# INVESTIGATING ANTIBIOTIC ACTIVITY OF THE GENUS *BACILLUS* STRAINS AND PROPERTIES OF THEIR BACTERIOCINS IN ORDER TO DEVELOP NEXT-GENERATION PHARMACEUTICALS

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**Abstract:** In recent years, we have witnessed a considerable growth in number of strains resistant to antibiotics. Therefore, research on new antimicrobial components that might be used for development of new-generation drugs is currently very important. We have studied antibiotic activity of *Bacillus safensis*, *Bacillus endopheticus*, *Bacillus subtilis strains*, isolated their bacteriocins, and evaluated their properties. The study was carried out in the scientific research institute for biotechnology, Kemerovo Institute of Food Science and Technology, in the city of Kemerovo. Strains of microorganisms were isolated from vegetables grown in Krasnodar region, namely, samples of Manas onions, Big Beef tomatoes, and Capia bell peppers. Antibiotic activity of the strains was evaluated in liquid nutrient medium. All test strains demonstrated some level of antimicrobial activity which varied from 18 to 91%. We established minimum inhibitory concentrations for the isolated strains based on measured optical density; MIC for *Bacillus safensis* was $1.5*10^6$ CFU/cm$^3$, for *Bacillus endopheticus*, $1.5*10^6$ CFU/cm$^3$, for *Bacillus subtilis*, $1.5*10^8$ CFU/cm$^3$. We then isolated respective bacteriocins and purified them by HPLC method. During disk diffusion tests, bacteriocin preparations proved active against *Micrococcus luteus* strain. Molecular weight was determined by PAGE electrophoresis. Molecular weight of bacteriocins varied from 3.6 through 4.21 kDa. Isolated bacteriocins were proved to belong to the lantibiotics class.

**Keywords:** antibacterial activity, *Bacillus* strains, bacteriocins, pathogenic strains

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## INTRODUCTION

This article considers some research that seems promising for future application in medicine and in developing the next generation of drugs. The aim of this work is to investigate antibiotic activity of the genus Bacillus strains and to study properties of their bacteriocins in order to create next-generation pharmaceuticals.

Bacteriocins are heterogeneous antibacterial complexes, rather different in their activity level, action spectrum and mechanisms, molecular weight, physical and chemical properties. The main biologically active part of any bacteriocin is a protein component. Action mechanism of bacteriocins is based on creation of unregulated pores in the membrane of targeted cells, which interferes with the membrane potential and kills the cells. Bacteriocins are normally synthesized by strains as a defense system, and they inhibit the development of microorganisms related to the producing strain. Bacteriocins were classified in accordance with their chemical, structural properties, and method of killing.

It is proposed to subdivide bacteriocins from lactic acid bacteria into three classes. The first class includes lantibiotics: small peptides with molecular weight below 4–5 kDa, such as nisin and subtilin. The second class is represented by thermostable proteins with molecular weight below 10 kDa, these include coagulin and turicin. The third class includes thermostable proteins with molecular weight below 30 kDa, representatives of this group being lacticin and helveticin. The fourth class of bacteriocins includes glycoproteins or lypoproteins, representatives of this group being lactocin and lacstrepticin [1].

Ample potential for bacteriocin synthesis is known to be present in numerous strains of various micro-organisms. Main bacteriocin-producing species are lactic acid bacteria and the genus *Bacillus* strains.

Lactic acid bacteria are in the focus of interest for many researchers, due to their high potential for

bacteriocins synthesis [2, 3]. The following strains are known as the main bacteriocin-producing lactic acid bacteria: *Lactococcu, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus, Carnobacterium*. Bacteriocins produced by lactic acid bacteria include the following: helveticin, lactacin, bavaricin, sacacin [4].

Some bacteriocins produced by lactic acid bacteria hold the GRAS status, which confirms their safety. Therefore, bacteriocins prooduced by lactic acid bacteria are widely used in food industry and in pharmaceutical industry [5].

*Bacillus* strains have a capacity to synthesize a wide spectrum of different bacteriocins. Main bacteriocins-producing strains are *Bacillus licheniformis, Bacillus pumilus, Bacillus circulans, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Bacillus amyloliquefaciens* [6, 7, 8].

Thus, *Bacillus* strains are producers of peptide and lypopeptide antibiotics [9] and they demonstrate capacity for synthesis of an ample spectrum of bacteriocins, such as: bacilin and fungumicin [10] plipastatin and sufractin, [11], coagulin [12], tochiin, [13], amylolychin [14].

The ample spectrum of antimicrobial activity demonstrated by *Bacillus* strains-produced bacteriocins allows them to inhibit not only gram-positive bacteria, but also gram-negative bacteria, yeast or fungi that are pathogenic for humans or animals.

Thus, this capacity of *Bacillus* strains to synthesize a great number of antimicrobial substances with an ample spectrum of antimicrobial activity, corroborates the importance of further research on new types of bacteriocins.

Bacteriocins are natural substances, harmless for humans, with a wide activity spectrum. All this makes their application in food industry and medicine rather promising. In food industry, bacteriocins are mostly used as biopreservatives to control growth of pathogenic microorganisms. Bacteriocin concentrates are both added into fermented products and used for treatment of foods during storage [14]. One of the bacteriocins that are widely applied in food industry is nisin, it is used as preservative for sauces and cheese production. [15].

Occurrence and distribution of antibiotics-resistance strains is a problem that has acquired global relevance and represents a significant threat for public health.

Resistance is a capacity of microorganisms to withstand greater concentration of antibiotics than other microorganisms of the same species, and to grow when exposed to high concentrations of antibiotics above their respective therapeutic values.

There are several reasons for resistance development in bacterial strains. One of the main reasons is the uncontrolled self-administration of antibiotics in daily life. Improper uses or early discontinuation of antibiotics results in development of resistant microorganisms. Resistant bacteria might be disseminated through direct contact with humans or animals [14].

Yes another reason is the use of antibiotics in agricultural industry. Antibiotics are included into nutrition supplements and used as drugs for animals and poultry [15]. Antibiotics do not accumulate in the body, however, whenever norms for their use are not respected, they remain in meat, milk, and eggs, and all these products are getting consumed. Nowadays, there are now measures that would allow to control antibiotics content in food products, and manufacturers are solely responsible for monitoring the compliance with applicable norms and standards.

Distribution of resistant bacteria leads to such consequences as rise of new untreatable infections, and resistance development in bacteria that cause severe diseases, such as tuberculosis, diarrhea, respiratory disorders, and malaria [16].

At present, a number of studies have centered on several antimicrobial agents that might become alternative to antibiotics, such as bacteriophages [17], probiotic bacteria [18], antimicrobial peptides [19], and bacteriocins [20]. Bacteriocins are one of the most promising components for further development of antibiotics.

Development of new antimicrobial components is an important direction for current research. Due to their capacity to produce bacteriocins with an ample activity spectrum, *Bacillus* strains represent a promising object of reseach in the field of new-generation drugs development.

## OBJECTS AND METHODS OF STUDY

We performed surface strain recovery in vegetables grown in Krasnodar region: onion Manas, tomato Big Beef, bell pepper Capia. The study was carried out in the scientific research institute for biotechnology, Kemerovo Institute of Food Science and Technology (University), in the city of Kemerovo.

At different stages of the study, the following chemicals were used: Bacto Peptone, meat extract, enzymatic dry peptone, yeast extract, dry nutrient agar (LLC Lab-Biomed, Russia); HPLC columns XK16 with Phenyl Sepharose 6 Fast Flow carrier, column Octyl HR 16/60 (GE Healthcare, USA); ENrich S (BioRad, USA); $(NH_4)_2SO_4$ (Reakhim, Russia); tris, tricine, N, N'-methylene-bis-acrylamid, acrylamid, 2-mercaptoethanol, sodium dodecylsulfate (Helicon, Russia); acetic acid and boric acid (LLC Component-Reactiv, Russia); hydrochloric acid, sodium chloride, sodium acetate (LLC Component-Reactiv, Russia; coomassie R250 (Amresco, USA); ammonia persulfate, acrylamid and TEMED (Bio-Rad, Great Britain); glycerin (LLC Belkhim, Belarus); bromphenol blue (LLC Cation, Russia); tris (Applichem, USA); tris, N,N'-methylene-bis-acrylamid, acrylamid, 2-mercaptoethanol, sodium dodecylsulfate (Helicon, Russia); DNA-marker (Sibenzyme, Novosibirsk); kitfor Gram coloring (LLC Lab-Biomed, Russia); chemical kit PROBA-NK for DNA purification from biologic materials (LLC DNA Technology, Moscow); tris, N,N'-methylene-bis-acrylamid, acrylamid, 2-mercaptoethanol, sodium dodecylsulfate (Helicon, Russia); DNA-marker (Sibenzyme, Novosibirsk); набор Gene clean (MP Biomedicals, USA); GFXTM PCR DNA and Gel Band Purification Kit (GE Healthcare, USA); purification kit for amplified DNA (Sigma, USA); ABI

Prism Big Dye Terminator Cycle Sequincing Ready Reaction Kits (Applied biosistems, CIIIA); GTG-agarose (Lonza Rockland, USA); crystal violet (MERCK, Germany); *BigDye Terminator v1.1/3.1* Sequencing Buffer (Applied biosystems, England); Terminator v3.1 cycle. Sequencing RR-100 (Applied biosystems, England); Sephadex G50 Superfine (Bio-sciences, Sweden).

All experiments were replicated three times. Data provided in the table represent an average of the obtained results.

**Strains recovery.** Three strains of microorganisms, *Bacillus safensis*, *Bacillus endopheticus*, *Bacillus subtilis*, were recovered from onions, tomatoes, and bell peppers. The recovery procedure was as follows: triturated vegetables were placed into a liquid nutrient broth medium (meat-peptone broth) and cultivated at 30 ± 2°C, 37 ± 2°C and 45 ± 2°C for 1–5 days. When sufficient biomass was grown, inoculation was performed onto nutrient agar (meat-peptone agar). In order to obtain pure cultures, streaks were inoculated daily, until single colonies were produced. Isolated colonies were identified by Gram coloring, spores formation, morphological properties, cell size, oxidase and catalase tests. For more precise identification, genetic analysis of the strains of performed, with 16S RNA sequencing.

**Determination of antimicrobial activity.** To evaluate antagonistic activity, we measured optical density by spectrophotometry. Test culture was grown onto the MPA for 24 hours, at optimal temperature for each strain. Cells from the agar surface were re-suspended in NaCL solution until $10^9$. Purified strains were grown into the MPB medium for 24 hours at 37 ± 2°C. Then, the cultural liquid was centrifuged at 7000 rpm for 10 minutes, and supernatant was separated. In order to isolate the cells, the supernatant was filtered with Millex-GV filters (0.22 μm, Millipore, USA). The supernatant was neutralized by adding sodium hydroxide. 150 μl of each *Bacillus* strain culture filtrate were introduced into a plate, then 150 μl of test strains culture solution were added to each culture. The plate was incubated at 37 ± 2°C for 24 hours. 150 μl of sterile water, and 150 μl of pathogenic strain solution were used as controls. Optical density of the mixture was measured during cultivation. All experiments for antagonistic activity evaluation were replicated three times.

**Determination of minimum inhibitory concentration.** Minimum inhibitory concentration of strains was determined by spectrophotometry. Test strains were cultivated onto the MPB nutrient medium, then cells were separated by centrifugation at 7000 rpm for 10 minutes. Supernatant was separated, and pH was made up to 7.0 by adding NaOH 1M solution. Turbidity of the cultural liquid was controlled, as it achieved the value of 0.5 in McFarland units (containing approx. $1.5 \cdot 10^8$CFU/cm$^3$). Optical density level was controlled by spectrophotometry: absorption was within the same range as 0.5 in the McFarland units (at OD 625 nm, optical density was within 0.08–0.13). Optical density of the obtained microbial suspension was regulated by adding sterile nutrient medium. Then, a series of consequent dilutions 1 : 10 was performed, thus reaching a metabolite concentration from $10^7$ to $10^1$ CFU/ cm$^3$. In order to prepare the microbial suspension of test cultures, the colonies were diluted by sterile nutrient broth up to 0.5 in the McFarland units.

500 μl of the diluted test culture were mixed with 500 μl of the microbial suspension obtained from the tested strains. Minimum inhibitory concentration was assumed to be minimum metabolite concentration that slows down the metabolite growth.

**Separation and purification of bacteriocins.** In order to obtain bacteriocins, cells were separated from the cultural liquid, prior to purification, by 30 minutes centrifugation at 4200 rpm. Bacteriocins were sedimented with ammonia sulfate up to 90% of saturating concentration. Sediment and cultural liquid were separated by centrifugation at 4200 rpm for 40 minutes. The sediment was dissolved in 20 mmol acetate buffer pH 5.0. Undissolved sediment was separated by centrifugation at 4200 rpm for 30 minutes. Then the sediment waswashed again in 20 mmol acetate buffer pH 5.0, and the undissolved part was separated againby centrifugation.

**Bacteriocin purification by HPLC.** Purification was performed with AKTAfplc system (Amersham Biosciense, Sweden). At the first stage of purification, XK16 column was used, with PhenylSepharose 6 FastFlow as a carrier. The column with Phenyl-sepharose was balanced by initial buffer: 20 mmol acetate buffer pH 5.0 + 1 mol $(NH_4)_2SO_4$. $(NH_4)_2SO_4$ concentration in the applied preparation was made up to 1 mol. The preparation was applied onto the column at 3 ml/min. The second stage of purification was performed at OctylHR 16/60 column (GEHealthcare, USA). The column was balanced by initial buffer: 20 mmol acetate buffer pH 5.0+, application rate 1 ml/min. The final stage of purification was performed at ENRichS column (BioRad, USA). The column was balanced with initial buffer: 20 mmol acetate buffer, pH 5.0, application rate 1 ml/min. Electrophoresis was performed in Tricine-SDS, PAGE 16%, in the Mini-PROTEAN II cell (BioRad, USA).

**Evaluation of antimicrobial properties in purified bacteriocin preparations.** Antimicrobial activity of the purified bacteriocin preparations was determined by disk diffusion test. Test culture was streaked onto the MPA. Bacteriocin preparations were applied onto disks placed on the nutriuent agar. Nutrient agar was left to dry for some time. After 24 hours of cultivating the test microorganism at 30ºC, inhibition zone diameter was evaluated around the wells.

**Protein analysis.** Electrophoresis was performed in Tricine-SDS, PAGE 16%, as per Schagger & von Jagow procedure (Schagger H., von Jagow G., 1987). The following markers were used: triosephosphate isomerase (26.625 kDa), myoglobin (16.95 kDa), alfa-lactalbumin (14.437 kDa), aprotinin (6.512 kDa), insulin chain B oxidized (3.496 kDa), bacitracin (1.423 kDa).

## RESULTS AND DISCUSSION

**Determination of antimicrobial activity.** We performed a study of antimicrobial properties in bacteriocins of *Bacillus* strains purified at the previous stages of our research. Antagonistic properties of these strains were evaluated through cultivation of test cultures together with the metabolites produced by the strains under consideration. Biomass growth was monitored in liquid medium for 24 hours. Results are presented in Fig. 1–3.

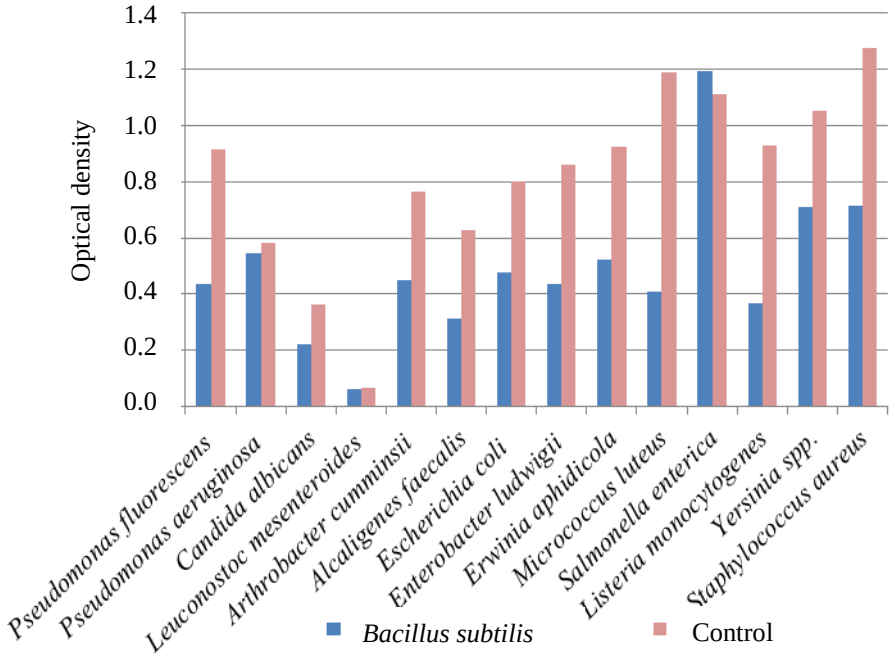


**Fig. 1.** Antagonistic activity of *Bacillus subtilis* strain.

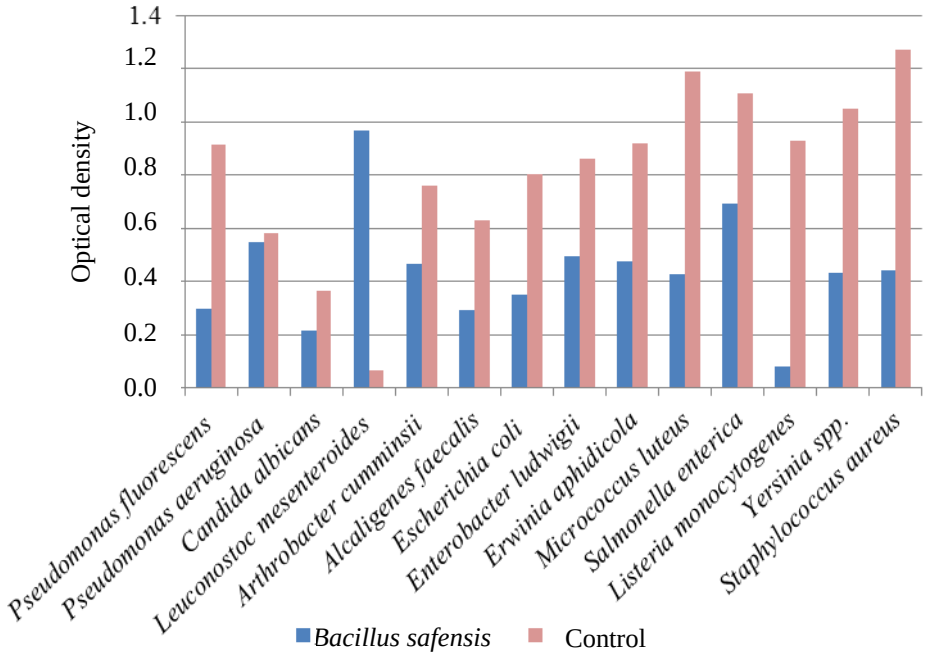


**Fig. 2.** Antagonistic activity of *Bacillus safensis* strain.

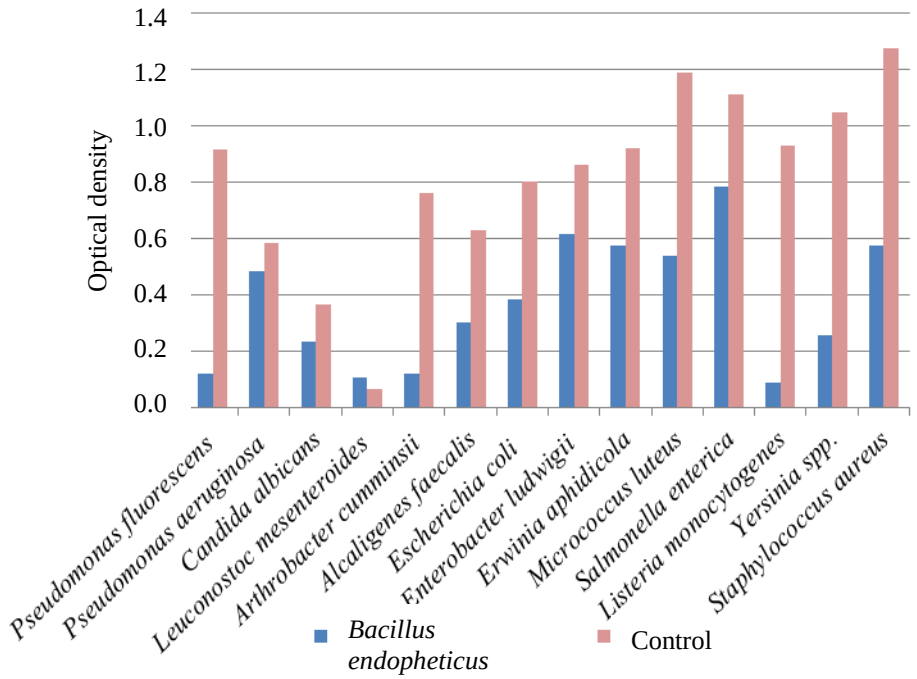


**Fig. 3.** Antagonistic activity of *Bacillus endopheticus* strain.

Purified cultures demonstrated high antagonistic activity again most pathogenic strains. Thus, during the study, *Pseudomonas fluorescens* growth was inhibited by *Bacillus safensis*, *Bacillus endopheticus* strains (from 53 to 88%). Activity against *Arthrobacter cumminsii* and *Staphylococcus aureus* was found in *Bacillus pumilus* and *Bacillus endopheticus* strains (from 55 to 85%). *Micrococcus luteus*, *Listeria monocytogenes* were inhibited by *Bacillus endopheticus*, *Bacillus safensis*, *Bacillus subtilis* strains (from 55 to 91%). Growth of *Yersinia spp.*, *Escherichia coli* was inhibited by *Bacillus endopheticus* and *Bacillus safensis* strains (from 54 to 76%). Growth of *Enterobacter ludwigii* and *Erwiniaa phidicola* strains was inhibited by the following bacterial cultures: *Bacillus safensis*, *Bacillus endopheticus*, (from 28 to 49%). Growth of *Salmonella enterica* was restrained by strains of *Bacillus safensis*, *Bacillus endopheticus* (from 25 to 38%), while antimicrobial activity against *Alcaligenes faecalis* was demonstrated by *Bacillus safensis*, *Bacillus endopheticus*, *Bacillus subtilis* (52–54%). *Pseudomonas aeruginosa* was inhibited by strains of *Bacillus endopheticus*, *Bacillus subtilis*, *Bacillus safensis* (48–55%).

**Determination of bacteriocin minimum inhibitory concentration.** Determination of minimum inhibitory concentration is an important step in studying the spectrum of antimicrobial activity of the strains and in further development of antibiotics. Findings are presented in Tables 1–3.

**Table 1.** Minimum inhibitory concentration of *Bacillus subtilis* strain

| Cultivation time, hours | Concentration of *Escherichia coli* metabolites, CFU/cm$^3$ | | | | |
|---|---|---|---|---|---|
| | $1.5*10^8$ | $1.5*10^7$ | $1.5*10^6$ | $1.5*10^5$ | control |
| | Optical density | | | | |
| 2 | 0.0725 ± 0.0036 | 0.0658 ± 0.0033 | 0.0507 ± 0.0025 | 0.0419 ± 0.0021 | 0.0755 ± 0.0038 |
| 4 | 0.0893 ± 0.0044 | 0.1013 ± 0.0051 | 0.0977 ± 0.00488 | 0.0905 ± 0.0045 | 0.1329 ± 0.0066 |
| 6 | 0.0948 ± 0.0048 | 0.1666 ± 0.0083 | 0.1974 ± 0.0099 | 0.1053 ± 0.0053 | 0.1843 ± 0.0092 |
| 24 | 0.1275 ± 0.0064 | 0.0802 ± 0.0041 | 0.3012 ± 0.0151 | 0.3433 ± 0.0172 | 0.2525 ± 0.0126 |

**Table 2.** Minimum inhibitory concentration of *Bacillus endopheticus* strain

| Cultivation time, hours | Concentration of *Escherichia coli* metabolites, CFU/cm$^3$ | | | | |
|---|---|---|---|---|---|
| | $1.5*10^8$ | $1.5*10^7$ | $1.5*10^6$ | $1.5*10^5$ | control |
| | Optical density | | | | |
| 2 | 0.0741 ± 0.0037 | 0.0629 ± 0.0031 | 0.0699 ± 0.0035 | 0.0456 ± 0.0023 | 0.0755 ± 0.0037 |
| 4 | 0.0859 ± 0.0043 | 0.0895 ± 0.0045 | 0.0882 ± 0.0044 | 0.0967 ± 0.0048 | 0.1329 ± 0.0066 |
| 6 | 0.0932 ± 0.0470 | 0.1889 ± 0.0094 | 0.1553 ± 0.0078 | 0.1034 ± 0.0052 | 0.1843 ± 0.0018 |
| 24 | 0.0544 ± 0.0027 | 0.0809 ± 0.0041 | 0.1051 ± 0.0053 | 0.1966 ± 0.0098 | 0.2525 ± 0.0126 |

**Table 3.** Minimum inhibitory concentration of *Bacillus safensis* strain

| Cultivation time, hours | Concentration of *Escherichia coli* metabolites, CFU/cm$^3$ | | | | |
|---|---|---|---|---|---|
| | 1.5*10$^8$ | 1.5*10$^7$ | 1.5*10$^6$ | 1.5*10$^5$ | control |
| Optical density | | | | | |
| 2 | 0.1251 ± 0.0062 | 0.0889 ± 0.00444 | 0.0767 ± 0.0038 | 0.0655 ± 0.0033 | 0.0755 ± 0.0038 |
| 4 | 0.1328 ± 0.0066 | 0.1323 ± 0.0066 | 0.1009 ± 0.0051 | 0.0938 ± 0.0047 | 0.1329 ± 0.0066 |
| 6 | 0.1596 ± 0.0079 | 0.1897 ± 0.0095 | 0.1102 ± 0.0055 | 0.2228 ± 0.0111 | 0.1843 ± 0.0092 |
| 24 | 0.0291 ± 0.0014 | 0.0913 ± 0.0046 | 0.1283 ± 0.0064 | 0.2515 ± 0.0126 | 0.2525 ± 0.0126 |

Our findings suggest that higher optical density, and, consequently, a higher biomass growth was observed when test culture was cultivated without adding the bacterial suspension of the strain under investigation. Growth of test culture was being inhibited during the 24 hours cultivation. The lowest biomass growth was observed during their joint cultivation, which means that the test culture growth was inhibited. Thus, for *Bacillus subtilis* strain, minimum concentration that inhibited the growth of *Escherichia coli* was 1.5*10$^8$ CFU/cm$^3$ (Table 1).

Findings show that optical density was decreasing - in comparison to the controls when metabolite concentration reached 1.5*10$^5$ CFU/cm$^3$. Thus, for *Bacillus endopheticus* minimum inhibitory concentration was 1.5*10$^5$ CFU/cm$^3$ (Table 2).

Decreasing optical density of the medium, and, as a result, decreasing text culture biomass concentration, in comparison to the controls, was observed at *Bacillus safensis* concentration of 1.5*10$^6$ CFU/cm$^3$ (Table 3).

**Bacteriocins purification.** Bacteriocins were obtained from cultural liquid by multi-step sedimentation with ammonia sulfate and washing in acetate buffer. Bacteriocins were purified by HPLC, and putification parameters were suitably adjusted. Purification in several steps proved to be optimal. The first stage was performed with XK16 column, with Phenyl Sepharose 6 Fast Flow used as a carrier, the second stage, with the column Octyl HR 16/60 (GE Healthcare, USA), and the third, with the column ENrich S (BioRad, USA). Application rate varied in dependence with the type of the column. Preparation was washed by initial and acetate buffers. Adjustment of purification parameters revealed the hydrophobic nature of bacteriocins. Bacteriocin preparations obtained by purification were analyzed for protein concentration and output. Findings are presented in Table 4.

Purification of bacteriocins produced by *Bacillus subtilis* resulted in a bacteriocin preparation with protein concentration of 13.900 ± 0.278 mg/cm$^3$, while the product output was 1.26 ± 2.00%.

Upon purification of bacteriocins produced by Bacillus safensis, protein concentration in the resulting preparation was 0.067 ± 0.001 mg/cm$^3$, with the product output of 16.60 ± 2.00%.

**Table 4**. Results of bacteriocins purification

| Purification stage | Protein concentration, mg/cm$^3$ | Output, % |
|---|---|---|
| *Bacillus endopheticus* | 1.750 ± 0.035 | 46.30 ± 2.00 |
| *Bacillus subtilis* | 13.900 ± 0.278 | 1.26 ± 2.00 |
| *Bacillus safensis* | 0.067 ± 0.001 | 16.60 ± 2.00 |

Purified preparations of *Bacillus endorpiticus* bacteriocins had a protein concentration of 1.750 ± 0.035 mg/cm$^3$, the end product output being 46.30 ± 2.00%.

**Protein analysis.** In order to determine molecular weight and to identify the isolated and purified bacteriocins, we performed electrophoresis in Tricine-SDS PAGE 16.50%. Findings are presented in Fig. 4.

Electrophoresis results detemined that molecular weight of *Bacillus subtilis* was 3.6 kDa, while for *Bacillus endorpiticus* strain molecular weight was 3.8 kDa. *Bacillus safensis* strain had molecular weight of 4.21 kDa (Table 5).

**Determination of antimicrobial activity in bacteriocins.** The next important step after bacteriocins purification was determination of their antimicrobial activity. We studied the capacity of the bacteriocin preparations to suppress the growth of *Micrococcus luteus* strain. The study was carried out on MPA, at cultivation temperature of 30°C. Specific activity of the bacteriocins under consideration was evaluated by changed in their inhibition areas. A relative unit of activity was assumed to be the amount of bactericin that creates a lysis area of 1 cm in diameter.

Bacteriocin activity is an important characteristic for describing bacteriocins in bacterial cultures. Antibiotic activity study in bacteriocins preparations revealed that *Bacillus safensis* strain demonstrated the highest specific activity, which amounted to 115.6 RU. A lower activity in relation to the test culture was observed in *Bacillus subtilis* strain, with specific activity of 13.64 RU. Activity value in *Bacillus endopheticus* strain was 13.64 RU.

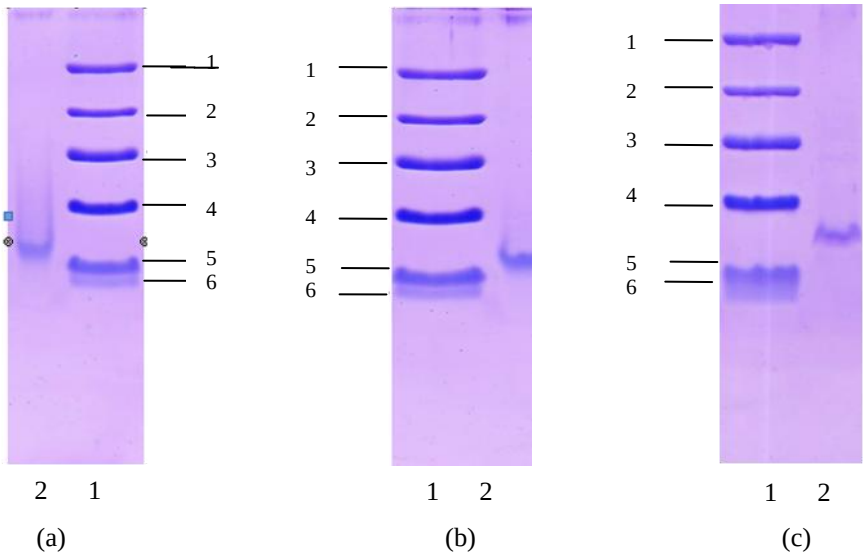


**Fig. 4.** Tris-tricine electrophorsis for bacteriocins after purification: (a) *Bacillus subtilis*, (b) *Bacillus safensis*, (c) *Bacillus endorpiticus*. Markers, kDa: (1) triosephosphate isomerase – 26.625; (2) myoglobin – 16.95; (3) alfa-lactalbumin – 14.437; (4) aprotinin – 6.512; (5) insulin chain B oxidized – 3.496; (6) bacitracin – 1.423.

**Table 5.** Results of activity evaluation in purified bacteriocin preparations

| Bacteriocins of strains | Volume, ml | Activity, RU/cm$^3$ | Overall activity, RU | Specific activity, RU/mg |
|---|---|---|---|---|
| *Bacillus safensis* | 3.40 ± 0.07 | 34.00 ± 0.68 | 115.60 ± 2.31 | 503.70 ± 10.07 |
| *Bacillus endopheticus* | 2.90 ± 0.06 | 28.00 ± 0.56 | 81.20 ± 1.62 | 4.48 ± 0.09 |
| *Bacillus subtilis* | 4.40 ± 0.09 | 3.10 ± 0.06 | 13.64 ± 0.28 | 7.30 ± 0.15 |

## CONCLUSIONS

In this work, we found strains of the following microorganisms present on the surface of fresh onions, tomatoes, and bell peppers: *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus*.

The studies we performed revealed antibiotic properties in bacteriocins produced by purified strains of *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus*. All strains under consideration demonstrated antibiotic activity against common pathogenic strains that provoke numerous human diseases. Antibiotic activity present in these strains allows us to consider them as bacteriocin producers.

We carried out an evaluation of minimum inhibitory activity for bacteriocins produced by *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus* strains. As a result of this study, we determined minimum concentrations of purified bacteriocins that are sufficient to inhibit the growth of bacterial cultures.

Adjustment of HPLC parameters targeting the purification of bacteriocins produced by *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus* strains allowed to select proper columns, to determine main purification stages and their respective specifications. As a result, we obtained bacteriocin preparations with a high protein concentration; besides, this specific purification procedure contributed to the increase of the end product output.

PAGE electrophoresis of the purified bacteriocin preparations was carried out in order to determine their molecular weight and, consequently, to perform their further identification. Specific activity present in purified preparations confirms that they belong to bacteriocins. Molecular weight of all bacteriocin preparations varied from 3.60 to 4.21 kDa. Thus, we found out that all the bacteriocins we obtained belong to the class of lanbiotics, the peptides with molecular weight below 5 kDa.

Lanbiotics comprise such well-known antibiotics as nisin, subtilin, epidermin, mercacidin, cinnamycin, mutacin II, and lacticin 481. It is considered that they kill cells by making pores in cell membrane, by interfering with the membrane potential, and by inhibiting the biosynthesis of enzymes within the cell wall.

Classifying the isolated protein products as lanbiotics, a group which includes many of the previously described antibiotics, together with their capacity to suppress the growth of pathogenic strains, makes their further research rather promising.

Development of new-generation drigs is an important direction for current medicine. Results that we have gathered at the present stage of research prove that the metabolites produces by test strains have a

wide spectrum of antimicrobial activity, and might be used in future for developing pharmaceutical products of the new generation.

Further studies of the isolated bacteriocins produced by the genus *Bacillus* strains shall provide a better understanding of their properties, which, in turn, will give way to opportunities for new-generation drugs development.

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