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Structure and properties of antimicrobial peptides produced by antagonist microorganisms isolated from Siberian natural objects

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Abstract:

Introduction. Public healthcare urgently needs new pharmaceuticals – alternative to traditional antibiotics – that pathogens develop no resistance to. Of special interest in this regard are antimicrobial, ribosomally synthesized bacterial peptides or bacteriocins. In this work, we aimed to study the structure and properties of antimicrobial peptides produced by antagonist microorganisms isolated from the natural objects of the Siberian region.

Study objects and methods. The study objects were bacteria isolated from the natural sources of Kuzbass. After culturing bacteria, total protein was precipitated from the culture fluid and separated into fractions by gel permeation HPLC. Their amino acid sequences were determined by MALDI-TOF mass spectrometry. The antibacterial (against *Bacillus pumilus* and *Escherichia coli*) and fungicidal (against *Aspergillus flavus* and *Aspergillus niger*) properties of the peptides were studied by the disk diffusion method.

Results and discussion. Seven peptides with different amino acid sequences were isolated from the culture fluid of bacteria, five of which had no analogues in the PepBank and Uniprot data banks. The peptide with an amino acid sequence of VMCLARKCSQGLIVKAPLM (2061.66 Da) was homologous to the cysteine membrane protein *Giardia lamblia* P15, and the peptide with an amino acid sequence of AVPSMKLCIQWSPVRASPCVMLGI (2587.21 Da) showed a homology with the *Planctomycetes bacterium* I41 peptides. We found antibacterial (against gram-positive and gram-negative bacteria) and fungicidal (against *Aspergillus*) properties in the peptide fractions.

Conclusion. Antimicrobial peptides produced by bacteria isolated from the natural objects of the Siberian region can be used to create pharmaceuticals as an alternative to traditional antibiotics to treat infectious diseases.

Keywords: Antimicrobial peptide, bacteriocin, fungicide, antagonistic properties, antibiotic resistance, amino acid sequence, mass spectrometry, bacteria

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INTRODUCTION

Pathogenic microorganisms resistant to traditional antibiotics are a serious problem of modern healthcare. There is evidence that over 70% of all pathogenic bacteria are resistant to at least one of the most commonly used antibiotics. Therefore, there is an urgent need for new drugs and therapeutic approaches to overcome their resistance [1–5].

Antimicrobial peptides produced by various organisms from bacteria to mammals are an ideal alternative to antibiotics due to their antimicrobial, anti-inflammatory, angiogenic, and immunomodulatory properties, as well as low bacterial resistance [6]. However, their use is limited by toxicity and stability *in vivo* [7].

Antimicrobial peptides act against various types of pathogens, including Gram-positive and Gram-negative

Table 1 Study objects

Microorganism	Reference	Source of isolation
Bacillus subtilis	Bs-1	Soil (Peshcherka village, Kemerovo district)
Lactobacillus plantarum	Lp-7	Rhizosphere of plants (Voznesenka village, Yaya district)
Leuconostoc mesenteroides	Lm-8	
Pediococcus acidilactici	Pa-9	Rhizosphere of plants (Ursk village, Guryevsk district)
Pediococcus pentosaceus	Pp-11	Plant waste at Sukhovsky farm (Kemerovo city)
Lactobacillus casei	Lc-12	
Lactobacillus fermentum	Lf-13	Plant waste at Niva farm (Gorskino village, Guryevsk district)
Pediococcus damnosus	Pd-16	Plant waste at Veles farm (Yaya village, Yaya district)
Geobacillus stearothermophilus	Bs-19	Bottom sediments of the Kara-Chumysh reservoir (Prokopyevsk district)
Bacillus caldotenax	Bc-20	Bottom sediments of Lake Udai (Mariinsk district)

bacteria, viruses, and microscopic fungi, through the destruction of the cytoplasmic membrane, intracellular penetration, and immunomodulation [8, 9]. Structurally, antimicrobial peptides are classified into linear cationic amphipathic peptides and macrocyclic peptides [10]. As a rule, antimicrobial peptides are short peptides consisting of 10–50 amino acids [11, 12]. They have common features despite differing in length, amino acid sequences, and conformation [13]. Typical antimicrobial peptides are composed of positively charged residues such as arginine, lysine, and histidine [14]. Cationic peptides with a positive charge ranging from +2 to +11 can interact with the membranes of microbial cells. Besides, a significant part of antimicrobial peptides is hydrophobic, contributing to the formation of amphipathic secondary or quaternary structures [15].

Antimicrobial peptides have several advantages over traditional antibiotics [16]. First of all, they have a broad spectrum of antimicrobial activity, against even multidrug-resistant pathogens [8, 16]. Secondly, antimicrobial peptides are highly active against gram-negative bacteria, which are more serious targets than gram-positive bacteria [17]. Another advantage is a rather low likelihood of drug resistance.

Bacteriocins are antimicrobial, ribosomally synthesized peptides of bacteria with a low molecular weight [18]. Mostly studied are bacteriocins produced by lactobacilli. They can be roughly divided into four categories: lantibiotics (e.g., nisin); non-antibiotic bacteriocins with good activity against *Listeria monocytogenes*, as well as pediocins, which make up the largest group; thermosensitive macromolecular proteinaceous bacteriocins; and complex bacteriocins with carbohydrates, lipids, and proteins [19–23]. Of all well-studied bacteriocins of lactobacilli, only nisin is produced commercially [24].

Potential sources of bacteria producing bacteriocins are dairy products, cow rumen, feed, as well as natural objects such as soils, plant waste, rhizosphere of plants, bottom sediments of water bodies, etc. [18, 25, 26].

In our previous studies, we isolated 19 microorganisms from the natural sources of Kemerovo Region (Siberian Federal District, Russia), including 10 species of bacteria (*Geobacillus*, *Bacillus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*) that showed high antimicrobial activity against *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus mycoides*, *Candida albican*s, and *Penicíllium citrinum* [27–29].

In this study, we aimed to examine the structure and properties of antimicrobial peptides produced by antagonist microorganisms isolated from the natural objects in Siberia.

STUDY OBJECTS AND METHODS

Our study objects were bacteria isolated from the natural sources of Kuzbass (Table 1).

Microorganism cultures. To obtain enrichment cultures of microorganisms, we crushed the samples of soil, bottom sediments, and plant waste under sterile conditions and rubbed their small amounts on Petri dishes with nutrient agar. The Petri dishes were incubated for three days at 26°C. Two nutrient media were used: lactobacilli were cultured on MRS agar; *Bacillus* and *Geobacillus* bacteria were cultured on a medium (pH 7.4 ± 0.2) containing 10.0 g/L casein hydrolysate, 2.5 g/L yeast extract, 5.0 g/L glucose, 2.5 g/L potassium hydrogen phosphate, and 12.0 g/L bacteriological agar.

Pure cultures of microorganisms were obtained from enrichment cultures by streaking. Microorganisms were cultivated on the media described above for 24 h: *Lactobacillus*, *Leuconostoc* and *Pediococcus* bacteria at 37°C, and *Bacillus* and *Geobacillus* at 30°C.

At the end of cultivation, cell debris was removed from all suspension cultures. The cultures were centrifuged at 3900 rpm in plastic flasks. The resulting supernatant was dried in a Labcocnco Triad freeze dryer (Labcocnco, USA) at a freezer temperature of –80°C, supernatant temperature of –20°C, and 0.05 mbar vacuum.

Protein fractions. To separate protein into individual fractions, the dried biomass was dissolved in 1 mL of 0.25 M phosphate buffer and the total protein was precipitated by adding 2 mL of concentrated

ammonium sulfate solution. The resulting protein suspension was separated by centrifugation at 8000 rpm. The protein precipitate was dissolved in 1 mL of 0.025 M Tris buffer solution (pH 4.5). The precipitate was applied to an Enrich 650 10 mm × 300 mm column (Biorad, USA) for a gel permeation high performance liquid chromatography (HPLC) at 280 nm using a direct injection system. Fractionation was performed using an NGC fraction collector (Biorad, USA).

Additionally, each protein fraction was purified on hydrophobic Amberlite XAD X-6 resins by chromatography. A glass column was filled with 10 g of Amberlite XAD-2 resin equilibrated with 10 mL of 20 mM trifluoroacetic acid solution. A protein solution in an acetate buffer was applied to the column and eluted in a methanol gradient from 0 to 15%, with a gradient rise of 5% for every 10 fractions. Fractions containing proteins were determined by taking 50 µL of each fraction and mixing it with a solution of Bradford's reagent in a 1:1 ratio. The resulting solution was measured on a Biorad SmartSpec Plus Spectrophotometer (USA). Fractions with an optical absorption of 0.06 or more were selected for further drying and identifying the amino acid sequence by the MALDI-TOF method using a MALDI TOF/TOF BRUKER Autoflex Speed mass spectrometer (Bruker Corporation, USA)

Trypsinolysis. Peptides were precipitated by adding an equal volume of methanol/chloroform mixture to an aliquot of a 200 µL fraction. The resulting precipitate was separated by centrifugation at 4000 rpm. The precipitate was dissolved in 100 µL of 6 M urea solution, to which 5 µL of dithiothreitol (DTT) solution was added to keep for 60 min at room temperature. Then, we added 20 µL of iodoacetamide solution and kept the mixture for 60 min at room temperature. After that, we added 20 µL of a DTT solution and kept the mixture again for 60 min at room temperature. After adding 775 µL of MiliQ H_2O and 50 µL of trypsin solution, the mixture was stirred by pipetting and kept in a thermostat at 37°C for 12 h. The enzyme was inactivated by adding 10 µL of trifluoroacetic acid. The peptides were purified by chromatography on C18 cartridges. The reaction mixture was applied to a cartridge and eluted with a solution of 0.1% trifluoroacetic acid in a 1:1 H_2O/acetonitrile mixture. Analysis and Top-Dawn sequencing were performed on 1 µL of a purified peptide solution.

Table 2 Peptides from the biomass of bacteria isolated from natural sources of Kuzbass

Microorganism	Isolated fractions	Microorganism	Isolated fractions
Bs-1	Bs-1_1	Lc-12	Lc-12_1
Lp-7	Lp-7_1	Lf-13	Lf-13_1 Lf-13_2 Lf-13_3
Lm-8	Lm-8_1 Lm-8_1	Pd-16	Pd-16_1 Pd-16_2 Pd-16_3 Pd-16_4
Pa-9	Pa-9_1 Pa-9_2	Bs-19	Bs-19_1 Bs-19_2
Pp-11	Pp-11_1 Pp-11_2 Pp-11_3 Pp-11_4 Pp-11_5 Pp-11_6 Pp-11_7 Pp-11_8	Bc-20	Bc-20_1

The antibacterial properties of the peptides against *Bacillus pumilus* and *Escherichia coli* were measured by the disk diffusion method. For this, we used suspensions of night cultures grown on a standard liquid nutrient LB medium with a titer of 0.5. The number of microorganisms (titer) in the suspension was determined by optical density at 595 nm. 200 µL of the pathogen culture was dropped onto a 90 mm Petri dish, rubbed with a sterile spatula by the spread plate method, and left to dry for 20 min under a laminar with the lid ajar. Then, 0.5 cm sterile filter disks soaked in the peptide solutions under study and dried at room temperature for 10 min were placed on the Petri dishes in the radial direction. The Petri dishes were left for 30 min at room temperature and then incubated in a thermostat at 37°C for 12 h. Then, we identified a bacterial inhibition zone around the disc and measured its diameter with a vernier caliper. Ampicillin at a concentration of 5 mg/mL was used as a positive control, and a disc soaked in a liquid medium was used as a negative control.

The fungicidal activity of the peptides against the microscopic fungi *Aspergillus flavus* and *Aspergillus niger* was measured by the disk diffusion method. The fungi were cultivated for 7 days, with an inoculation density of 6×10^7 conidia per 1 mL of medium. The results were analyzed with time intervals (3, 9, 12, 24, 48, 72 h, etc.) and by the fungus growth phase (stationary, accelerated growth, logarithmic), i.e., during the periods of exponential cell growth, decreased growth, and death or autolysis. At the end of the incubation, the inhibition zone around the disc was measured with a vernier caliper (mm), which indicated the degree of biocidal activity or its absence. A negative control was the samples with filters impregnated with the medium, and a positive control was the pharmaceutical preparation Irunin® (Veropharm, Russia) with itraconazole as an active ingredient.

Statistical data were analyzed in Microsoft Office Excel 2007. All the experiments were carried out in triplicate. Statistical analysis was performed using a one-sample Student's t-test. The differences were considered statistically significant at $P < 0.05$.

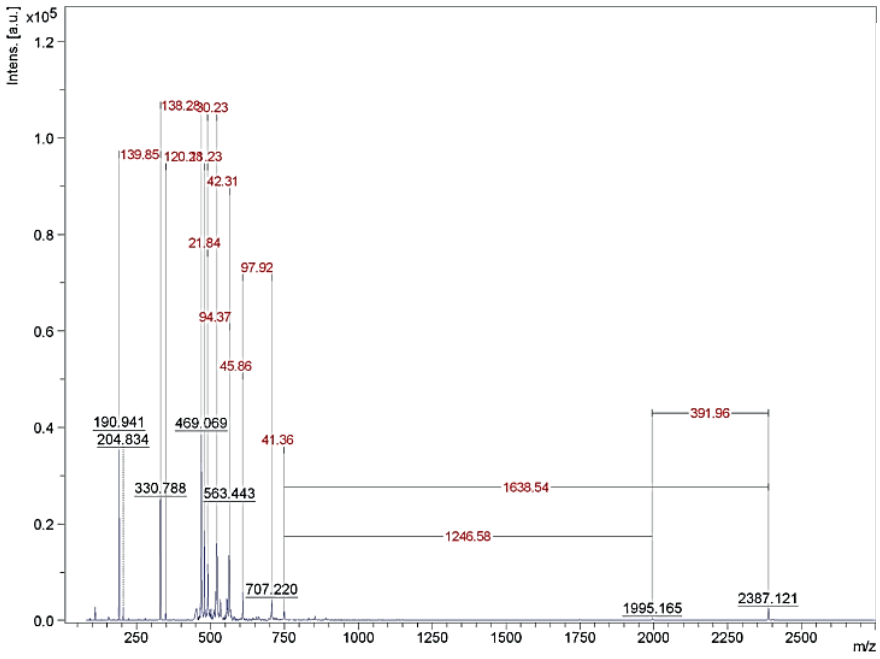


Figure 1 Mass spectrum of Bs-1_1 fraction

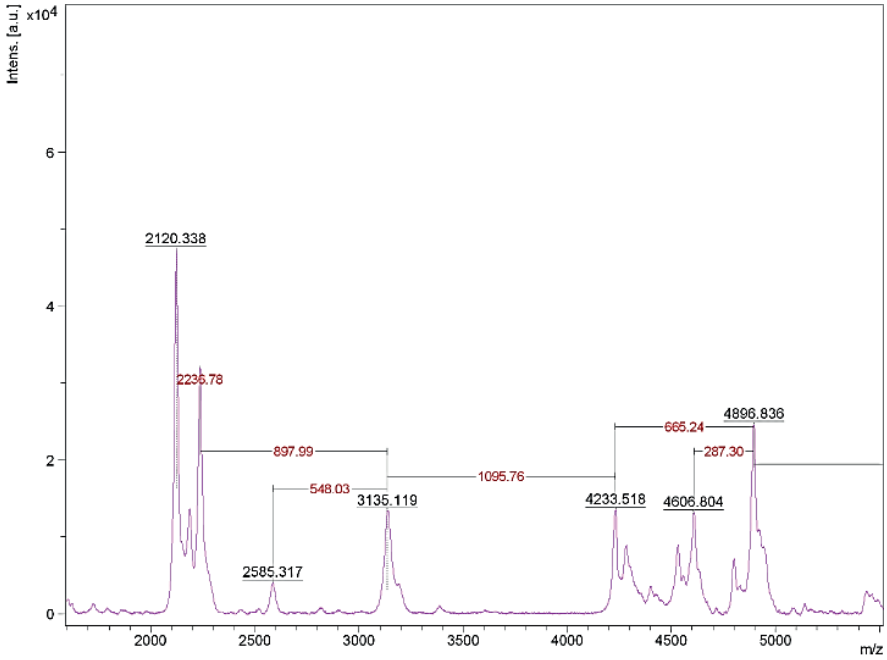


Figure 2 Mass spectrum of Bc-20_1 fraction (Lf-13_1, Lf-13_2, Lf-13_3)

RESULTS AND DISCUSSION

Several protein fractions were isolated from the culture fluid of all the studied samples (Table 2).

According to Table 2, one protein fraction was isolated from the culture fluid of *Bacillus subtilis*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Bacillus caldotenax*; two protein fractions from *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, and *Geobacillus stearothermophilus*; three protein fractions from *Lactobacillus fermentum*; four protein fractions from *Pediococcus damnosus*; and eight protein fractions from the *Pediococcus pentosaceus* culture fluid.

The results of the MALDI TOF mass spectrometry of protein fractions are presented in Figs. 1–7. We found some identical mass spectra of protein fractions synthesized by different bacteria.

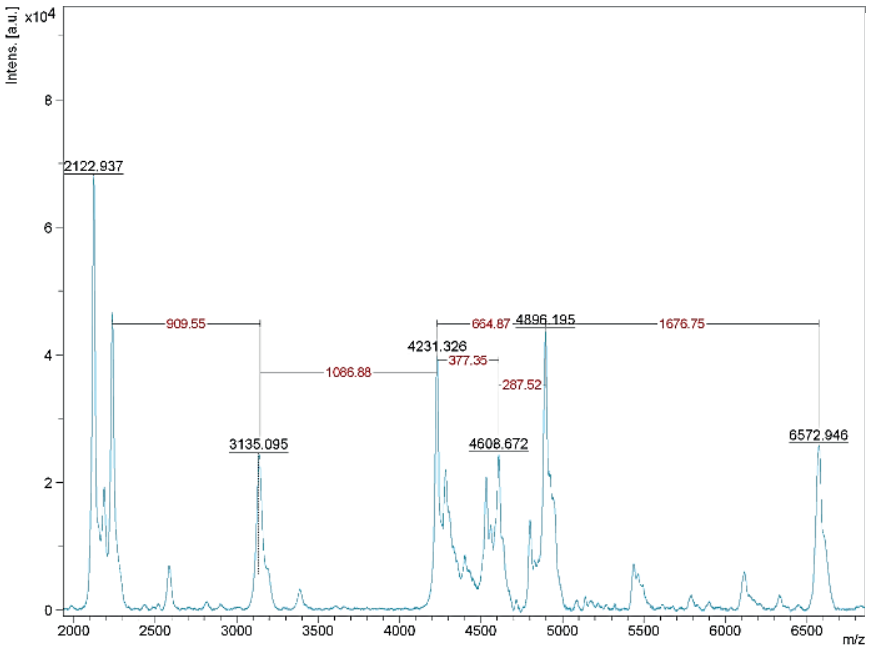


Figure 3 Mass spectrum of Bs-19_1 fraction (Lc-12_1)

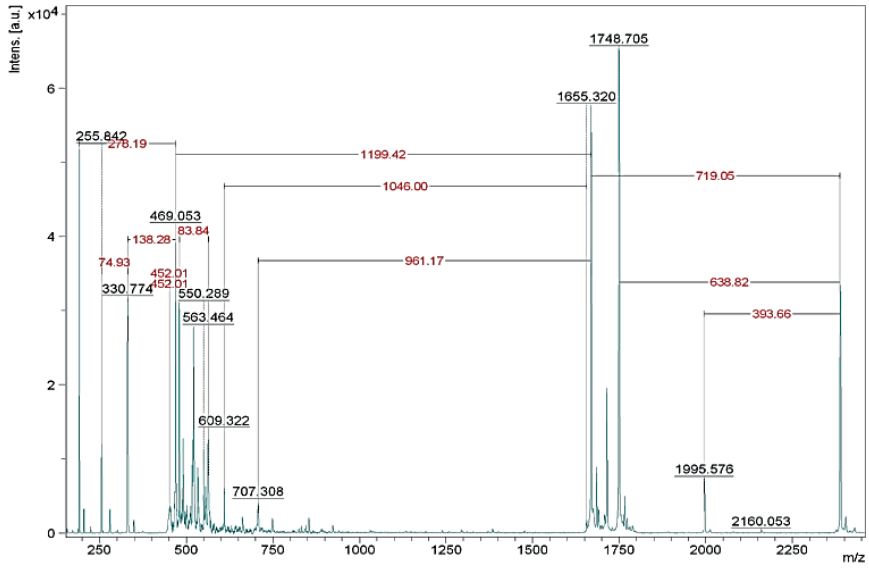


Figure 4 Mass spectrum of Bs-19_2 fraction

Having analyzed the mass spectra, we determined the molecular masses and amino acid sequences of seven peptides (Table 3).

Table 3 also shows the presence of analogues for the studied peptides in the PepBank and Uniprot databases. We established a homology of fractions Pp-11_1, Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, Pp-11_7, Pp-11_8, Lp-7_1, Pd-16_1, Pd-16_2, Pd-16_3, and Pd-16_4 with the cysteine membrane protein *Giardia lamblia* P15 (Fig. 8), as well as a homology of peptides Pa-9_1 and Pa-9_2 with the *Planctomycetes bacterium* I41 peptides (Fig. 9). The rest of the peptides had no analogues in the PepBank and Uniprot databases.

The antibacterial properties of the studied peptides against gram-positive (*Bacillus pumilus*) and gram-negative (*Escherichia coli*) bacteria, as well as their fungicidal properties against the microscopic fungi *Aspergillus niger* and *Aspergillus flavus* are presented in Tables 4–5 and Figs. 10–11.

According to Table 4 and Fig. 10, of the seven peptides under study, only one (Bs-19_2) exhibited no antagonistic activity against *E. coli* and *B. pumilus* strains. Peptide fraction Pp-11_1 (and peptides with identical amino acid sequences Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, Pp-11_7, Pp-11_8, Pd-16_1, Pd-16_2, Pd- 16_3, Pd-16_4, and Lp-7_1) showed

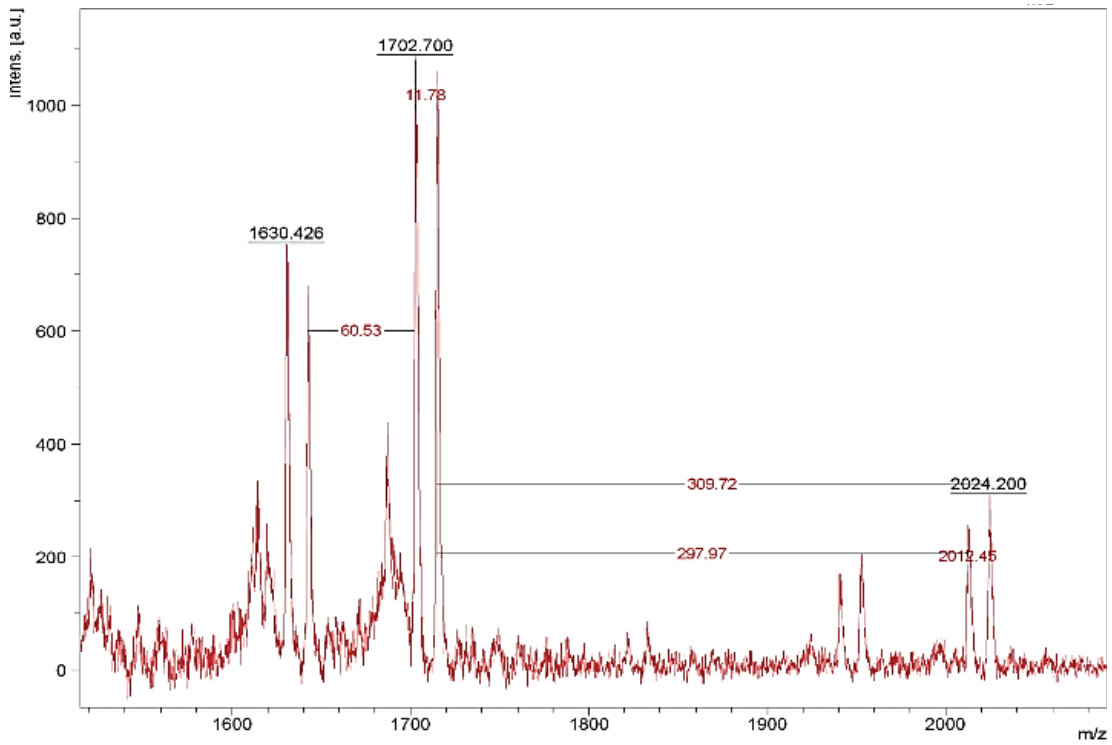


Figure 5 Mass spectrum of Pp-11_1 fraction (Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, Pp-11_7, Pp-11_8, Lp-7_1, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4)

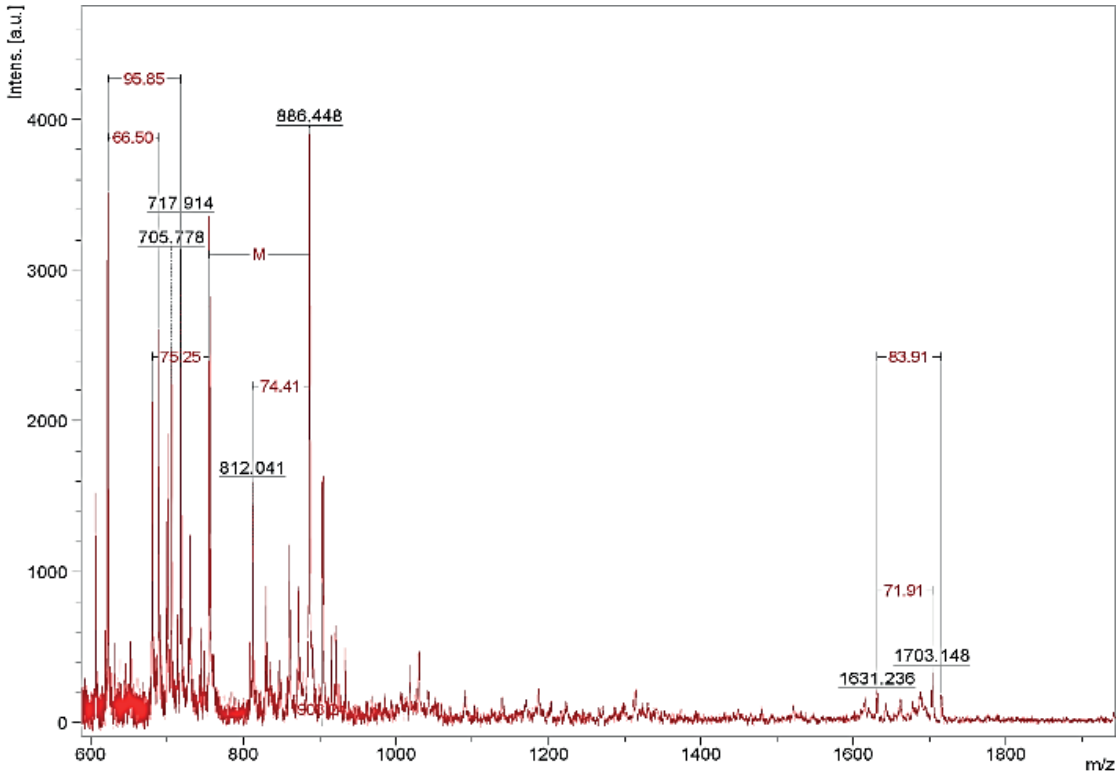


Figure 6 Mass spectrum of Lm-8_1 fraction (Lm-8_2)

high antagonism against *B. pumilus* and pronounced antibacterial activity against *E. coli*. Peptides Bs-1_1 and Bc-20_1 (identical Lf-13_1, Lf-13_2, and Lf-13_3), Lm-8_1 (identical Lm-8_2), and Pa-9_1 (identical Pa-9_2) had moderate and pronounced antagonistic activity against *B. pumilus*, but no activity against *E. coli*. Finally, peptide Bs-19_1 (identical Lc-12_1) showed bacteriostatic activity only against *E. coli*.

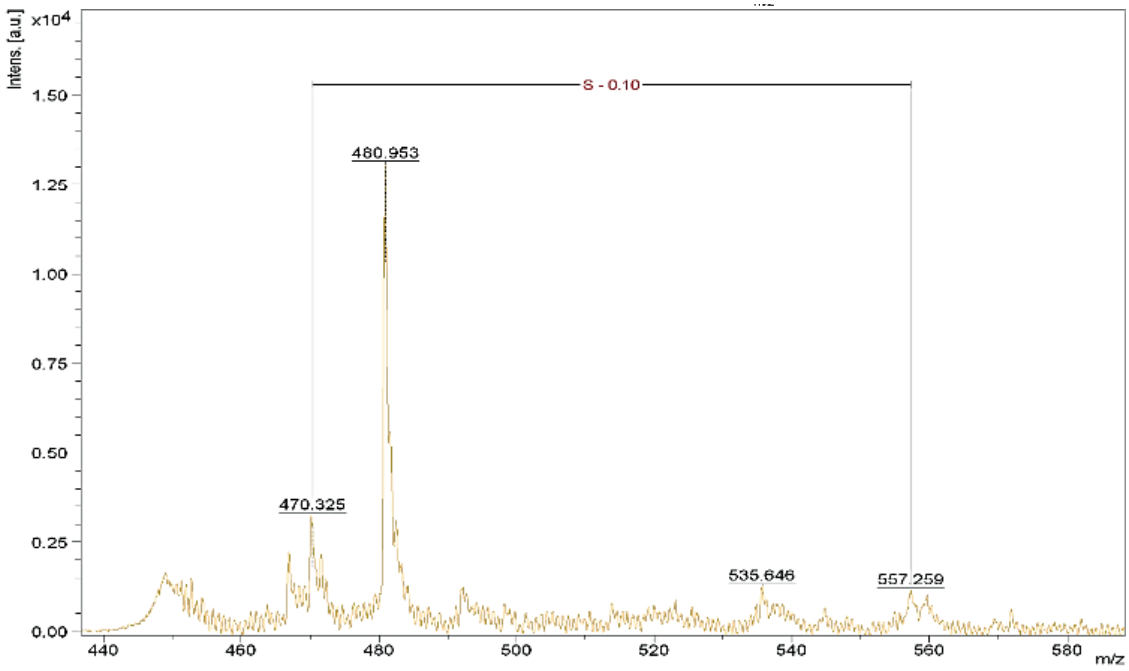


Figure 7 Mass spectrum of Pa-9_1 fraction (Pa-9_2)

Table 3 Molecular masses and amino acid sequences of peptides from the culture fluid of bacteria isolated from the natural sources of Kuzbass

Code of peptide	Molecular mass of peptide, Da	Amino acid sequence	Analogues in PepBank or Uniprot
Bs-1_1	13140.97	AFGKHVLIPVSCGFTYVWKCTLIPHISARPHYCFH RQHCDYKINQVSFEDAWHTPC	No analogues
Bc-20_1 Lf-13_1 Lf-13_2 Lf-13_3	6577.63	FLAFAYLPIPGWHPDYNGRAMKWANRPFTYICHGR DLKLRQMLYSGATIGHAEMR	No analogues
Bs-19_1 Lc-12_1	6572.00	PHQGHAFNFSCDMETAGFKGTQFWTFKSV SPHLATFKLGHMSTYAILGFAGCH	No analogues
Bs-19_2	6290.80	FVKGFHPSMTARGVVSDEADGRCDRFV KGFHPSMTARGVVSDEADGRCDR	No analogues
Pp-11_1 Pp-11_2 Pp-11_3 Pp-11_4 Pp-11_5 Pp-11_6 Pp-11_7 Pp-11_8 Lp-7_1 Pd-16_1 Pd-16_2 Pd-16_3 Pd-16_4	2061.66	VMCLARKCSQGLIVKAPLM	High homology with cysteine membrane protein *Giardia lamblia* P15
Lm-8_1 Lm-8_2	35571.18	MOPRKLCQSP VAILKMCVPA RQKVPSILKM OPRKLCQSPV AILKMCVPAR QKVPSILKMO PRKLCQSPVAILKMCVPARQ KVPSILKMOP RKLCQSPVAI LKMCVPARQK VPSILKMOPR KLCQSPVAIL KMCVPARQKV PSILKMOPRK LCQSPVAILK MCVPARQKVP SILKMOPRKL CQSPVAILKM CVPARQKVPS ILKMOPRKLC QSPVAILKMC VPARQKVPSILKMOPRKLCQ SPVAILKMCV PARQKVPSIL KMOPRKLCQS PVAILKMCVP ARQKVPSILK MOPRKLCQSP VAILKMCVPA RQKVPSILK	No analogues
Pa-9_1 Pa-9_2	2587.21	AVPSMKLCIQWSPVRASPCVMLGI	High degree of homology with *Planctomycetes bacterium* I41 peptides

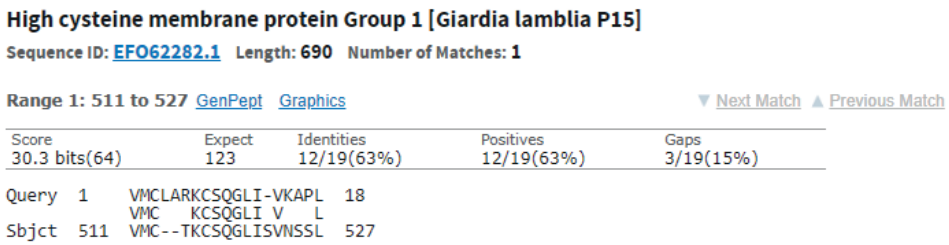


Figure 9 The closest analogues for peptides Pa-9_1 and Pa-9_2 according to BLAST NCBI

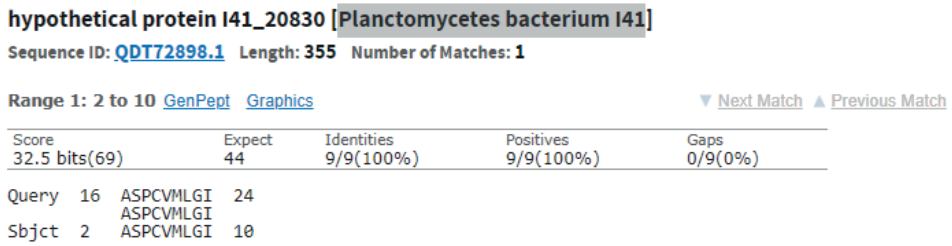


Figure 8 The closest analogues for peptides Pp-11_1, Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, Pp-11_7, Pp-11_8, Lp-7_1, Pd-16_1, Pd-16_2, Pd-16_3, and Pd-16_4 according to BLAST NCBI

Table 4 Antibacterial properties of peptides from the culture fluid of bacteria isolated from the natural sources of Kuzbass (M ± m)

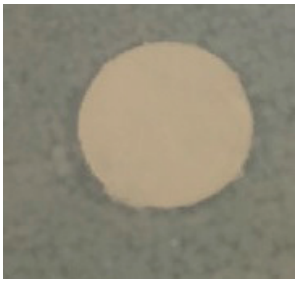
Peptide code	Test strain	Lysis zone diameter, cm	Degree of activity
Negative control	*Escherichia coli*	0	–
	Bacillus pumilus	0	–
Ampicillin (positive control) 0.5 mg/mL	*Escherichia coli*	0.90 ± 0.05	–
	Bacillus pumilus	2.40 ± 0.10	–
Bs-1_1	*Escherichia coli*	0	Absent
	Bacillus pumilus	0.60 ± 0.03	Moderate
Bc-20_1 Lf-13_1 Lf-13_2 Lf-13_3	*Escherichia coli*	0	Absent
	Bacillus pumilus	0.80 ± 0.04	Pronounced
Bs-19_1 Lc-12_1	*Escherichia coli*	0.60 ± 0.03	Moderate
	Bacillus pumilus	0	Absent
Bs-19_2	*Escherichia coli*	0	Absent
	Bacillus pumilus	0	Absent
Pp-11_1 Pp-11_2 Pp-11_3 Pp-11_4 Pp-11_5 Pp-11_6 Pp-11_7 Pp-11_8 Pd-16_1 Pd-16_2 Pd-16_3 Pd-16_4 Lp-7_1	*Escherichia coli*	0.70 ± 0.04	Pronounced
	Bacillus pumilus	1.00 ± 0.05	High
Lm-8_1 Lm-8_2	*Escherichia coli*	0	Absent
	Bacillus pumilus	0.70 ± 0.04	Pronounced
Pa-9_1 Pa-9_2	*Escherichia coli*	0	Absent
	Bacillus pumilus	0.60 ± 0.03	Moderate

Unlike biocidal properties, which do not depend on the pathogen growth phase and naturally decrease over time, fungicidal properties need to be determined at each stage of the fungus life cycle since fungal pathogens have a complex growth cycle. We found that the peptide fractions under study did not stop fungal growth, but only inhibited it, which was indicated by a change in the mycelium color. The results were analyzed with time intervals (3, 9, 12, 24, 48, 72 h, etc.) and by the fungus growth phase (stationary, accelerated growth, logarithmic), i.e., during the periods of exponential cell growth, decreased growth, and death or autolysis. The samples with filters impregnated with a nutrient medium were used as a control.

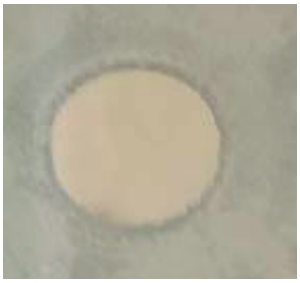
Having analyzed the peptides' fungicidal activity (Table 5, Fig. 11), we identified those peptides which could inhibit *Aspergillus* growth, rather than stop it completely. They were Bs-1_1, Bc-20_1 (identical Lf-13_1, Lf-13_2, and Lf-13_3) and Bs-19_2, with a lysis zone diameter of 0.1–0.2 mm. The maximum fungicidal activity against *A. niger* (0.3–0.5 mm lysis zone) was demonstrated by peptides Bs-19_1 (identical Lc-12_1), Pp-11_1 (identical Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, p-11_7, Pp-11_8, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4, and Lp-7_1), and Pa-9_1 (Pa-9_2). The highest activity against *A. flavus* (0.3–0.4 mm lysis zone) was revealed by peptides Pp-11_1 (identical Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, p-11_7, Pp-11_8, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4, and Lp-7_1), Lm-8_1 (identical Lm-8_2), and Pa-9_1 (identical Pa- 9_2).

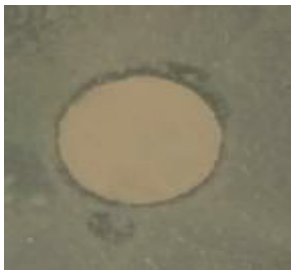
Based on the study of antimicrobial activity, we selected peptides with maximum antibacterial (against *B. pumilus*) and fungicidal (against *A. niger* and *A. flavus*) properties: Pp-11_1 (identical Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, p-11_7, Pp-11_8, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4, and Lp-7_1), Lm-8_1 (identical Lm-8_2), and Pa-9_1 (identical Pa-9_2).

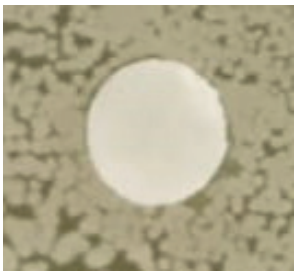

Bs-1_1 *Escherichia coli*


Bs-1_1 *Bacillus pumilus*

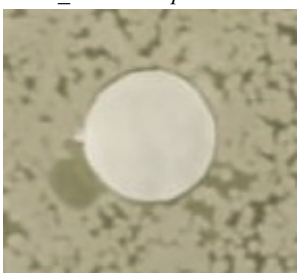

Bc-20_1, Lf-13_1, Lf-13_2, Lf-13_3 *Escherichia coli*

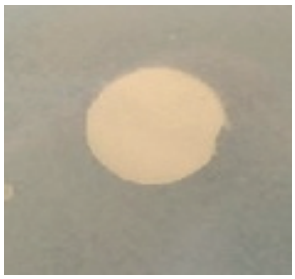

Bc-20_1, Lf-13_1, Lf-13_2, Lf-13_3 *Bacillus pumilus*

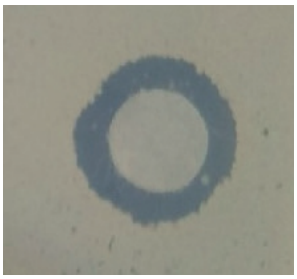

Bs-19_1, Lc-12_1 *Escherichia coli*


Bs-19_1, Lc-12_1 *Bacillus pumilus*


Bs-19_2 *Escherichia coli*


Bs-19_2 *Bacillus pumilus*


Pp-11_1, Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, Pp-11_7, Pp-11_8, Lp-7_1, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4 *Escherichia coli*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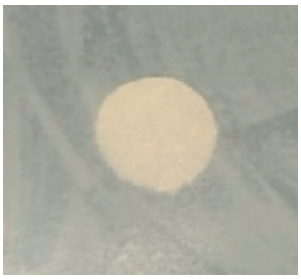
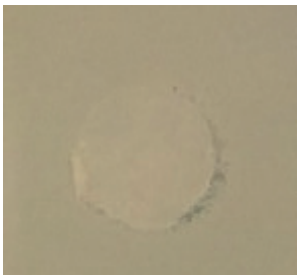
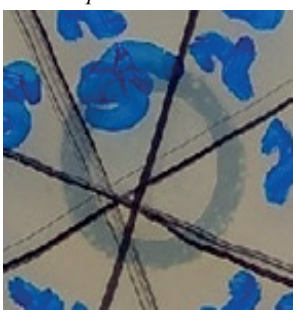
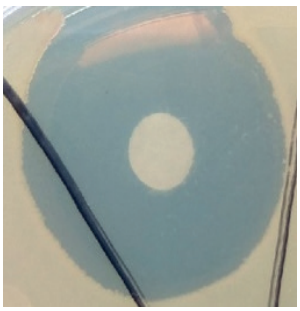

Pa-9_1, Pa-9_2 *Escherichia coli*

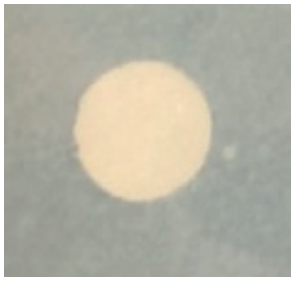

Pa-9_1, Pa-9_2 *Bacillus pumilus*


Lm-8_1, Lm-8_2 *Escherichia coli*


Lm-8_1, Lm-8_2 *Bacillus pumilus*


Amp *Escherichia coli*


Amp *Bacillus pumilus*


– *Escherichia coli*

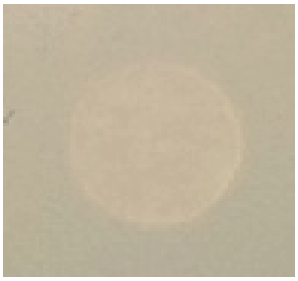

– *Bacillus pumilus*

Figure 10 Antibacterial properties of peptides from the culture fluid of bacteria isolated from the natural sources of Kuzbass

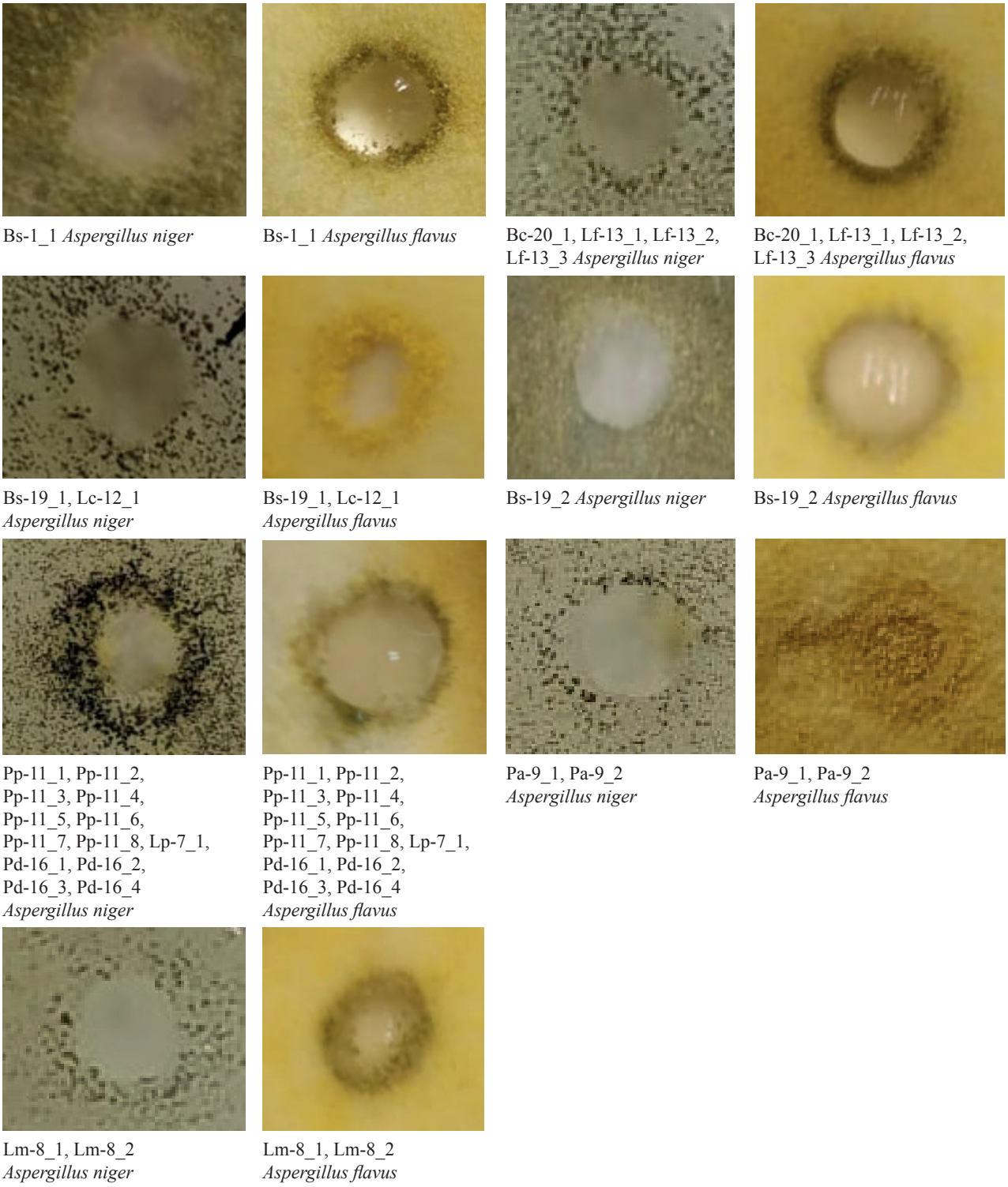


Figure 11 Fungicidal properties of peptides from the culture fluid of bacteria isolated from the natural sources of Kuzbass

Thus, the fact that peptides produced by microorganisms inhabiting the natural ecosystems of Kuzbass exhibit antagonistic activity against opportunistic strains opens up prospects for their use in the production of pharmaceutical substances with antimicrobial action, alternative to traditional antibiotics.

CONCLUSION

We identified amino acid sequences and molecular masses of peptide fractions produced by bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bacillus*, and *Geobacillus*) isolated from the natural objects of the Siberian region (soil, rhizosphere of plants, bottom

Table 5 Fungicidal properties of peptides from the culture fluid of bacteria isolated from the natural sources of Kuzbass (M ± m)

Peptide code	Lysis zone diameter by growth phase, mm						
	Exponential cell growth, h			Decreased growth, h		Death or autolysis, days	
	3	9	12	48	72	6	12
Aspergillus niger							
Bs-1_1	+	+	+	0.100 ± 0.005	0.200 ± 0.010	0.100 ± 0.005	0.100 ± 0.005
Bc-20_1, Lf-13_1, Lf-13_2, Lf-13_3	+	+	+	0.200 ± 0.010	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005
Bs-19_1, Lc-12_1	+	+	+	0.500 ± 0.025	0.500 ± 0.025	0.400 ± 0.020	0.400 ± 0.020
Bs-19_2	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.200 ± 0.010	0.200 ± 0.010
Pp-11_1, Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, p-11_7, Pp-11_8, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4, Lp-7_1	+	+	+	0.100 ± 0.005	0.200 ± 0.010	0.400 ± 0.020	0.400 ± 0.020
Lm-8_1, Lm-8_2	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005
Pa-9_1, Pa-9_2	+	+	+	0.100 ± 0.005	0.300 ± 0.015	0.400 ± 0.020	0.400 ± 0.020
Aspergillus flavus							
Bs-1_1	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005
Bc-20_1, Lf-13_1, Lf-13_2, Lf-13_3	+	+	+	0.200 ± 0.010	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005
Bs-19_1, Lc-12_1	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005
Bs-19_2	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005
Pp-11_1, Pp-11_2, Pp-11_3, Pp-11_4, Pp-11_5, Pp-11_6, p-11_7, Pp-11_8, Pd-16_1, Pd-16_2, Pd-16_3, Pd-16_4, Lp-7_1	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.300 ± 0.015	0.400 ± 0.020
Lm-8_1, Lm-8_2	+	+	+	0.100 ± 0.005	0.300 ± 0.015	0.300 ± 0.015	0.400 ± 0.020
Pa-9_1, Pa-9_2	+	+	+	0.100 ± 0.005	0.300 ± 0.015	0.400 ± 0.020	0.400 ± 0.020
Positive control	+	+	+	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005	0.100 ± 0.005

sediments of reservoirs, and plant waste). In total, we isolated 25 protein fractions, some with identical mass spectra. Thus, we obtained seven peptides with different amino acid sequences, five of which have no analogues in the PepBank and Uniprot databases. One of the peptides (VMCLARKCSQGLIVKAPLM, 2061.66 Da) was homologous to the cysteine membrane protein *Giardia lamblia* P15, and another one (AVPSMKLCIQWSPVRASPCVMLGI, 2587.21 Da) was homologous to the *Planctomycetes bacterium* I41 peptides.

The peptides obtained from the culture fluid of bacteria isolated from natural sources of the Siberian Federal District were analyzed for antibacterial properties against *Bacillus pumilus* and *Escherichia coli*. We identified one peptide that exhibited no antagonistic activity against either gram-negative or gram-positive bacteria. One peptide fraction showed high antibacterial properties against both *B. pumilus* and *E. coli*. One peptide was active against *E. coli*, but not against *B. pumilus* (gram-positive bacteria). Finally, four out of seven peptides under study exhibited moderate and pronounced antagonism against *B. pumilus*, but no antibacterial activity against *E. coli*.

Our study of the peptides' antifungal activity revealed three peptides that could inhibit the growth of the microscopic fungi *Aspergillus niger* and *Aspergillus flavus*, without stopping it completely (0.1–0.2 mm lysis zone). Four peptide fractions showed high fungicidal activity against *Aspergillus* (0.3–0.5 mm lysis zone).

According to our results, antimicrobial peptides produced by bacteria isolated from the natural objects of the Siberian region can be used as promising agents in the production of pharmaceutical substances and drugs (after safety trials) to treat infectious diseases, such as gastrointestinal, respiratory, blood and skin, as well as fungal infections.

CONTRIBUTION

The authors are equally responsible for the research results and the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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