Research Article

Antimicrobial Properties of the Probiotic Strain Bacillus Subtilis 534

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ARTICLE INFO

Received Date: December 06, 2018
Accepted Date: January 07, 2019
Published Date: January 10, 2019

KEYWORDS

Antibiotic resistance
Pathogenic microorganisms
Antimicrobial activity
Bacillus subtilis 534
Probiotic Sporobacterin

Citation for this article: Olga Efremenkova, Nina Gabrielyan, Irina Malanicheva, Mariya Demiankova, Tatiana Efimenko, Eugene Rogozhin, Sofiya Sharapchenko, Tatyana Krupenio, Dmitrij Davydov and Maksim Kornilov. Antimicrobial Properties of the Probiotic Strain Bacillus Subtilis 534. SL Gastroentrology. 2019; 2(1):119

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ABSTRACT

The effectiveness of antibiotic therapy is constantly decreasing due to the increasing spread of resistant pathogens. One alternative treatment for infections is the use of probiotics – living microorganisms that contribute to the normalization of the human microbiota. Strain Bacillus subtilis 534 is a transient probiotic, which is the basis of the drug Sporobacterin. Under submerged cultivation, the strain 534 produces antimicrobial substances that suppress the growth of Gram-positive bacteria and fungi. The Culture Liquid (CL) of the strain 534 inhibits the growth not only the test strains, but also the multidrug-resistant clinical isolates of the following Gram-positive bacteria and fungi: Staphylococcus aureus, S. epidermidis, S. capitissbsp. urealyticus, Candidaalbicans, C. glabrata, C. lusitaniae, Cryptococcus neoformans, Prototheca sp., Trichosporon sp. Although no activity against Gram-negative bacteria was detected in the CL, component # 1 with such activity was detected after its isolation from CL and concentration of approximately 800 times. Component #1 has been found to be active against some clinical isolates of Gram-negative multidrug-resistant bacteria of the Klebsiella pneumonia and Acinetobacter baumannii species.

INTRODUCTION

The use of antibiotics in medicine began during the Second World War, but over the past decades, the effectiveness of antibiotic therapy is constantly decreasing due resistance development of pathogenic microorganisms [1-3]. The current situation requires the development and introduction of new antibiotics into the medical practice, as well as other antimicrobial drugs. The latter include probiotics - drugs, which are based on living microorganisms that contribute to the normalization of the human microbiota. The term "probiotic" is also applied directly to probiotic microorganisms. The action of probiotics is multifactorial in nature, and one of the active principles is the formation of antibiotic substances that help restore the balance of microbiota. A valuable property of many probiotics is the lack of toxicity [4].

Probiotic microorganisms include such inhabitants of the intestine as streptococci, lactobacilli and bifidobacteria, as well as transient microorganisms, i.e. temporarily present in the human body. In particular, hay bacillus (Bacillus subtilis), which is widely distributed in nature and enters the human body with water and food, gives grounds for some researchers to consider this bacterium as a normal intestinal microbiota

inhabitant [5-7]. The first bacillary probiotic described was the B. subtilis strain isolated from the wound in 1945 [8]. The result of subsequent research was the isolation from bacilli many antibiotics of different structure, active against both Gram-positive and Gram-negative microorganisms, as well as fungi [9-13]. In medical practice of different countries, biological preparations based on bacteria of the genus Bacillus are used. In Western Europe apply probiotic Baktisubtil (the company Marion Merrell, France) and its analogue Flonivin BS (the company Galenika, Slovenia). The basis of these preparations is B. cereus IP 5832 strain from the collection of the Institute Pasteur (France). Also known is the biological preparation Cereobiogen (Xing Jian, China), the active agent of which is B. cereus strain DM-423. The Vietnamese drug Biosubtil as an active ingredient contains the strain B. subtilis. Three bacilli-based drugs have been registered in Russia: Biosporin, Sporobacterin, Baktisporin. Biosporin is the only complex drug, which is based on two strains - B. subtilis 2335 and B. licheniformis 2336. Sporobacterin and Bactisporin are based on B. subtilis strains – 534 and 3H respectively.

The drug sporobacterin is a spore suspension of probiotic strain B. subtilis 534 in 7% aqueous sodium chloride solution. In Russia, probiotic Sporobacterin has been used for last thirty years, in particular, for the prevention and treatment of postoperative bacterial and fungal infections in high-tech surgery, including transplantation [14-16]. To date, the results of long-term clinical and laboratory studies have been published, according to which the use of Sporobacterin in the technology of early postoperative management of patients with cardio-surgical and transplant profiles opens up possibilities for optimizing the clinical condition of patients, and also significantly reduces the duration of antibiotic therapy courses [17-20]. The purpose of this study was to determine the ability of strain 534 produce antimicrobial substances under in vitro cultivation conditions. Collection test strains and clinical isolates with different antibiotic resistance were used as test organisms.

MATERIALS AND METHODS

The object of the study: Bacillus subtilis 534 is the basis of the probiotic Sporobacterin. The strain was deposited in the Microbiological Collection of the Gause Institute of New Antibiotics under the number INA 01122 (Russia).

Test organisms and determination of antimicrobial activity: As tests for determining antimicrobial activity, we used international collection strains of Gram-positive and Gram-negative bacteria, fungi (Table 1), as well as clinical isolates of pathogenic microorganisms with different antibiotic resistance (Table 2-5). The species identification of clinical isolates of pathogenic microorganisms, as well as their susceptibility/resistance to antibiotics was carried out on an automated bacteriological analyzer for the identification of microorganisms Siemens MicroScan Walk Away - 96 Plus System. The antimicrobial activity of the Culture Liquid (CL) of B. subtilis 534 and its active component extracted was determined by agar-diffusion method. The culture liquid was introduced into the wells in the agar medium contaminated with the test organism. The selected antimicrobial component was applied to the disks and placed on the surface of the agar medium.

Table 1: Antimicrobial spectrum against collection strains and the highest established level of antimicrobial activity in Bacillus subtilis 534 Cultural Liquid (CL).

Test-strains	Growth inhibition zones of CL,mm
Bacillus mycoides 537	16-18
Bacillus pumilus NCTC 8241	22-24
Bacillus subtilis ATCC 6633	10-12
Leuconostoc mesenteroides VKPM B-4177	16-18
Micrococcus luteus NCTC 8340	23-25
Staphylococcus aureus FDA 209P (MSSA)	22-30
Staphylococcus aureus INA 00761 (MRSA)*	25-27
Staphylococcus epidermidis INA 01121*	22-23
Comamonas terrigena VKPM B-7571	0
Escherichia coli ATCC 25922	0
Pseudomonas aeruginosa ATCC 27853	0
Aspergillus niger INA 00760*	18-22
Saccharomyces cerevisiae RIA 259	19-25

Cultivation conditions: Submerged cultivation of B. subtilis 534 strain was carried out using modified medium #2 Gause of the following composition (%): glucose - 1, peptone - 0.5, tryptone - 0.3, NaCl - 0.5, tap water; pH 7.2-7.4. Spores of the strain 534 in an amount of 106/ml were injected into 750 ml Erlenmeyer flasks with 150 ml of the medium, after which the flasks were placed on the rotary shaker with rotation speed 220 rpm and incubated at 28°C. Antimicrobial activity of the Culture Liquid (CL) was determined at 2, 4 and 7 days of cultivation. For surface cultivation of all microorganisms, the agarized variant of the modified same medium was used with the addition of 2% agar. Fungal cultures, C. terrigena VKPM B-7571 and L. mesenteroides VKPM B-4177 were grown at

28°C, all other strains, including clinical isolates and the probiotic strain B. subtilis 534, at 37°C.

Table 2: Antimicrobial activity of the Bacillus subtilis 534 Cultural Liquid (CL) against clinical isolates of *Staphylococcus* spp.

Species, clinical isolates	Growth inhibition zones of CL, mm	The susceptibility/resistance of clinical isolates against 22 medical antibiotics*			
		R	I	S	Details:**
S. aureus 2476	0	3	0	18	3R: P, AM, LVX
S. capitis sbsp. *urealyticus* 1133	17	2	0	20	2R: P, AM
S. epidermidis 2480	0	16	0	6	6S: DAP, E, RA, SYN, TE, VA
S. epidermidis 2432	0	16	0	6	6S: DAP, E, RA, SYN, TE, VA
S. epidermidis 2624	25	15	0	5	5S: DAP, LZD, RA, SYN, VA
S. epidermidis 2688	0	16	0	4	4S: DAP, RA, TE, VA
S. epidermidis 1217	(17)***	11	1	10	10S: C, CC, DAP, LZD, OX, RA, SYN, TE, SXT, VA 1I: MXF
S .epidermidis 1259	0	12	0	10	10S: C, CC, DAP, LZD, MXF, RA, SYN, TE, SXT, VA
S .epidermidis 1306	0	6	0	16	6R: AM, E, LVX, P, TE, SXT
S. epidermidis 1319	0	3	1	18	3R: AM, E, P 1I: C
S. epidermidis 1401	0	14	1	7	7S: C, CC, DAP, LZD, RA, SYN, VA 1I: MXF
S. epidermidis 1109	0	11	0	10	10S: C, CC, DAP, LZD, MXF, RA, SYN, TE, SXT, VA
S. haemolyticus 4126	0	17	0	5	5S: DAP, RA, SYN, SXT, VA
S. hominis sbsp. *novobiosepticus* 4221	0	14	0	8	8S: SAM, DAP, MXF, OX, RA, SYN, SXT, VA
S. xylosus 1298	0	15	0	6	6S: C, GM, LVX, MXF, TE, SXT

*Antibiotics: AM - ampicillin, AN - amikacin, ATM - aztreonam, C - chloramphenicol, CC - clindamycin, DAP - daptomycin, E - erythromycin, GM - gentamicin, IPM - imipenem, LZD - linezolid, LVX - levofloxacin, MEM - meropenem, MXF - moxifloxacin, NN - tobramycin, OX - oxacillin, P - penicillin, RA - rifampin, SAM - ampicillin/sulbactam, SXT - trimethoprim/sulfamethoxazole, SYN - synercid, TE - tetracyclin, VA - vancomycin.

**R – resistance, I – intermediate susceptibility, S – susceptibility.

***Growth oppression.

Table 3: The activity of the component #1 of the Bacillus subtilis 534 antibacterial complex against clinical isolates of Acinetobacter baumannii.

Clinical isolates, ##	Growth inhibition zones of component #1, mm	The susceptibility/resistance of clinical isolates against 15 medical antibiotics*		
		R	I	S
1630	9	15	0	0
1839	9	14	1(SAM)	0
2050	9	15	0	0
2455	8	15	0	0
2617	12	15	0	0
3037	10	14	1(GM)	0
3050	0	13	2(GM, PIP)	0
3122	7	15	0	0
3164	0	13	2(GM, LVX)	0
3166	7	15	0	0
3208	8	15	0	0
3238	7	12	3(GM, FEP, SAM)	0
3255	Trace***	15	0	0
3275	Trace***	3	5(PIP, GM, CRO)	7(AN, FEP, SAM, CIP, LVX, MEM, NN)
3613	Trace***	15	0	0
4006	Trace***	15	0	0
4066	Trace***	15	0	0
4074	Trace***	15	0	0
4165	9	13	1(MEM)	1(TE)
4200	Trace***	15	0	0
4315	Trace***	15	0	0
4354	8	15	0	0
4372	0	15	0	0
4374	0	13	1(MEM)	1(TE)

*Antibiotics: AN - amikacin, SAM - ampicillin/sulbactam, GM - gentamicin, IPM - imipenem, LVX - levofloxacin, MEM - meropenem, PIP - piperacillin, TE - tetracycline, NN - tobramycin, SXT trimethoprim/sulfamethoxazole, FEP - cefepime, CTX - cefotaxime, CAZ - ceftazidime, CRO - ceftriaxone, CIP - ciprofloxacin.

R – resistance, I – intermediate susceptibility, S – susceptibility.

*** "Trace" – the thickness of the ring of the growth inhibition zone around the hole in agar medium with the sample which is tested for antimicrobial activity does not exceed 1 mm.

Table 4: Antimicrobial activity of the component #1 of the Bacillus subtilis 534 antibacterial complex against clinical isolates of Klebsiella pneumoniae.

Clinical isolates, ##	Growth inhibition zones, mm		The susceptibility/resistance of clinical isolates against 24-28 medical antibiotics*			
	CL	Component #1	R	I	S	Details:**
320	0	0	19	1	6	6S: FOX, IPM, MEM, TGC, PMB, CL 1I: TE
634	0	0	17	1	8	8S: AN, FOX, IPM, MEM, LVX, TGC, PMB, CL 1I: CIP
692	0	0	20	1	7	7S: AN, FOX, ETP, IPM, MEM, PMB, CL 1I: TGC
775	0	0	16	1	9	9S: AN, GM, IPM, MEM, TE, TGC, SXT, PMB, CL 1I: FOX
821	0	11	23	0	3	3S: TGC, PMB, CL
1050	0	0	17	2	5	5S: AN, GM, IPM, PMB, CL 2I: TGC, NN
1054	0	(11)**	19	1	4	4S: FOX, IPM, MEM, TGC 1I: LVX
1071	0	(9)**	20	0	4	4S: ATM, FEP, CAZ, CRO
1074	0	(11)**	23	3	0	3I: PIP/TAZ, PMB, CL
1114	Trace***	7	20	2	4	4S: AN, GM, PMB, CL 2I: TGC, NN
1878	0	8	23	1	0	1I: TGC
2080	Trace***	Trace***	23	1	0	1I: TGC
2266	0	0	23	0	3	3S: CAZ, TGC, PMB
2427	0	0	23	0	2	2S: TGC, PMB
2663	0	0	23	0	2	2S: TGC, PMB
3168	-	(9)**	22	1	3	3S: TGC, PMB, CL 1I: TE
3186	-	0	23	0	3	3S: TGC, PMB, CL
3266	-	0	22	0	4	4S: CIP, LVX, PMB, CL
4095	-	(8)**	23	1	2	2S: PMB, CL 1I: TGC
4261	-	0	19	0	7	7S: ATM, FEP, CAZ, CIP, LVX, PMB, CL
4353	-	0	14	1	11	11S: ATM, FEP, CTX, CAZ, CRO, IPM, MEM, CIP, LVX, PMB, CL 1I: TGC
4375	0	0	17	0	8	8S: AN, ETP, GM, IPM, MEM, TGC, PMB,CL

*Antibiotics: AN – amikacin, ATM – aztreonam, C - chloramphenicol, CAZ - ceftazidime, CIP - ciprofloxacin, CL - colistin, CRO - ceftriaxone, CTX - cefotaxime, E - erythromycin, ETP - ertapenem, FEP - cefepime, FOX - cefoxitin, GM - gentamicin, IPM - imipenem, LVX - levofloxacin, MEM - meropenem, MXF - moxifloxacin, NN - tobramycin, OX - oxacillin, P - penicillin, PIP - piperacillin, PIP/TAZ - piperacillin/tazobactam, PMB - polymyxin B, RA - rifampin, SXT - trimethoprim/sulfamethoxazole, TE - tetracycline, TGC - tigecycline.

R – resistance, I – intermediate susceptibility, S – susceptibility.

** - Growth oppression

*** "Trace" - the thickness of the ring of the growth inhibition zone around the hole in agar medium with the sample which is tested for antimicrobial activity does not exceed 1 mm.

Table 5: Growth inhibition zones of the clinical isolates of pathogenic fungi under the action of the Culture Liquid (CL) of the strain B. subtilis 534 (diameters in mm).

Species, strains	Growth inhibition zones of CL, mm	Medical antimycotics*					
		Pyrimidine	Polyene	Imidazoles		Triazoles	
		5 FC	AB	MCZ	KET	ITR	FLU
Candida albicans 58	18	S	R	R	R	R	R
C. albicans 182	19	S	S	S	S	S	S
C. albicans 265	29	S	R	S	S	I	S
C. albicans 317	20	S	S	R	R	R	R
C. albicans 456	23	S	R	I	I	I	S
C. albicans 458	17	S	R	R	R	R	R
C. albicans 848	28	S	R	R	R	R	R
C. albicans 922	24	S	I	R	R	R	R
C. albicans 1187	19	S	I	I	S	I	S
C. albicans 1294	31	S	R	R	R	R	R
C. albicans 1610	0	S	S	R	I	S	S
C. albicans 1815	36	S	R	I	S	I	S
C. albicans 2122	0	S	S	I	I	I	I
C. albicans 2356	12	R	R	R	R	R	R
C. albicans 4166	25	R	I	R	R	I	R
C. albicans 4244	25	R	R	R	R	I	R
C. albicans 4438	24	R	S	I	S	I	R
C. albicans 4895	24	S	R	I	R	R	R
C. albicans 4897	24	S	S	S	I	R	R
C. albicans 5228	14	R	R	I	R	R	R
C. albicans 5721	24	S	R	I	R	R	R
C. albicans 5963	18	I	R	R	R	R	R
C. albicans 6107	17	R	R	R	I	R	S
C. catenulata 1507	0	R	I	I	I	I	R
C. catenulata 6093	0	S	R	I	I	R	I
C. glabrata 13	0	S	R	I	I	I	I
C. glabrata 212	18	S	R	R	R	R	R
C. krusei 247	0	S	S	R	S	I	S
C. lusitaniae 5254	22	S	R	I	S	I	S
C. parapsilosis 1380	0	R	S	S	S	I	S
C. tropicalis 455	0	S	R	R	R	R	R
Cryptococcus neoformans 25	0	S	R	I	I	R	I
Cr. neoformans 245	25	R	R	S	I	I	I
Prototheca sp. 110**	27	S	R	R	R	R	R
Prototheca sp. 1376	0	R	S	S	S	I	S
Prototheca sp. 6017	0	S	R	I	R	R	I
Prototheca sp. 6047	24	S	R	R	R	R	R
Trichosporon sp. БМ1.5	24	I	S	I	I	I	S

*Antimycotics: 5 FC – 5-flucytosine, AB – amphotericin B, MCZ – miconazole, KET – ketoconazole, ITR – itraconazole, FLU – fluconazole.

R – resistance, I – intermediate susceptibility, S – susceptibility.

** - representatives of the genus Prototheca are chlorophyll-free algae; antimycotics are used to treat Prototheca infections.

Analysis of the strain 534 DNA sequence of the 16S rRNA gene: For the analysis of the nucleotide sequences of the 16S rRNA gene of strain 534 by the Polymerase Chain Reaction (PCR), we used universal primers 27f (agagtttgatcctggctcag) and 1492r (tacggytaccttgttacg act t). The mode of PCR was selected: (1) 95°C - 3 min, (2) 25 cycles with alternating temperature intervals 95°C-30 sec, 51°C-30 sec, 72°C-90 sec, (3) 72°C-7 min. Analysis of PCR products was performed by electrophoresis in 1% agarose gel with an electric field strength of 5 V/cm. Purification of PCR products was carried out by direct DNA reprecipitation under mild conditions (0.125 M ammonium acetate in 70% ethanol). Sequencing of the purified DNA fragments was performed on Genetic Analyzer 3500 automated sequencer (Applied Biosystems). To determine the sequences, the GenBank databases (www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (http://www.cme.msu.edu) were used.

Isolation and purification of antimicrobial components: Through the column filled with 80 ml of Amberlite XAD-2 sorbent, 0.8 l of culture liquid of Bacillus subtilis 534 strain was passed. Antimicrobial components were desorbed with "n-butanol-acetone-water" (1:1:1) at neutral pH. The resulting eluates were evaporated in vacuum to dryness at 37°C and the dry residue was dissolved in 60% aqueous ethanol. Further purification of the antimicrobial components was performed in the column filled with Kieselgel 60 silica gel (Merck) using

stepwise elution with chloroform-methanol solvents (9:1, 8:2, 7:3, 6:4 and 5:5). The fractions from the column were tested for biological activity against test strains of Staphylococcus aureus INA 00761 (MRSA) and Saccharomyces cerevisiae RIA 259. The active fractions was evaporated to dryness, dissolved in 1 ml of methanol each (800-fold concentration) and analyzed by Thin Layer Chromatography (TLC) on silica gel plates in a solvent system ethyl acetate-methanol (1:4) followed by bioautography. Of the three selected active components the component #1 with Rf=0.7 was investigated in the subsequent work.

RESULTS

Since the strain 534 was isolated more than 30 years ago, we were decided to confirm with modern methods identification of this strain. For this, the sequence of the 16S rRNA gene (1413 bp) of strain 534 was determined and was compared with sequences of genus Bacillus type strains from the GenBank database (the sequence of strain 534 is deposited in the GenBank database under the number KU051696). It is shown that the closest sequence to the 16S rRNA gene sequence of strain 534 is the sequence of the type strain B. subtilis DSM10 (99.4% coincidence) [21]. Thus, it was confirmed that the strain 534 belongs to the species Bacillus subtilis.

When analyzing Culture Liquid (CL), it is shown that the strain 534 forms antibiotic components with different antimicrobial spectrum, the maximum activity of which are achieved for different time (Figure 1). From Figure 1 it follows that in the CL there are at least four different compounds or groups of compounds that differ in antimicrobial activity:

- antibacterial activity only in respect of B. subtilis ATCC 6633 strain and only on the second day of cultivation; such activity may be due to bacteriocin;
- antifungal activity, the peak of which falls on the second day and gradually decreases by the seventh day;
- antibacterial activity against M. luteus NCTC 8340 and S. aureus FDA 209P (MSSA), which is manifested on the third day of cultivation and increases on the seventh day;
- antibacterial activity against B. pumilus NCTC 8241 and S. aureus INA 00761 (MRSA).

The maximum possible levels of antimicrobial activity in the above-described culture conditions are given in Table 1. From Figure 1 and Table 1 it follows that strain 534 inhibits the growth of Gram-positive bacteria and fungi. It is important to note that CL is active against Methicillin-Resistant Staphylococcus Aureus(MRSA) and the Leuconostocmesenteroides VKPM B-4177 test strain with a high level of natural resistance to vancomycin.

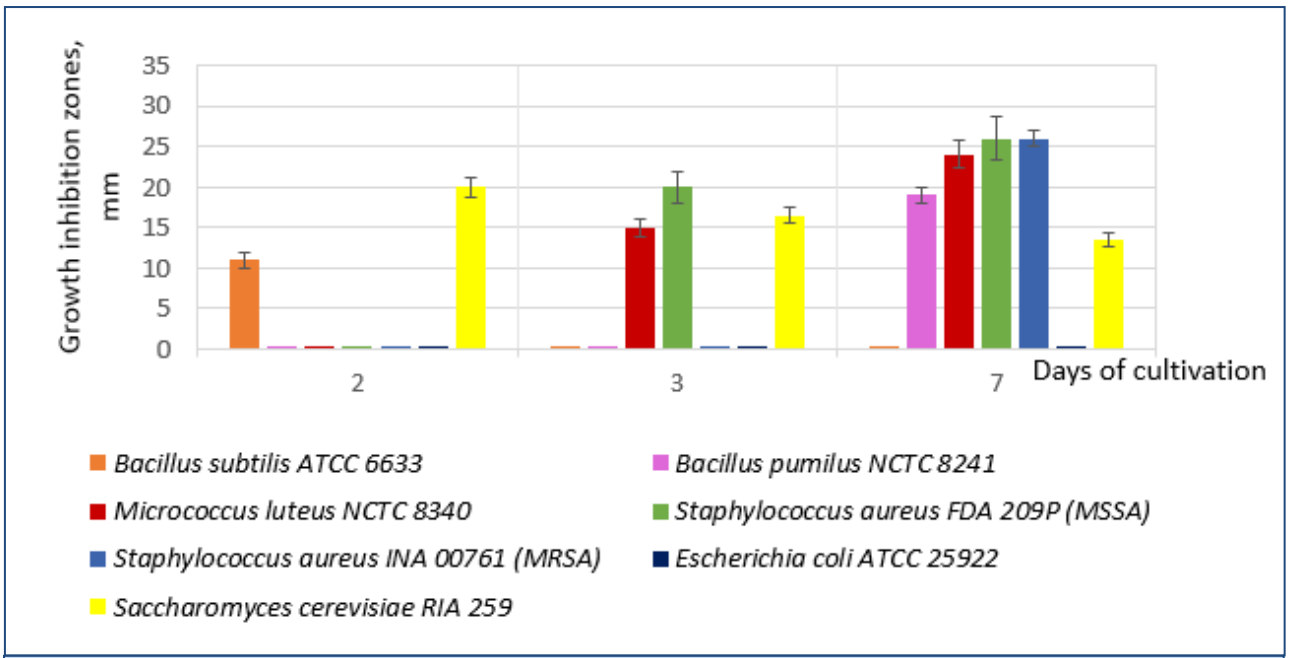

Figure 1: Dynamics of the strain B. subtilis 534 antimicrobial activities in during submerged cultivation.

Clinical isolates

At the next stage, the antimicrobial activity of the culture liquid of strain 534 against 15 clinical isolates of the genus Staphylococcus was tested (Table 2). For two clinical isolates of S. capitissbsp. urealyticus 1133 and S. epidermidis 2624 it was showed pronounced antimicrobial activity. It is important to note that strain 2624 is resistant to 15 medical antibiotics out of 20. Subsequently, in a chemical study of the culture liquid of strain 534, microbiological control with the release of the active components was carried out using the MRSA test strain.

The culture liquid of B. subtilis 534 showed no activity against collection strains of Gram-negative bacteria (Table 1). However, when isolating and, accordingly, increasing the concentration of component #1, which according to preliminary estimates consisted of polypeptide compounds, such activity was observed against a number of clinical isolates of Gram-negative bacteria (Table 3,4). Although the level of activity of component #1 in relation to Acinetobacterbaumannii and Klebsiellapneumoniae clinical isolates is low, further biotechnological and chemical research is reasonable, since multidrug-resistant bacteria are susceptible to component #1.Since CL of the strain 534 is active against collection test-strains of the fungi Aspergillusniger INA 00760 and Saccharomyces cerevisiae RIA 259, we have investigated its activity against clinical isolates of fungi with different susceptibility to antimycotics (Table 5). Of the 38 clinical isolates, CL of the strain 534 inhibits the growth of 27, including 12 isolates with resistance to 5 out of 6 used medical antimycotics.

DISCUSSION

The drug Sporobacterin is a suspension of B. subtilis 534 spores in 7% aqueous solution of sodium chloride that is intended for oral administration. We have previously shown that Sporobacterin itself does not possess antibiotic properties in vitro [21]. On the contrary, in the conditions of submersed cultivation of the strain 534 in a nutrient medium, a broad spectrum of antimicrobial activity is manifested. On the other hand, it is known that the stay of B. subtilis in the intestines of warm-blooded animals lasts from 10 to 30 days, and the bacteria are present both in the form of vegetative cells and spores. The process of germination and sporulation of spores is repeated several times before excretion from the body with feces [22, 23].

Based on this data, we assumed that in the intestine and in the nutrient medium in flasks developmental processes of B. subtilis are similar and bacterial biosynthesis of antimicrobial substances in the digestive tract is possible. The ability of strain 534 to secrete antibacterial and antifungal substances may be the main explanation for the effectiveness of Sporobacterin in preventing the development and in treatment of bacterial and fungal infections along with such properties as enhancing immunity, stimulating the growth of normal intestinal microflora and secretion of digestive enzymes. It is especially valuable that the probiotic strain 534 is capable to produce substances that overcome the antibiotic resistance of clinical isolates of a number of Gram-positive bacteria and fungi. As for Gram-negative bacteria, it is also possible the manifestation of antimicrobial activity in vitro, albeit to with low intensity. We are currently considering the B. subtilis 534 strain as a producer of antibiotics and are working on identifying the produced antimicrobial compounds.

Is it possible to detect new antibiotics in B. subtilis? Among the bacilli, the B. subtilis species takes the first place in the number of described antibiotics of various chemical nature, among which are polyketide antibiotics [24], phospholipids [25], polyenes [25-28], macrolactins [29] and various peptides that differ by chemical nature, by the mechanism of biosynthesis and by antimicrobial spectra. Some B. subtilis antibiotics are used as modern medicines. The peptide antibiotic bacitracin, one of the first antibiotics described in 1945 and formed by the B. subtilis strain isolated from a human wound, is still used in medicine [8]. A number of close antibiotics forming the bacitracin complex are currently known [30]. Due to the low toxicity in 2010, bacitracin was approved in the United States for the treatment of staphylococcal infections in newborns [9]. The combination of bacitracin with neomycin is effective against most clinical isolates of resistant staphylococcus (MRSA) that is very important for modern antimicrobial therapy [31]. Among the B. subtilis peptides, relatively large peptide antibiotics are bacteriocins, having a molecular weight of tens of thousands of Daltons, characterized by a narrow spectrum of antibiotic activity and acting on other strains of B. subtilis, i.e. acting as

an intraspecific regulatory factor [8, 32,33]. Among bacteriocins, antibiotics with a relatively small molecular weight of 5-10 kilo Dalton, which are called microcins, are isolated into an independent group [30]. Among the peptide antibiotics of B. subtilis, there is also a group of lantibiotics compounds, which include the rare thioether amino acid lanthionine and its derivatives, due to which internal thioester bonds are formed in the antibiotic molecule. Lantibiotics of B. subtilis, for example, subtilin and ericin, unlike bacteriocins, have a broad antimicrobial spectrum of action. Subtilin, consisting of 32 amino acids and having a pentacyclic structure, is similar in structure to the preservative nisin, widely used in the food industry and formed by Lactococcuslactis [9]. From fermented soybeans, traditional Korean food, B. subtilis strain the antibiotic SC-8 was isolated, which exhibits a narrow spectrum of antimicrobial activity against bacteria of the Bacillus cereus group and contributes to food preservation [31]. In B. subtilis also was described a large group of broad-spectrum lipopeptide antibiotics. These are oligopeptides synthesized on multienzyme complexes, to which fatty acids are then added. The amino acid chain and fatty acid bacillarlipopeptides are divided into three families. Representatives of the iturin family are heptapeptides with a lipophilic β-amino acid. They have a pronounced efficacy against mycelial and yeast phytopathogenic fungi [34,35]. Decapeptides with lipophilic β-hydroxy acid belong to the fenicine family and are characterized by the presence of ornithine. They are characterized by efficiency against filamentous fungi [36,37]. The third family includes surfactins - heptapeptides with β-hydroxy fatty acid, which are less effective against fungi, but exhibiting the most pronounced hydrophobic properties among these lipopeptides, which allow the formation of stable biofilms on the surface of plants and prevent their colonization by other microorganisms. In this case, B. subtilis antibiotics play an important role in ecosystems, contributing to the formation of biofilms and the colonization of area [38,39]. The oligopeptides of the lowest molecular weight, containing only two or three amino acids, include rhizocticins, which were isolated as antifungal phosphono-oligopeptides from the CL of collection strain B. subtilis ATCC 6633. These peptides contain a non-protein amino acid (Z)-2-amino-5-phosphono-3-pentenoic acid [33].

Since B. subtilis is a cosmopolitan species and is found on a wide variety of substrates, it is characterized by wide adaptability to various environmental conditions, which, in particular, is reflected in the wide variety of antibiotics produced, and we should expect further description of new antibiotics B. subtilis.

CONCLUSIONS

Strain XXXX 534 refers to probiotics and has long been successfully used in medicine, being the basis of the drug Sporobacterin. The use of Sporobacterin is not toxic and not burdensome to patients, but can be effective. Since it was established that strain 534 exhibits antimicrobial activity under conditions of submerged cultivation, we consider it as a potential producer of antimicrobial substances that overcome the multidrug resistance of pathogenic microorganisms. It is advisable to continue further chemical research and clinical trials of antimicrobial substances with the prospect of their use as medical antibiotics.

ACKNOWLEDGMENTS

The authors thank Svetlana Dolgorukova for Technical assistance.

REFERENCES

1. Butler MS, Blaskovich MA, Cooper MA. (2013). Antibiotics in the clinical pipeline in 2013. J. Antibiot. 66:571-591.
2. Butler MS, Blaskovich MA, Cooper MA. (2017). Antibiotics in the clinical pipeline at the end of 2015. J. Antibiot. 70:3-24.
3. O'Neill J. The Review on Antimicrobial Resistance. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, 2016.
4. Duc LH, Hong HA, Barbosa TM, Henriques AO, Cutting SM. (2004). Characterization of Bacillus Probiotics Available for Human Use. Appl Environ Microbiol. 70: 2161-2171.
5. Pokhilenko VD, Perelygin VV. (2007). Probiotics on the basis of spore-forming bacteria and their safety. Chemical and biological safety. 2-3: 20-41.
6. Skrypnik IN, Maslova AS. (2009). Modern spore-forming probiotics in clinical practice. Modern gastroenterology (Ukrain). 3: 81-90.
7. Savustianenko AV. (2016). Mechanisms of action of probiotics based on Bacillus subtilis. Actual Infectology. 2: 35-44.

8. Johnson B, Anker H, Meleney F. (1945). Bacitracin: a new antibiotic produced by a member of the B. subtilis group. Science. 102: 376-377.
9. Fickers P. (2012). Antibiotic compounds from Bacillus: why are they so amazing? Am. J. Biochem. and Biotech. 8:38-43.
10. Stein T. (2005). Bacillus subtilis antibiotics: structures, syntheses and specific functions. Molecular Microbiology 56: 845-857.
11. Baruzzi F, Quintieri L, Morea M, Caputo L. (2011). Antimicrobial Compounds Produced by Bacillus spp. and Applications in Food. In: Vilas AM. (ed.). Science against Microbial Pathogens: Communicating Current Research and Technological Advances. Formatex, Badajoz, Spain, P. 1102-1111.
12. Cotter PD, Ross RP, Hill C. (2013). Bacteriocins - a viable alternative to antibiotics? Nat. Rev. Microbiol. 11: 95-105.
13. Sumi CD, Yang BW, Yeo IC, Hahm YT. (2015). Antimicrobial peptides of the genus Bacillus: a new era for antibiotics. Can. J. Microbiol. 61:93-103.
14. Nikitenko VI, Polyakova BC, Nikitenko MV. (2001). The drug Sporobacterin. New data on the mechanism of action of this and other living bacterial preparations. Scientific Bulletin of the Tyumen Medical Academy 2: 70-72. (In Russian)
15. Nikitenko VI, Nikitenko IK. (1988). The bacterial strain Bacillus subtilis used to obtain the drug for the prevention and treatment of inflammatory processes and allergic diseases. Patent SU 1723116 A1. USSR.
16. Nikitenko MV, Nikitenko VI. (2001). Drug Sporobacterin liquid. Patent RU2217154C2. Russia.
17. Gabrielyan NI, Suskova VS, Suskov SI, Vologodskaya NL. (2012). Study of the effect of probiotic Sporobacterin on the functional state of granulocyte-macrophage cells of blood donors in vitro. Bulletin of experimental biology and medicine. 153: 653-655. (In Russian)
18. Kazakov EN, Gabrielyan NI, Senchenko OR, Petrakov KV, Arefieva LI, et al. (2013). To the question of the prevention of infectious complications after cardiac surgery in cardiopulmonary bypass. Russian medical journal. 2:13-16. (In Russian)
19. Arefieva LI, Gorskaya EM, Savostiyanova OA, Senchenko OR, Gabrielyan NI. (2013). Infectious complications of a bacterial nature in cardiovascular surgery. Russian medical journal. 3:36-42. (In Russian)
20. Gabrielyan NI, Arefieva LI, Gorskaya EM, Kornilov MN, Moysyuk YG, et al. (2013). The use of biological products in abdominal surgery and liver transplantation. Bulletin of transplantology and artificial organs. 2:148-155.
21. Gabrielyan NI, Gorskaya EM, Krupenio TV, Zenkova VA, Efimenko TA, et al. (2016). Evaluation of the antimicrobial activity of bacillary probiotic Bacillus subtilis (strain 534). Epidemiology and infectious diseases. Current issues. 1:41-47. (In Russian)
22. Osipova IG, Sorokulova IB, Vasilieva EA, Budanova EV. (2005) Preclinical testing of new spore probiotics. Vestn. RAMS 12:36-40.
23. Leser TD, Knarreborg A, Worm J. (2008). Germination and outgrowth of Bacillus subtilis and Bacillus licheniformis spores in the gastrointestinal tract of pigs. J. Appl. Microbiol. 104:1025-1033.
24. Bostian M, McNitt K, Aszalos A, Berdy J. (1977). Antibiotic identification: a computerized data base system. J. Antibiot., 30: 633-634.
25. Egorov ES, Baranova IN. (1999). Bacteriocins: formation, properties, and application. Antibiot. Khimioter. 44: 33-40.
26. Rea MC, Ross RP, Cotter PD, Hill C. (2011). Classification of bacteriocins from gram-positive bacteria. In: Prokaryotic Antimicrobial Peptides: From Genes to Applications. Drider, D. and Rebuffat, S, Eds., New York, Springer, pp. 29-53.
27. Abriouel H, Franz CMAP, Ben Omar N, Galvez A. (2011). Diversity and applications of Bacillus bacteriocins. FEMS Microbiol. Rev. 35: 201-232.
28. Severinov K, Semenova E, Kazakov T.(2011). Class I microcins: Their structures, activities, and mechanisms of resistance. In: Prokaryotic Antimicrobial Peptides: From Genes to Applications. Drider, D. and Rebuffat, S., Eds., New York: Springer, pp. 289-308.
29. Ross RP, Morgan S, Hill C. (2002). Preservation and fermentation: past, present and future. Int. J. Food Microbiol., 79: 3-16.

30. Ikai Y, Oka H, Hayakawa J, Harada KI, Suzuki M. (1992). Structural characterization of bacitracin components by frit-fast atom bombardment (FAB) liquid chromatography/mass spectrometry (LC/MS). J. Antibiot. 45: 1325–1334.
31. Suzuki M, Yamada K, Nagao M, Aoki E, Matsumoto M, et al. (2011). Antimicrobial ointments and methicillin-resistant Staphylococcus aureus USA300. Emerg. Infect. Dis. 17: 1917-1920.
32. Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M. (2010). Natural functions of lipopeptides from Bacillus and Pseudomonas: more than surfactants and antibiotics. FEMS Microbiol Rev. 34: 1037-1062.
33. Fredenhagen A, Angst C, Peter HH. (1995). Digestion of rhizocticins to (Z)-L-2-amino-5-phosphono-3-pentenoic acid: revision of the absolute configuration of plumbemycins A and B. J. Antibiot., 48: 1043-1045.
34. Thimon L, Peypoux F, Wallach J, Michel G. (1995). Effect of the lipopeptide antibiotic, iturin A, on morphology and membrane ultrastruture of yeast cell. FEMS Microbiol. Lett., 128: 101-106.
35. Tsuge K, Akiyama T, Shoda M. (2001). Cloning, sequencing, and characterization of the iturinA operon. J. Bacteriol., 183: 6265-6273.
36. Steller S, Vollenbroich D, Leenders F, Stein T, Conrad B, et al. (1999). Structural and functional organization of the fengycinsynthetasemultienzyme system from Bacillus subtilis b213 and A1/3. Chem. Biol., 6: 31-41.
37. Vanittanakom N, Loeffer W, Koch U, Jung G. (1986). Fengycin – A novel antifungal lipopeptide antibiotic produced by Bacillus subtilis F-29-3. 39: 888-901.
38. Magnet-Dana R, Thimon L, Peypoux F, Ptak M. (1992). Surfactin/iturinA interactions may explain the synergistic effect of surfactin on the biological properties of iturin A. 74: 1047-1051.
39. Bais HP, Fall R, Vivanco JM. (2004). Biocontrol of Bacillus subtilis against infection of Arabidopsis roots by Pseudomonas syringae is facilitated by biofilm formation and surfactin production. 134: 307-319.