opportunistic pathogenic microflora - *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris*.

The study was performed with the use of the method of serial dilutions in a liquid nutrient medium, and diffusion in agar, with the use of agarized nutrient medium according to Bannikova<sup>[9]</sup> and Solovyeva *et al.*<sup>[10]</sup>

## RESULTS AND DISCUSSION

Table 1 shows the sources of selecting strains of lactic acid bacteria, their morphological parameters, speed of milk fermentation, and indicators of acid formation limit, which significantly affects the shelf life of probiotic lactic acid products.

As follows from the data in Table 1, the studied strains of lactic bacteria have been isolated from raw self-ripening milk and varied in morphology. Thus, strains RNCIM V-11174 and RNCIM V-11177 have coccoid shape, while strains RNCIM V-11175 and RNCIM V-11176 are rod-shaped bacteria.

It has been established that the studied cultures differed in their cultural characteristics. Hence, strains RNCIM V-11174 and RNCIM V-11177 form circular colonies, have small size and smooth surface. Strains RNCIM V-11175 and RNCIM V-11176 form round colonies, have medium size and smooth and shining surface. The color of colonies formed by strain RNCIM V-11175 is grayish, whereas strains RNCIM V-11174, RNCIM V-11176, and RNCIM V-11177 are white. Colonies of all studied cultures of strains of lactic acid microorganisms had smooth edges and homogeneous structure. Consistency of the colonies is viscous for strains RNCIM V-11175 and RNCIM V-11176, while RNCIM V-11174 had pasty consistency, and strain RNCIM V-11177 - soft consistency.

For studying the antagonistic properties of lactic acids of microorganisms, the test microbes were representatives of pathogenic and opportunistic pathogenic microflora: *S. aureus*, *E. coli*, and *P. vulgaris*.

*S. aureus* is the most pathogenic kind of staphylococci, a persistent, highly virulent, easily acquiring resistance to antimicrobial agents, and the exiter of staphylococcal infections.

*E. coli* belongs to the group of facultative anaerobes. Virulent strains of *E. coli* must not dwell in the digestive tract, and the disease occurs as they enter the gut with food and water.

*P. vulgaris* are rod-shaped non-spore-bearing motile bacteria that actively decompose proteins. Some species of *Proteus* form toxins capable of causing poisoning when ingested.

**Table 1:** Characteristics of strains of lactic acid bacteria bred at the Research Institute of Biotechnology of the Gorsky State Agrarian University

Strain number	Source of isolation	Microscopic picture	Milk ripening rate, hours	Acid formation limit °T
RNCIM V-11174	Self-ripening raw milk	Chains of coccoid cells	6	119
RNCIM V-11175	Self-ripening raw milk	Long intertwined chains of rod-shaped large cells	6	311
RNCIM V-11176	Self-ripening raw milk	Chains of large rod-shaped cells	5	325
RNCIM V-11177	Self-ripening raw milk	Chains of coccoid cells	6	125

When studying the antagonistic activity of strains of lactic acid microorganisms bred at the Research Institute of Biotechnology to the above test microbes by the serial dilution method with the use of meat-and-peptone broth, the minimum concentration of lactic acid bacteria inhibiting growth and development of test microbes was determined.

The results of the studies aimed at determining the minimum inhibitory concentration of lactic acid microorganisms to some types of pathogenic and opportunistic pathogenic microflora are shown in Table 2.

Analysis of Table 2 shows that the lack of growth in the representatives of pathogenic and opportunistic pathogenic microflora is an indicator of high sensitivity to pure cultures of the lactic acid bacteria bred at the Research Institute of Biotechnology of the Gorsky State Agrarian University. The presence of test microbe growth is the evidence of resistance to cultures of lactic acid microorganisms.

Growth of *S. aureus* was absent when exposed to cultures of the strains diluted as follows: RNCIM V-11174 -  $10^{-4}$ , RNCIM V-11175 -  $10^{-7}$ , RNCIM V-11176 -  $10^{-6}$ , and RNCIM V-11177 -  $10^{-5}$ .

The degree of diluting cultures of strains RNCIM V-11174, RNCIM V-11175, RNCIM V-11176, and RNCIM V-11177 that suppresses the growth of *E. coli* was  $10^{-5}$ ,  $10^{-8}$ ,  $10^{-7}$ , and  $10^{-7}$ , respectively.

Growth of *P. vulgaris* was not observed when exposed to cultures of strains RNCIM V-11174, RNCIM V-11175, RNCIM V-11176, and RNCIM V-11177 in dilutions of  $10^{-4}$ ,  $10^{-6}$ ,  $10^{-6}$ , and  $10^{-5}$ , respectively.

Antagonism of the studied strains to *S. aureus*, *E. coli*, and *P. vulgaris* using the method of diffusion into agar was performed. The results of the study are shown in Table 3.

**Table 2:** Sensitivity of representatives of pathogenic and opportunistic pathogenic microflora to lactic acid bacteria bred at the Research Institute of Biotechnology of the Gorsky State Agrarian University

Strain	The degree of lactic bacteria dilution									
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$	$10^{-7}$	$10^{-8}$	$10^{-9}$	$10^{-10}$
<b><i>Staphylococcus aureus</i></b>										
RNCIM V-11174	-	-	-	-	+	+	+	+	+	+
RNCIM V-11175	-	-	-	-	-	-	-	+	+	+
RNCIM V-11176	-	-	-	-	-	-	+	+	+	+
RNCIM V-11177	-	-	-	-	-	+	+	+	+	+
<b><i>Escherichia coli</i></b>										
RNCIM V-11174	-	-	-	-	-	+	+	+	+	+
RNCIM V-11175	-	-	-	-	-	-	-	-	+	+
RNCIM V-11176	-	-	-	-	-	-	-	+	+	+
RNCIM V-11177	-	-	-	-	-	-	-	+	+	+
<b><i>Proteus vulgaris</i></b>										
RNCIM V-11174	-	-	-	-	+	+	+	+	+	+
RNCIM V-11175	-	-	-	-	-	-	+	+	+	+
RNCIM V-11176	-	-	-	-	-	-	+	+	+	+
RNCIM V-11177	-	-	-	-	-	+	+	+	+	+

-: Means absence of test microbe growth; +: Means good growth of test microbe

**Table 3:** Antagonistic activity of lactic bacteria of local breeding

Strain	Test microbe											
	<i>Staphylococcus aureus</i>				<i>Escherichia coli</i>				<i>Proteus vulgaris</i>			
	Sterility zone, mm											
	1	2	3	mm	1	2	3	mm	1	2	3	mm
RNCIM V-11174	20	19	20	20 0.31	23	25	24	24 0.32	17	19	16	17 0.63
RNCIM V-11175	26	27	25	26 0.31	27	28	28	28 0.31	24	25	23	24 0.31
RNCIM V-11176	25	26	25	25 0.31	26	28	27	27 0.63	22	21	22	22 0.31
RNCIM V-11177	23	21	22	22 0.63	24	25	24	25 0.31	18	20	19	19 0.63

The data in Table 3 show that sterility zone of *S. aureus* is 20–26 mm, which corresponds to the average sensitivity of the test microbe to the studied strains of lactic bacteria.

When culture *E. coli* was exposed to strain RNCIM V-11174, the sterility zone was 24 mm. For strain RNCIM V-11175, this indicator was 28 mm, for strain RNCIM V-11176 - 27 mm, and for strain RNCIM V-11177 - 25 mm.

When studying the influence of cultures of lactic bacteria strains on *P. vulgaris*, the sterility zone varied between 17 mm and 24 mm, which was the evidence of low sensitivity of the test culture to the strains of lactic acid bacteria.

Thus, all studied strains of lactic acid bacteria bred at the Research Institute of Biotechnology of the Gorsky State Agrarian University have significant antagonism to test strains of pathogenic and opportunistic pathogenic microorganisms.

The highest index of inhibition was observed in cultures of strains of lactic bacteria *L. helveticus* RNCIM V-11175 and *L. helveticus* RNCIM V-11176.

Because the microbiology of food products required accurate identification during their testing for the presence of undesirable organisms, such as pathogens, as indicated by Beasley,<sup>[11]</sup> Hartman *et al.*,<sup>[12]</sup> and Tsugkiev *et al.*,<sup>[13]</sup> physiologically highly active strains of microorganisms obtained at the Research Institute of Biotechnology, Gorsky State Agrarian University (Republic of North Ossetia-Alania, Vladikavkaz) and deposited at the National Bioresource Center All-Russian Collection of Industrial Microorganisms of FSBI GosNIIGenetika, Moscow, were used, and their antagonistic activity was determined against pathogenic and conditionally pathogenic microflora.

The results of these studies confirm the data of some authors who consider it necessary to search for and develop scientific approaches to the production of probiotic products with the use of physiologically active strains of microorganisms that are able to suppress the development of pathogenic and conditionally pathogenic microorganisms in the organism.<sup>[14]</sup>

## CONCLUSION

The obtained results prove the feasibility of using studied strains of lactic acid bacteria in the preparation of starter cultures for probiotic lactic acid bacteria due to their antagonistic activity to pathogenic and opportunistic pathogenic bacteria.

The studied cultures of lactic acid bacteria strains have various abilities to suppress the growth of pathogenic and conditionally pathogenic microorganisms, which should be considered in choosing lactic bacteria for preparing starter cultures and producing probiotic dairy products and functional food products.

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