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Determination of the level of Production of Bacteriocin-Like Compounds by a Strain of Lactic Acid Bacteria Lactobacillus Plantarum ATCC 8014

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Abstract

This article presents the results of studying the potential for the synthesis of bacteriocins in a promising strain of Lactobacillus plantarum ATCC 8014, which is the basis of a probiotic preparation for the prevention of zooanthroponic infections. Lactobacillus delbrueckii strain B-13108 was used as a control culture with known antagonistic activity. The localization of bacteriocin-like compounds of L. plantarum and L. delbrueckii strains was assessed: in the thickness of the MRS-3 medium, in a cell-free culture medium, and in cell lysate. Under experimental (using L. plantarum strains) and control (using L. delbruecki strain) experimental conditions, we compared the antimicrobial activity of lactic acid bacteria, incl. the antimicrobial activity of the culture liquid and cell lysate was determined. It has been experimentally proven that the strain L. plantarum ATCC 8014 has a higher antimicrobial activity.

Discipline: Bacteriology

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1 Introduction

Lactic acid bacteria (LAB) constitute a heterogeneous group of microorganisms; produce lactic acid as the main product in the fermentation process; have antagonistic activity against a wide range of pathogenic and opportunistic microorganisms. As a rule, the development of microbial antagonism is observed in the natural habitats of a large number of species confined to a variety of taxonomic and ecological-physiological groups, but with the same nutritional and energy needs. The mechanisms of antagonistic activity of microorganisms are extremely diverse and can be associated with the inhibitory effect of organic acid molecules, the synthesis of exometabolites in the form of toxins, antibiotic-like compounds, lytic enzymes, or bacteriocins (bacteriocin-like inhibitory substance, BLIS) [1]. Bacteriocins are protein antimicrobial molecules of various origins, incl. and mediated by post-translational modification.

The long-term use of antibiotics in industrial animal husbandry as inhibitors of the growth of enteric pathogens in animals has led to the emergence and spread of antibiotic-resistant zooanthroponic strains of Escherichia coli around the world. A pandemic of extraintestinal infectious diseases caused by antibiotic-resistant zooanthroponotic strains of E. coli persisting in the human intestine was first reported in 2008 and continues to develop [2–5]. Pathogenic multiresistant strains of E. coli isolated from broilers belonging to clone ST 131 have genetic similarity and common virulence genes with E. coli isolates circulating in the human intestine and causing extraintestinal infectious diseases (cystitis, pyelonephritis, meningitis, sepsis) [6].

The rapid growth of infectious morbidity and mortality suggests a new level of preventive measures aimed at reducing the spread of socially significant antibiotic-resistant zoonotic infections (C. jejuni, S. enterica var. Enteritidis, E. coli) transmitted to humans through animal products.

In connection with the above, it is relevant to create innovative probiotics, prebiotics, metabiotics and their consortiums for targeted correction of the intestinal microbiome in farm animals in industrial animal husbandry, which have high antagonistic activity against C. jejuni, S. enterica var. Enteritidis, E. coli, as well as improving the functions of the immune and digestive systems and the productivity of animals [7-11].

The study aimed to characterize the potential for the synthesis of bacteriocins in a promising strain of Lactobacillus plantarum ATCC 8014, which is the basis of a probiotic preparation for the prevention of zooanthroponic infections.

2 Materials and Methods

In the experiment to determine the production of bacteriocins, strains B-148 of Lactobacillus plantarum ATCC 8014 were used (storage condition - sublimation; source - All-Russian Collection of Industrial Microorganisms (VKPM); products synthesized by the strain - organic acids and antibiotic substances).

To assess the viability, all the studied cultures were restored by the method of double subculturing using liquid nutrient media. The recultivated suspension contained 1% of the applied inoculum. The strains were incubated in a thermostat at 37 ± 2 °C for 24 hours. The number of viable cells was determined by seeding 0.1 ml of the suspension on agar media. Petri dishes with crops were kept in a thermostat at 37 ± 2 °C for 24 hours, followed by counting the number of grown colonies. The growth rate and the time to reach the maximum density of the recovered LAB strains were evaluated in a liquid MRS medium (deMan, Rogosa, and Sharpe) under static conditions in an RTS-1C personal bioreactor with software and a real-time microorganism growth control function. The strains were incubated using sterile 50 ml TPP TubeSpin 50 flasks for aerobic cultivation with a membrane filter.

Lactobacillus delbrueckii strain v-13108 was used as a control culture with known antagonistic activity (storage condition - sublimation; source (VKPM); products synthesized by the strain - organic acids and antibiotic substances). The localization of bacteriocin-like compounds of L. plantarum and L. delbrueckii strains was assessed: in the thickness of the MRS-3 medium, in a cell-free culture medium, and in cell lysate.

The physiological activity of the studied cultures of lactic acid bacteria was assessed by the time to reach the maximum population density and growth rate on the liquid MRS medium under static conditions of the RTS-1C bioreactor.

As indicator strains for testing bacteriocin-like compounds of LAB, the following were used: Escherichia coli O75 (storage condition - cryopreservation; source - Collection of microorganisms of the MIP "Microbiom"), Staphylococcus aureus ATCC 25923 (storage condition - sublimation; source - VKPM), Bacillus subtilis KR-1 (storage condition - cryopreservation; source - Collection of microorganisms of the MIP "Mirobiom").

3 Result and Discussion

The results of measurements of the physiological activity of lactic acid bacteria are reflected in typical graphs (Fig. 1 and 2). Adaptation of L. plantarum to cultivation conditions lasted for 9 hours. From 10 a.m. to 8 p.m., an exponential growth stage was noted, followed by a transition to a stationary growth stage or “plateau”, which corresponded to 3.39 f.u. and values μ (h^{-1}) – 0.02 at the 135th measurement point. Adaptation of L. delbrueckii to cultivation conditions lasted for 32 hours, the exponential phase had 2 peaks at 40 and 60 hours of the experiment, followed by a transition to the "plateau" stage, which corresponded to 0.98 f.u. and values μ (h^{-1}) - 0.01. This OD value turned out to be the maximum for the L. delbrueckii population and did not change significantly until the end of the experiment, as did the growth rate.

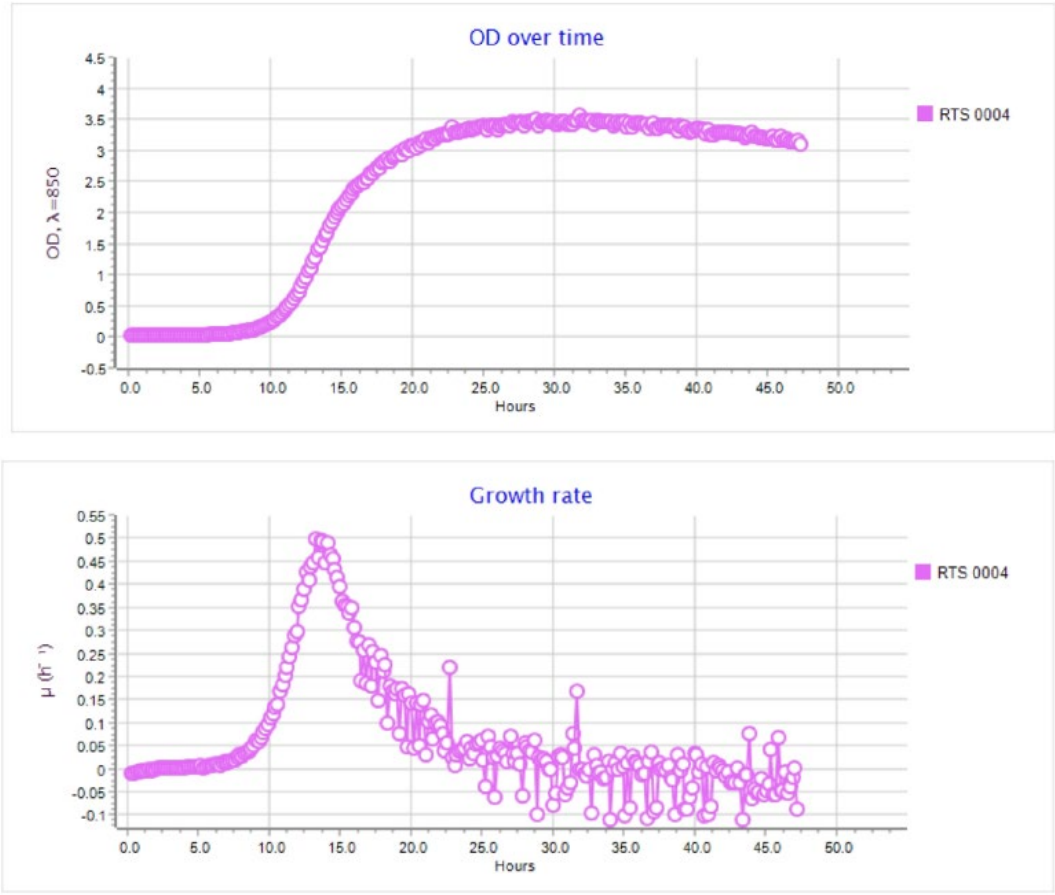
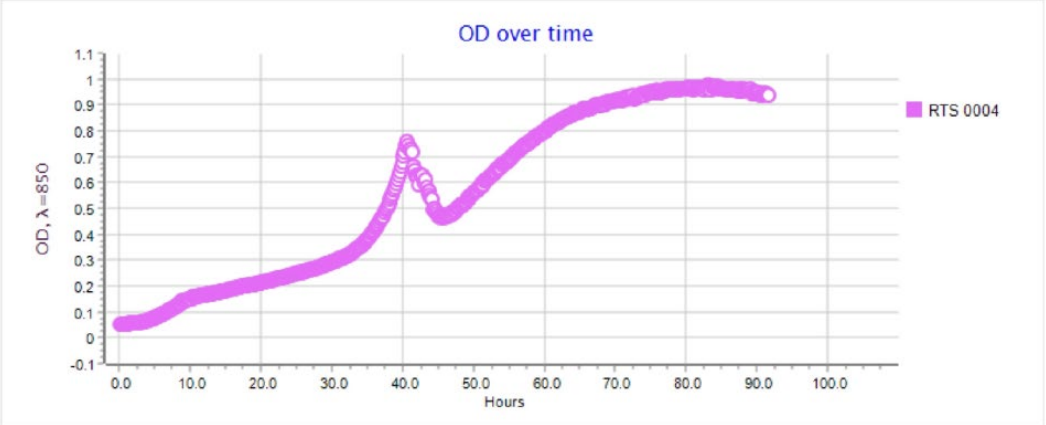


Figure 1: Physiological activity of the experimental L. plantarum strain: a. - optical density; b – growth rate.



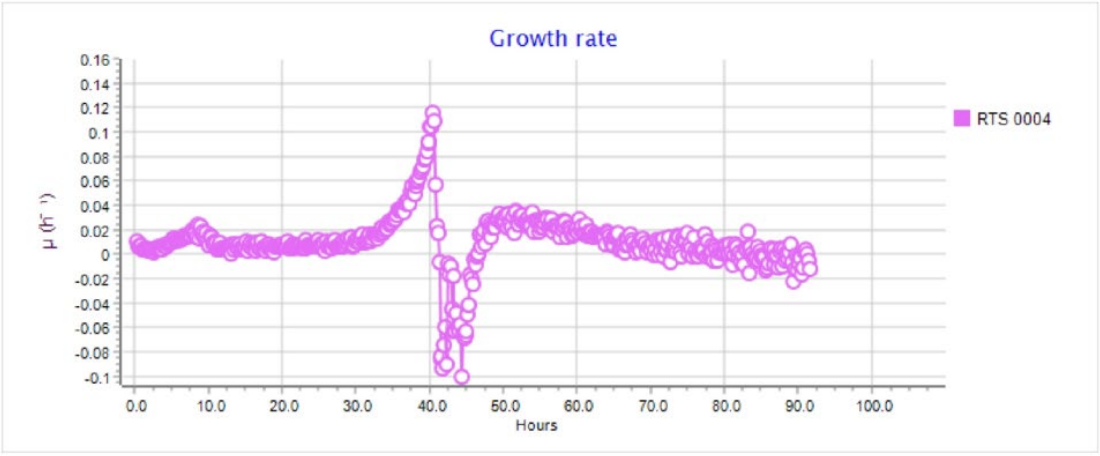


Figure 2: Physiological activity of the L. delbrueckii control strain: a. - optical density; b - growth rate

It was found that in the L. plantarum strain, only the cell-free culture medium possesses antimicrobial activity, as evidenced by large zones of growth inhibition for Bac. subtilis (19 ± 0.4 mm) and complete suppression of development for E. coli (21 ± 0.7 mm) and S. aureus (23 ± 0.7 mm).

In all cases, in the zone of antagonistic activity of L. delbrueckii, cultural growth was observed, which indicates a bacteriostatic effect of the control strain L. delbrueckii on the cells of Escherichia coli and staphylococcus.

As a result of determining the level of production of bacteriocin-like compounds in strains of lactic acid bacteria L. plantarum, it was found that the cell-free culture medium of L. plantarum has a selective bactericidal effect on asporogenic indicator microorganisms (E. coli, St. aureus) and a bacteriostatic effect on spore indicator microorganisms (Bac. subtilis) in the areas of application of the cell-free extract of L. plantarum. Antagonistic activity is characteristic of cultures with a stable stationary phase of growth, capable of reaching the optical density of the culture medium up to 3 fu and more and maintaining such activity for a long period. It is possible to assume that the preservation of the activity of the studied L. plantarum strain occurs due to the fact that the culture does not form extracellular proteases.

A similar result was obtained for the control strain L. delbruecki, which causes suppression of the growth of E. coli. According to the available data, such antibiotic activity may be associated with the phenotypic characteristics of the strains and the ability, depending on the cultivation conditions, to synthesize organic acids and bacteriocins, such as plantaricin F, during the period of active growth [12].

4 Conclusion

Thus, in the course of the studies, for the first time to study the physiological activity of potential producers of bacteriocins, a personal bioreactor RTS-1C with software and a function for monitoring the growth of microorganisms in real time was used. Given the peculiarity of LAB to synthesize bacteriocins at different periods of micropopulation growth, the use of this approach allows us to analyze and predict the physiological state of the microorganism with high accuracy using a large number of specified parameters for objective monitoring of its antibiotic / antagonistic activity.

Using the above approach, it was found that the L. plantarum strain is the most promising producer of bacteriocins, with a period of adaptation of the microorganism to cultivation conditions of 9 hours, a long phase of stationary growth, and the ability to achieve high optical density values - up to 3.39 f.u. Under experimental (using L. plantarum strains) and control (using L. delbruecki strain) experimental conditions, we compared the antimicrobial activity of lactic acid bacteria, incl. the antimicrobial activity of the culture liquid and cell lysate

was determined. It has been experimentally proven that the strain L. plantarum ATCC 8014 has a higher antimicrobial activity.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

6 Acknowledgement

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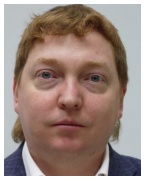

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