AGRICULTURAL BIOLOGY,
2020, V. 55, № 3, pp. 816-829
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 579.62:579.852.11:615.33:575.113

doi: 10.15389/agrobiology.2020.4.816eng
doi: 10.15389/agrobiology.2020.4.816rus

GENOMIC AND PHENOTYPICAL POTENTIAL OF ANTIMICROBIAL ACTIVITY OF A BACILLUS STRAIN *Bacillus megaterium* B-4801

G.Yu. LAPTEV[1], E.A. YILDIRIM[2], T.P. DUNYASHEV[1], L.A. ILYINA[2], D.G. TYURINA[2], V.A. FILIPPOVA[2], E.A. BRAZHNIK[2], N.V. TARLAVIN[2], A.V. DUBROVIN[2], N.I. NOVIKOVA[2], V.K. MELIKIDI[2], S.N. BIKONYA[2]

[1]*Saint Petersburg State Agrarian University,* 2, lit A, Peterburgskoe sh., St. Petersburg—Pushkin, 196601 Russia, e-mail laptev@biotrof.ru (corresponding author ✉), timur@biotrof.ru;
[2]*JSC «Biotrof+»,* 19, korp. 1, Zagrebskii bulv., St. Petersburg, 192284 Russia, e-mail deniz@biotrof.ru, ilina@biotrof.ru, bea@biotrof.ru, dumova@biotrof.ru, novikova@biotrof.ru, tiurina@biotrof.ru, tarlav1995@biotrof.ru, dubrovin@biotrof.ru, veronika@biotrof.ru, svetlana@biotrof.ru

ORCID:
Laptev G.Yu. orcid.org/0000-0002-8795-6659
Yildirim E.A. orcid.org/0000-0002-5846-4844
Dunyashev T.P. orcid.org/0000-0002-3918-0948
Ilyina L.A. orcid.org/0000-0003-2490-6942
Tyurina D.G. orcid.org/0000-0001-9001-2432
Filippova V.A. orcid.org/0000-0001-8789-9837
Brazhnik E.A. orcid.org/0000-0003-2178-9330
Tarlavin N.V. orcid.org/0000-0002-6474-9171
Dubrovin A.V. orcid.org/0000-0001-8424-4114
Novikova N.I. orcid.org/0000-0002-9647-4184
Melikidi V.K. orcid.org/0000-0002-2883-3974
Bikonya S.N. orcid.org/0000-0002-3900-6341

The authors declare no conflict of interests

Acknowledgements:
Supported financially from the Russian Foundation for Basic Research, grant No. 19-316-90041 "Whole-genome sequencing of bacilli strains isolated from the cicatricial contents of various ruminants"

Received June 13, 2020

Abstract

The genetic determinants of bacterial strains *Bacillus* sp., which determine the possibility of biosynthesis of various antimicrobial compounds, are of particular scientific interest, since thanks to them these microorganisms are widely used as the basis of probiotics. An important stage in the systemic analysis of the mechanisms of probiotic action, in particular the antimicrobial activity of microorganisms, is the reconstruction of its metabolic map, that is, the collection and visualization of all potential cell processes. In this work, for the first time, the potentially inherent genetic mechanisms for the synthesis of a number of biologically active substances in the bacterial strain *Bacillus megaterium* are described, in particular, the possibility of synthesizing kanosamine, a bacteriocin belonging to the aminoglycoside group, which can play an important role in the implementation of probiotic properties due to its pronounced antimicrobial activity. Our goal was to study the antimicrobial activity of the strain *Bacillus megaterium* B-4801 against pathogenic and opportunistic bacteria, as well as to search for genes associated with antimicrobial activity based on whole genome sequencing. The *B. megaterium* B-4801 strain deposited in the collection of OOO BIOTROF+, possesses a pronounced probiotic activity. Its antimicrobial activity against *Staphylococcus aureus*, *Candida tropicalis*, *Clostridium* sp., and *Escherichia coli* was assessed by the method of delayed antagonism using wells. A DNA library for whole genome sequencing was generated using Nextera XT kit (Illumina, Inc., USA). Nucleotide sequences were determined using a MiSeq instrument (Illumina, Inc., USA) and MiSeq Reagent Kit v3 (300-cycle) (Illumina, Inc., USA). Invalid sequences and adapters were removed using the Trimmomatic-0.38 program. Filtered in length from 50 to 150 bp pair-terminal sequences were assembled de novo using genomic assembler SPAdes-3.11.1. Functional annotation of the genome was performed with PROKKA 1.12 and RAST 2.0 programs. The pool of genes associated with antimicrobial activity was assessed and the metabolic map was constructed using the KEGG Pathway database (http://www.genome.jp/kegg/). The antagonistic activity of *B. megaterium* B-4801 against pathogenic and opportunistic microorganisms was revealed by cultural methods. The growth inhibition zones of the test strains ranged from 2±0.15 to 25±1.4 mm. The genome of the *B. megaterium* B-4801 strain is a single circular chromosome with a size of 6,113,972 bp, containing 37.5 % GC pairs. More than 45 % of *B. megaterium* B-4801 genes are involved in the transport and metabolism of amino acids, transcription, translation, transport and metabolism of carbohydrates and proteins. The key genetic loci that determine the synthesis of antimicrobial metabolites have been identified. The sequenced genome of the strain contains genes (*FabD*, *FabF*, *FabG*, *FabZ*, *FabI*, etc.) associated with the production of proteins involved in the synthesis of aliphatic unsaturated C_3-C_{18} carboxylic acids, in particular, butyric, nylon, caprylic,

capric, lauric, myristic, palmitic, stearic, oleic. According to the information accumulated by world science, all these substances have pronounced antimicrobial properties. The whole-genome sequencing also discovered a cluster of genes (*Asm22-24*, *Asm43-45*, and *Asm47*) associated with the biosynthesis of bacteriocin kanosamin, which belongs to the aminoglycoside group, and polyketide ansamycin antibiotics from the macrolide group. The established probiotic potential indicates the role of the investigated strain as a potential probiotic candidate, in particular for use in animal husbandry. The performed genomic analysis revealed new systems of operons that control the metabolic pathways for the synthesis of antimicrobial substances, which were not previously described for *B. megaterium*.

Keywords: whole-genome sequencing, *Bacillus megaterium*, acid biosynthesis, bacteriocins, antimicrobial activity, kanosamine, ansamycin antibiotics, probiotics

Nowadays, animal husbandry and poultry farming, due to the entry into force of Federal Law No. 280-FZ "On organic products and on amendments to certain legislative acts of the Russian Federation" from January 1, 2020, show a vital interest to natural biological products. According to the law, the use of antibiotics is prohibited in the organic farming, with the exception of those permitted for use by the national, interstate and international standards of organic production in force in Russia.

Genetic determinants of *Bacillus* sp. encoding biosynthesis of various antimicrobial compounds are of particular interest, since wide application of these microorganisms as biopesticides in crop production and as a basis for therapeutic agents and probiotics in animal husbandry. According to some researchers [1], strains of the genus *Bacillus* are more promising for probiotics preparations as compared to traditionally used lactobacilli, as *Bacillus* strains form endospores in the life cycle and, therefore, are more resistant to aggressive factors in the digestive tract. *Bacillus* strains are typical commensal bacteria of the rumen and intestines of farm animals [2], where as an increase in the abundance of lactobacilli in the rumen is associated with the occurrence of lactic acidosis, a decrease in pH and a decrease in the number of bacteria synthesizing cellulases [2].

On average, 87% of total pool of antimicrobial metabolites of bacilli are organic acids, alcohols, ketones, alkanes, aldehydes, alkenes, and 13% are other substances, the ribosomal peptides (bacteriocins and enzymes), polyketides, non-ribosomal peptides [3]. A case study of a *B. subtilis* strain as an example has been shown that at least 4-5% of its genome are operons associated with the synthesis of antimicrobial compounds [4].

Interest in studying the ability of *Bacillus* sp. to synthesize bacteriocins, peptide and lipopeptide antibiotics is currently rising [5, 6], as information about previously unknown substances appears [7-9]. Many of the *Bacillus* bacteriocins are classified as lantibiotics, the post-translationally modified peptides widely distributed among various bacterial taxa.

Bacteria of genus Bacillus also produce many unmodified bacteriocins, some of which are similar to pediocin-like bacteriocins of lactobacilli while peptide sequences of other bacteriocins are completely new [10] Bacteriocins of *Bacillus* sp. are of practical interest due to the ability to inhibit various pathogenic forms, including gram-negative and gram-positive bacteria, yeast and micromycetes [7-9].

The synthesis of organic acids by *Bacillus* microorganisms is studied to a much lesser extent. There are indications of *Bacillus* sp. ability to produce lactic acid [11] known for its antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, etc. [12]. *Bacillus megaterium* ELI 24 strain isolated from soil in Mexico produced significant amounts of succinic acid, which was identified by spectroscopic methods [13]. Chromatographic analysis showed that *Bacillus megaterium* P1 isolated from soil, depending on the composition of the culture medium, synthesized volatile organic acids (formic, acetic,

propionic, and butyric) and non-volatile organic acids (oxalic, malic, succinic, tartaric, and citric) in various combinations [14].

Whole genome sequencing is currently recognized as the most effective technology for detailed genetic characterization of microbial strains, their properties, and metabolic processes [15)]. An important stage in the analysis of mechanisms of probiotic action, in particular the antimicrobial activity of microorganisms, can be the re-construction of its metabolic map, that is, the collection and visualization of all processes potentially occurring in the cell. Experimental data on antimicrobial activity combined with whole genome sequencing allow researchers to reconstruct models that are as close to virtual organisms as possible. This also allows predicting the relationship of a microorganism with various pathogenic and conditionally pathogenic forms in animal digestive system in order to create effective probiotic products.

Currently, work is underway to study the genomes of *Bacillus* sp. strains which are promising for the creation of biological products. P. Li et al. [15] used whole genome sequencing to assess the probiotic potential of the *Bacillus* sp. DU-106, an active producer of L-lactic acid. Other researchers [16] used this technique to study the genome of *Bacillus clausii* ENTPro as the base for the production of the probiotic Enterogermina (Sanofi Synthelabo Pvt. Ltd., India). whole genome sequencing in B. *megaterium* was performed by M. Eppinger et al. [17] who analyzed the strains QM B1551 and DSM319. J.I. Vílchez et al. [18] sequenced genome of TG1-E1 strain which is promising for producation of fertilizers. L. Liu et al. [19] studied the genome of the WSH-002 strain promising for the creation of recombinant proteins and vitamins.

This work is the first to disclose in a *Bacillus megaterium* strain potentially inherent genetic mechanisms for synthesis of a number of bioactive substances, in particular, the ability to synthesize kanosamine, a bacteriocin of the group of aminoglycosides, which can play an important role in probiotic properties due to a pronounced antimicrobial activity.

Our goal was to measure antimicrobial activity of *Bacillus megaterium* B-4801 strain against pathogenic and opportunistic bacteria, and to search for genes associated with antimicrobial activity based on whole genome sequencing.

Materials and methods. B. megaterium B-4801 a strain with a pronounced probiotic activity (the collection of OOO BIOTROF+) was used in the study. Test cultures for estimation of antagonistic properties were obtained from the All-Russian collection of non-pathogenic microorganisms for agricultural purposes (the All-Russian Research Institute for Agricultural Microbiology, St. Petersburg—Pushkin).

Antimicrobial activity against pathogenic and opportunistic microorganisms (*Staphylococcus aureus*, *Candida tropicalis*, *Clostridium* sp., *Escherichia coli*) was assessed in vitro by the delayed antagonism method (wells method). A Petri dish with agar medium GRM (State Research Center for Applied Micro-biology and Biotechnology, Russia) containing 7 g/l glucose and culture of opportunistic test strains was dried for 24-48 hours, then *B. megaterium* B-4801 culture suspension (10^7 CFU/ml, 100 μl) was poured in a well in the center of the dish. After 48 h of incubation at 37±1 °C, the zones of growth inhibition of the test strains were measured.

DNA was extracted by standard procedures using the Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) according to the attached instructions [2]. The analysis is based on selective detergent-mediated precipitation of DNA using solutions for lysis of cell walls and DNA precipitation, a solution of 1.2 M sodium chloride, and chloroform.

DNA library for whole genome sequencing was constructed Nextera

XT kit (Illumina, Inc., USA). Nucleotide sequences were determined using a MiSeq instrument (Illumina, Inc., USA) with MiSeq Reagent Kit v3 (300-cycle) (Illumina, Inc., USA). Invalid sequences and adapters were removed using the Trimmomatic-0.38 program (https://www.osc.edu/book/ex-port/html/4385) [20]. Paired-end sequences filtered by length not less than 50 to 150 bp were assembled de novo (the SPAdes-3.11.1 genomic assembler, http://cab.spbu.ru/software/spades/) [21]. Functional genome annotation was performed using programs PROKKA 1.12 (https://github.com/kbase-apps/ProkkaAnnotation) [22] and RAST 2.0 (https://rast.nmpdr.org) [23] The KEGG Pathway database (http://www.genome.jp/kegg/) was used to assess the pool of genes associated with antimicrobial activity for constructing a metabolic map [24, 25].

Mathematical and statistical processing was carried out using the software packages Microsoft Office Excel 2003, R-Studio (Version 1.1.453) (https://rstudio.com). The mean value for each sample (M) and the standard deviation (±SD) were estimated.

Results. *B. megaterium* B-4801 strain had a pronounced antagonistic effect against the test cultures of *Staphylococcus aureus* and *Candida tropicalis*, with the size of growth inhibition zones of 25±1.4 and 10±0.7 mm, respectively (Fig. 1). *Clostridium* sp. proved to be the most resistant to *B. megaterium* B-4801 (2±0.15 mm). For *Escherichia coli*, the size of the growth retardation zone was 5±0.3 mm. This suggests that the *B. megaterium* B-4801 culture liquid contains antimicrobial substances that diffuse into agar.

These results are of great practical importance, since *S. aureus* is associated with the occurrence of diseases in cattle, primarily mastitis [26]. Previously, we have proved the relationship between the increased abundance *of Staphylococcus* sp. in the rumen and an increase in the number of somatic cells in the milk of dairy cows [2]. *C. tropicalis* is also pathogenic for cattle, in particular, it can spread through the bloodstream to peripheral organs, moreover, its association with abortion has been found [27, 28]. These facts allow us to conclude that the *B. megaterium* B-4801 strain is a promising biocontrol agent for suppressing pathogenic microbiota, in particular, through its introduction into the digestive system of animals.

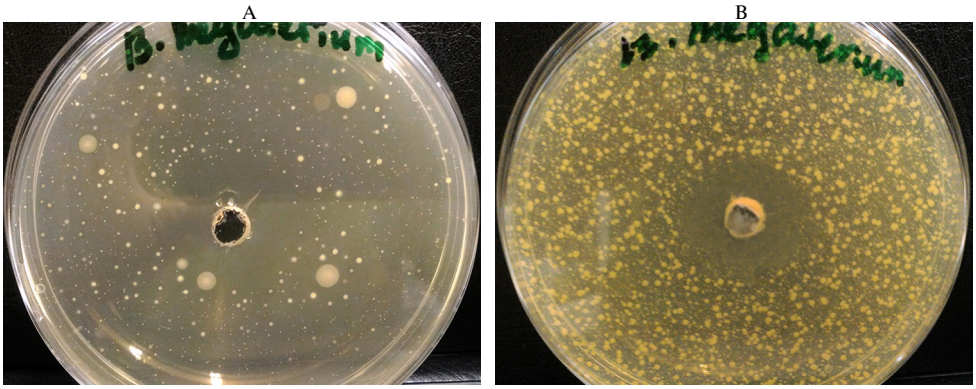

Fig. 1. Antagonistic properties of *Bacillus megaterium* B-4801 towards *Staphylococcus aureus* (A) **and *Candida tropicalis*** (B). Petri dishes with colonies of pathogen test cultures, in the center zones of growth suppression are visualized.

The *B. megaterium* B-4801 genome was annotated using the RAST toolkit under a unique genomic identifier 1404.252. The genome is organized into a single circular chromosome of 6,113,972 bp in size, containing 37.5% GC pairs. The chromosome contains 6324 open reading frames capable of determin-

ing polypeptide synthesis, 129 for tRNA synthesis, and 6 for rRNA. The plasmid of 78379 bp in size contains 23.5% GC pairs, which was 14% less than in the chromosome.

Comparison of the contigs of the studied strain with the NCBI nucleotide sequence database (https://www.ncbi.nlm.nih.gov/genome/microbes/) using the PATRIC database (https://www.patricbrc.org) showed its high similarity with the genome of *B. megaterium* QM B1551 545693.3 (Fig. 2). Both of these strains were similar to the other two *B. megaterium* strains. Interestingly, the above-mentioned four *B. megaterium* strains turned out to be rather closely related to the *Clostridium* sp. Earlier S. Porwal et al. [29] showed that the *B. megaterium* strain (with the GC-pair level from 38 to 39%) is only distantly related to the *B. cereus* and *B. subtilis* species, which contradicts the traditional concepts.

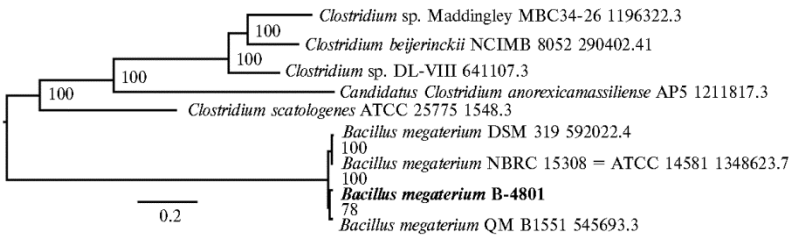

Fig. 2. Phylogenetic relationship between *Bacillus megaterium* B-4801 and the closest representative bacterial genomes from the NCBI database (https://www.ncbi.nlm.nih.gov/genome/microbes/). Genetic distances were estimated using the Mash/MinHash technology [30]. The dendrogram was constructed using PATRIC tools [31].

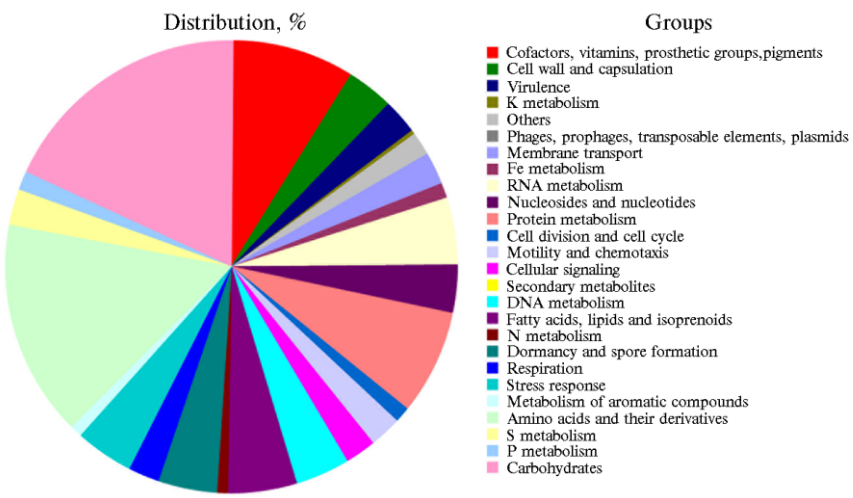

Fig. 3. Metabolic systems of *Bacillus megaterium* B-4801 based on functional annotation according to the RAST database (https://rast.nmpdr.org).

Analysis of metabolic subsystems, that is, groups of proteins that jointly realize a certain biological process, revealed that more than 45% of the *B. megaterium* B-4801 genes are involved in the transport and metabolism of amino acids, transcription, translation, transport and metabolism of carbohydrates, proteins (Fig. 3). This strain possesses products for the functioning of a complete set of metabolic pathways, including glycolysis, the tricarboxylic acid cycle, and the pentose phosphate pathway.

The fact that in *B. megaterium* B-4801 a significant number of genes (17.5%) was associated with the metabolism of carbohydrates is quite logical, since it has been proven that *Bacillus* sp. produce many antibiotic-like com-

pounds of a nonribosomal nature and organic acids, the synthesis of which requires active carbohydrate metabolism [4, 13]. Among synthesized proteins, an important role is played by enzymes that determine the entry of sugars into the cell and their oxidation, the permeases and hydrolases. These processes resulted, in particular, in production of pyruvate, 2-oxo-glutarate, oxaloacetate and acetyl-CoA, which serve as precursors for the synthesis of fatty acids, amino acids, polyketides and other vital metabolites.

It is important that the genome contains a noticeable amount (more than 20%) of genes that implement interactions with the environment, in particular those associated with cell membrane formation and encapsulation, motility and chemotaxis, cellular signaling, and stress response, which indicates a high potential for probiotic activity. Probably, this set of genes can contribute to the survival of the strain in the aggressive environment of the gastrointestinal tract, and facilitates adhesion to the host's epithelial cells. A significant part of the genome was annotated as associated with the synthesis of vitamins, in particular B_1, B_2, B_9 and biotin, which play an important role in many metabolic processes in macroorganisms [32]. Earlier L. Liu et al. [19], due to whole genome sequencing, revealed the potential ability of the *B. megaterium* strain to synthesize vitamins.

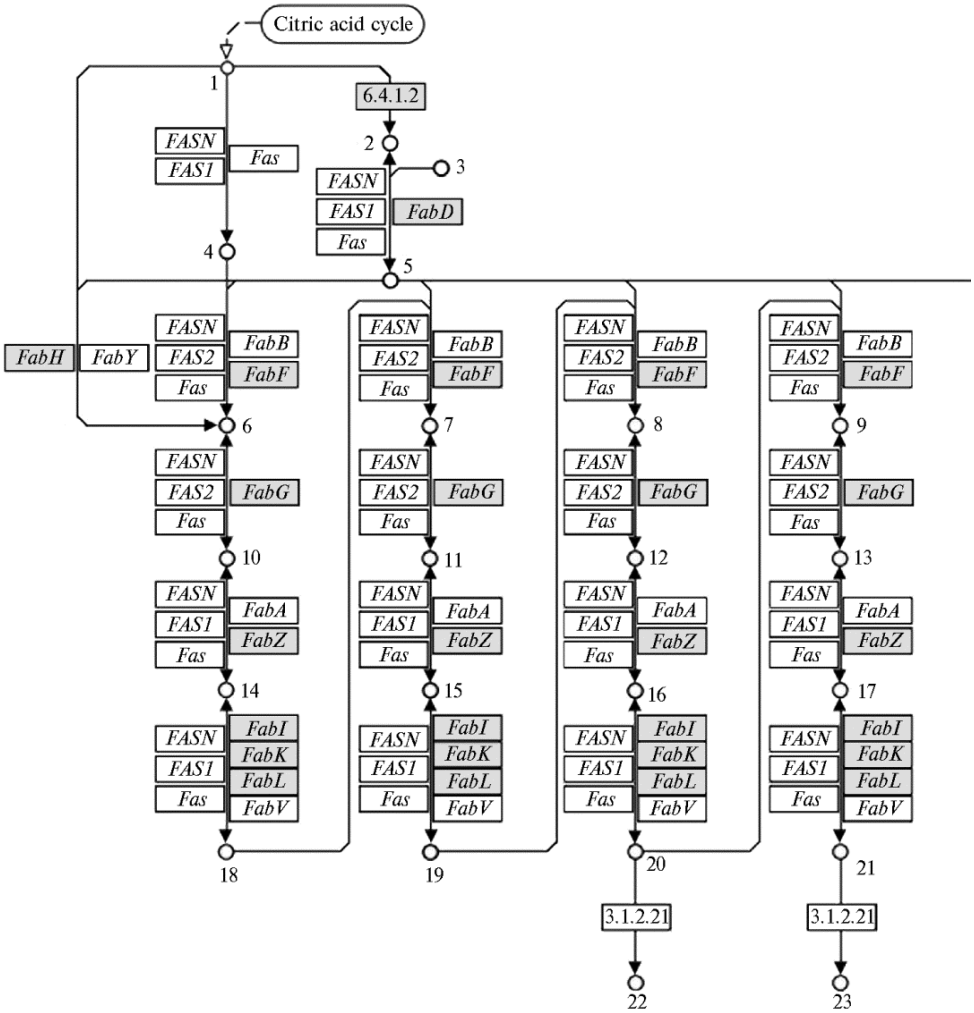

Fig. 4. Fatty acid metabolic pathways and synthesis of precursors in *Bacillus megaterium* B-4801 (built using the KEGG Pathway database, http://www.genome.jp/kegg/): 1 — acetyl-CoA, 2 —

malonyl-CoA, 3 — acyl carrier protein (ACP), 4 — acetyl-[ACP], 5 — malonyl-[ACP], 6 — acetoacetyl-[ACP], 7 — 3-oxohexanoyl-[ACP], 8 — 3-oxooctanoyl-[ACP], 9 — 3-oxodecanoyl-[ACP], 10 — (R)-3-hydroxibutanoyl-[ACP], 11 — (R)-3-hydroxihexanoyl-[ACP], 12 — (R)-3-hydroxioctanoyl-[ACP], 13 — (R)-3-hydroxidecanoyl-[ACP], 14 — but-2-enoyl-[ACP], 15 — trans-hex-2-enoyl-[ACP], 16 — trans-oct-2-enoyl-[ACP], 17 — trans-dec-2-enoyl -[ACP], 18 — butyryl-[ACP], 19 — hexanoyl-[ACP], 20 — octanoyl-[ACP], 21 — decanoyl-[ACP], 22 — caprylic acid, 23 — capric acid. The numbers in the boxes are the enzyme codes in the IUPAC nomenclature; genes present in the genome are marked in gray; arrows pointing to circles indicate the direction of steps in the reaction.

We identified in *B. megaterium* B-4801 the key genetic loci that determine the synthesis of a number of antimicrobial metabolites, including fatty acids, kanosamine, which belongs to the aminoglycoside group, and polyketide ansamycin bacteriocins from the group of macrolides. The identification of the key products involved in the pathway for the synthesis of antimicrobial metabolites in *B. megaterium* B-4801 strain was performed using the Kegg Pathway database (Fig. 4).

The *B. megaterium* B-4801 genome contained genes (*FabD*, *FabF*, *FabG, FabZ*, *FabI*, etc.) associated with the production of proteins that are involved in the synthesis of aliphatic unsaturated carboxylic acids C3-C18, in particular, butyric, nylon, caprylic, capric, lauric, myristic, palmitic, stearic, oleic acids. Metabolic maps of the synthesis of some carboxylic acids in the *B. megaterium* B-4801 strain are shown in Figure 4 as an illustration.

According to the KEGG Pathway database (see Fig. 4), the precursors for the biosynthesis of fatty acids in *B. megaterium* B-4801 were formed from the acetyl-CoA pool. At the first stage, the acetyl group was transferred from acetyl-CoA to an acyl carrier protein (ACP) molecule. Acetyl-ACP acts as an initiator to which the C2 fragment is attached. The malonyl-ACP molecule also synthesized from acetyl-CoA is a donor of the C2 fragment. This reaction is catalyzed by FabD enzyme (transacylase, EC 2.3.1.39, the malonyl CoA-acyl carrier protein), which was previously shown for *Streptomyces coelicolor* as, but not described for *Bacillus* sp. [33].

Further transformations which in *B. megaterium* B-4801 differed for specific fatty acids we will describe on the example of butyric acid. The C2 fragment binding to acetyl-ACP resulted in acetoacetyl-ACP. According to the KEGG Pathway database, FabF protein catalyzed the condensation of acetyl-CoA with malonyl-ACP to form acetoacetyl-ACP. A similar pathway was previously described for *Escherichia coli* [34]. According to these authors, the synthetic pathway leading to the formation of fatty acids in *E. coli* requires two specialized proteins, FabA and FabB. However, the genes associated with the synthesis of FabA and FabB are found only in gram-negative *Proteobacteria*. It was shown [35] that the gram-positive *Enterococcus faecalis* has a protein annotated as FabZ1, which functionally replaces the *E. coli* FabA protein. Probably, FabF in *B. megaterium* B-4801 is similar in function to FabA and FabB of *E. coli*.

The next stage in the *B. megaterium* B-4801 involved reduction of oxidized carbon atoms in aceto-acetyl-ACP via a series of enzymatic reactions with the participation of FabG, FabZ, FabI (FabK, FabL) proteins. This, through the stages of precursors, resulted in formation of butyryl-ACP, the butyric acid radical. The final stage in the biosynthesis of fatty acids was catalyzed by the enzyme FabI (synonyms FabK, FabL) which initiates the hydration of the 2,3-double bond in the derivatives of enoyl-ACP previously shown for *E. coli* [36].

Thus, by genome sequencing we identified in *B. megaterium* B-4801 almost all the main enzymes responsible for the formation of fatty acids (C3-C18). Earlier, in the *Bacillus* sp. DU-106 [15] enzymes were discovered which

were involved in the synthesis of organic acids, in particular, lactate production. These enzymes were L-lactate dehydrogenase, D-lactate dehydrogenase, lactaldehyde dehydrogenase and malate/lactate dehydrogenase. Note that such enzymes were not detected in *B. cereus* [37]. Nevertheless, in a later study [15], genes associated with lactate synthesis were identified in the genome of *Bacillus cereus* DU-106.

The data we obtained at the genomic level additionally confirm that the *B. megaterium* B-4801 strain has potential probiotic properties, since the antimicrobial activity of these acids has been proven. The pronounced antimicrobial activity of butyric acid against *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Campylobacter jejuni*, *Clostridium perfringens*, *Streptococcus pneumoniae*, and *Str. suis* [38]. A number of fatty acids, in particular nylon, caprylic, capric, lauric and myristic, showed antagonism against *Streptococcus mutans*, *Str. gordonii*, *Str. sanguis*, *Candida albicans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* [39]. V. Prabhadevi et al. [40] noted the antimicrobial activity of stearic acid, and M.M. Rahman et al. [41] of palmitic acid.

It worth noting that that we revealed in *B. megaterium* B-4801 a cluster of genes (*Asm22-24*, *Asm43-45*, *Asm47*) associated with biosynthesis of bacteriocin kanosamine of the aminoglycoside group, and polyketide ansamycin antibiotics of the macrolide group. The *Asm43-45* genes are involved in biosynthesis of antimicrobial substance kanosamine (3-amino-3-deoxy-D-glucose) through the intermediate UDP-α-D-kanosamine. S. Umezawa et al. [42] demonstrated a similar pathway for kanosamine biosynthesis in *B. pumilus* (formerly known as *B. aminoglucosidicus*), although the genes involved have not been identified. The ability to synthesize kanosamine was also found in *Bacillus cereus* [43]. Later, in *B. cereus*, a set of genes involved in the kanosamin synthesis were described, although the pathway itself was not determined [44). N.D. Vetter et al. [45] discovered genes, in particular *NtdA*, *NtdB* and *NtdC* in *Bacillus subtilis* 168, associated with the biosynthesis of this antimicrobial substance. We are the first to reveal genetic potential of *Bacillus megaterium* strains to produce kanosamine.

Kanosamine and UDP-α-D-canosamine also are intermediates in the biosynthesis of 3-amino-5-hydroxybenzoic acid [46]. 3-Amino-5-hydroxybenzoic acid in microbial cells is a starting block for assembling the main carbon chain of anamycin precursors by modular polyketide synthase I. Ansamycins are a class of bacterial macrocyclic polyketides produced mainly by the phylum *Actinobacteria* and the genus *Bacillus* [47, 48]. It has been proven [49] that ansamycins exhibit a wide spectrum of antimicrobial activity. We discovered in *B. megaterium* B-4801 the transketolase enzyme, as well as genes, in particular *Asm47*, *Asm23* and *Asm24*, that can provide synthesis of 3-amino-5-hydroxybenzoic acid. For example, it is well known [50] that *Asm23* encodes dehydroquinate dehydratase II (DHQase II) which catalyzes dehydration of 5-deoxy-5-amino-3-dehydroquinate, a precursor of 3-amino-5-hydroxybenzoic acid. Product of *Asm24* gene presumably catalyzes the dehydration of 5-deoxy-5-amino-3-dehydroximate to 3-amino-5-hydroxybenzoic acid. Previously, genes *Asm22-24*, *Asm43-45*, and *Asm47* were found in *Actinosynnema pretiosum* ssp. *auranticum* ATCC 31565, which were associated with the synthesis of 3-amino-5-hydroxybenzoic acid [50].

The probability of bacteriocin production by *B. megaterium* B-4801 is expectable, since bacteria of the genus *Bacillus* are known producers of such substances [8]. For example, gene for gallidermin which effectively prevents the formation of biofilms in pathogenic *S. aureus* and *S. epidermidis* was identified in *B. clausii* genome [51]. Lacticin 3147 A2 and leukocyclin Q found in *B. amyloliquefaciens* are broad-spectrum bacteriocins. Lacticin has been effectively used

in treatment of bacterial mastitis, staphylococcal and enterococcal infections, including pathologies caused by vancomycin-resistant enterococci [9]. Lichenicidin VK21A2 found in *B. paralicheniformis* shows antimicrobial activity against several pathogenic strains, e.g. *Listeria monocytogenes*, methicillin-resistant *S. aureus*, and vancomycin-resistant enterococcus [7]. A biolactive compound similar to bacitracin was found in *B. megaterium* KC246043.1 [52]. I.A. Malanicheva et al. [53] showed the ability of *B. megaterium* to produce antibacterial antibiotics that differ in their spectrum of action. Three of them are peptide antibiotics, and three more are compounds not described previously. All substances were active against the methicillin-resistant strain *Staphylococcus aureus* INA 00761, as well as against *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

To summarize, the whole genome sequences of different *B. megaterium* strains were determined in several studies [19, 18], but genetic mechanisms ensuring production of fatty acids and bacteriocins in these microorganisms were not detected. We are the first to specify here these mechanisms.

Thus, we have revealed antagonistic activity of *Bacillus megaterium* B-4801 against pathogenic and opportunistic microorganisms. *B. megaterium* B-4801 genome is organized into a single circular 6,113,972 bp chromosome with 37.5% GC. More than 45% of the *B. megaterium* B-4801 genes are involved in the transport and metabolism of amino acids, transcription, translation, transport and metabolism of carbohydrates and proteins. Whole genome sequencing identified the key loci that determine synthesis of antimicrobial metabolites. The *B. megaterium* B-4801 genome contains genes (*FabD*, *FabF*, *FabG*, *FabZ*, *FabI*, etc.) associated with production of proteins that are involved in the synthesis of aliphatic unsaturated carboxylic acids C3-C18, in particular butyric, nylon, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic. All these substances are known to express pronounced antimicrobial properties. In *B. megaterium* B-4801, we found a cluster of genes (*Asm22-24*, *Asm43-45*, *Asm47*) associated with the biosynthesis of bacteriocin kanosamine of the aminoglycoside group and polyketide ansamycin antibiotics of the macrolide group. *B. megaterium* B-4801 genetic passport that we created based on whole genome sequencing is of fundamental interest and also contains valuable commercial information. The probiotic potential of *B. megaterium* B-4801 that we revealed in this study indicates the role of *B. megaterium* B-4801 strain as a candidate producer for biologicals, including those for animal husbandry. This research must be followed by experimental analytical study of fatty acids and bacteriocins produced by *B. megaterium* B-4801 for a deeper understanding of the mechanism of their probiotic action. Also, since the presence of operons that encode products potentially involved in various metabolic pathways does not unconditionally mean their functioning. Their functionality should be confirmed by proteomic and metabolomic methods and by targeted analysis of specific compounds.

REFERENCES

1. Hong H.A., Duc L.H., Cutting S.M. The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews*, 2010, 29(4): 813-835 (doi: 10.1016/j.femsre.2004.12.001).
2. Il'ina L.A. *Izuchenie mikroflory rubtsa krupnogo rogatogo skota na osnove molekulyarno-biologicheskogo metoda T-RFLP s tsel'yu razrabotki sposobov ee optimizatsii. Kandidatskaya dissertatsiya* [Study of the microflora of the cattle rumen based on the molecular biological method T-RFLP in order to develop methods for its optimization. PhD Thesis]. St. Petersburg, 2012 (in Russ.).
3. Caulier S., Nannan C., Gillis A., Licciardi F., Bragard C., Mahillon J. Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis*. *Frontiers in Microbiology*, 2019, 10: 302 (doi: 10.3389/fmicb.2019.00302).

4. Stein T. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Molecular Microbiology*, 2005, 56(4): 845-857 (doi: 10.1111/j.1365-2958.2005.04587.x).
5. Cotter P.D., Hill C., Ross R.P. What's in a name? Class distinction for bacteriocins. *Nature Reviews Microbiology*, 2006, 4(2): 160 (doi: 10.1038/nrmicro1273-c2).
6. Gabrielsen C., Brede D.A., Nes I.F., Diep D.B. Circular bacteriocins: biosynthesis and mode of action. *Applied and Environmental Microbiology*, 2014, 80(22): 6854-6862 (doi: 10.1128/AEM.02284-14).
7. Begley M., Cotter P.D., Hill C., Ross R.P. Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for LanM proteins. *Applied and Environmental Microbiology*, 2009, 75(17): 5451-5460 (doi: 10.1128/AEM.00730-09).
8. Saising J., Dube L., Ziebandt A.-K., Voravuthikunchai S.P., Nega M., Götz F. Activity of gallidermin on Staphylococcus aureus and Staphylococcus epidermidis biofilms. *Antimicrobial Agents and Chemotherapy*, 2012, 56(11): 5804-5810 (doi: 10.1128/AAC.01296-12).
9. Field D., Cotter P.D., Hill C., Ross R.P. Bioengineering lantibiotics for therapeutic success. *Frontiers in Microbiology*, 2015, 6: 1363 (doi: 10.3389/fmicb.2015.01363).
10. Abriouel H., Franz C.M., Ben Omar N., Gálvez A. Diversity and applications of *Bacillus bacteriocins*. *FEMS Microbiology Reviews*, 2011, 35(1): 201-232 (doi: 10.1111/j.1574-6976.2010.00244.x).
11. Poudel P., Tashiro Y., Miyamoto H., Miyamoto H., Okugawa Y., Sakai K. Direct starch fermentation to L-lactic acid by a newly isolated thermophilic strain, *Bacillus* sp. *Journal of Industrial Microbiology and Biotechnology*, 2015, 42: 143-149 (doi: 10.1007/s10295-014-1534-0).
12. Alakomi H.L., Skyttä E., Saarela M., Mattila-Sandholm T., Latva-Kala K., Helander I.M. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, 2000, 66(5): 2001-2005 (doi: 10.1128/aem.66.5.2001-2005.2000).
13. Ortiz A., Sansinenea E. Succinic acid production as secondary metabolite from *Bacillus megaterium* ELI24. *The Natural Products Journal*, 2020, 10(2): 154-157 (doi: 10.2174/2210315509666190410153811).
14. Chuanqing Z., Jiang A.U., Huang A., Qi W. Cao X. Guangxiang studies on the acid-production characteristics of *Bacillus megaterium* strain P17. *AIP Conference Proceedings*, 2017, 1839(1): 020054 (doi: 10.1063/1.4982419).
15. Li P., Tian W., Jiang Z., Liang Z., Wu X., Du B. Genomic characterization and probiotic potency of *Bacillus sp.* DU-106, a highly effective producer of L-lactic acid isolated from fermented yogurt. *Frontiers in Microbiology*, 2018, 9: 2216 (doi: 10.3389/fmicb.2018.02216).
16. Khatri I., Sharma G., Subramanian S. Composite genome sequence of *Bacillus clausii*, a probiotic commercially available as Enterogermina®, and insights into its probiotic properties. *BMC Microbiology*, 2019, 19(1): 307 (doi: 10.1186/s12866-019-1680-7).
17. Eppinger M., Boyke B., Mitrick A.J., Janaka N.E., Kirthi K.K., Koenig S.S.K., Creasy H.H., Rosovitz M.J., Riley D.R., Daugherty S., Martin M., Elbourne L.D.H., Paulsen I., Biedendieck R., Braun C., Grayburn S., Dhingra S., Lukyanchuk V., Ball B., Ul-Qamar R., Seibel J., Bremer E., Jahn D., Ravel J., Vary P.S. Genome sequences of the biotechnologically important *Bacillus megaterium* strains QM B1551 and DSM319. *Journal of Bacteriology*, 2011, 193(16): 4199-4213 (doi: 10.1128/JB.00449-11).
18. Vílchez J.I., Tang Q., Kaushal R., Wang W., Suhui L., Danxia H., Zhaoqing C., Heng Z., Renyi L., Huiming Z. Complete genome sequence of *Bacillus megaterium* strain TG1-E1, a plant drought tolerance-enhancing bacterium. *Microbiology Resource Announcements*, 2018, 7(12): e00842-18 (doi: 10.1128/MRA.00842-18).
19. Liu L., Li Y., Zhang J., Zou W., Zhou Z., Liu J., Li X., Wang L., Chen J. Complete genome sequence of the industrial strain *Bacillus megaterium* WSH-002. *Journal of Bacteriology*, 2011, 193(22): 6389-6390 (doi: 10.1128/JB.06066-11).
20. Bolger A.M., Lohse M., Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 2014, 30(15): 2114-2120 (doi: 10.1093/bioinformatics/btu170).
21. Nurk S., Bankevich A., Antipov D., Gurevich A., Korobeynikov A., Lapidus A., Prjibelsky A., Pyshkin A., Sirotkin A., Sirotkin Y., Stepanauskas R., McLean J., Lasken R., Clingenpeel S.R., Woyke T., Tesler G., Alekseyev M.A., Pevzner P.A. Assembling genomes and mini-metagenomes from highly chimeric reads. In: *Research in Computational Molecular Biology. RECOMB 2013. Lecture Notes in Computer Science, vol. 7821.* Springer, Berlin, Heidelberg, 2013: 158-170 (doi: 10.1007/978-3-642-37195-0_13).
22. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, 2014, 30(14): 2068-2069 (doi: 10.1093/bioinformatics/btu153).
23. Aziz R.K. The RAST server: rapid annotations using subsystems technology. *BMC Genomics*, 2008, 9(75) (doi: 10.1186/1471-2164-9-75).
24. Kanehisa M., Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 2000, 28(1): 27-30 (doi: 10.1093/nar/28.1.27).
25. Kanehisa M., Goto S., Sato Y., Furumichi, M. Tanabe M. KEGG for integration and interpretation of large-scale molecular datasets. *Nucleic Acids Research*, 2012, 40(D1): D109-D114

(doi: 10.1093/nar/gkr988).
26. Boss R., Cosandey A., Luini M., Artursson K., Bardiau M., Breitenwieser F., Hehenberger E., Lam Th., Mansfeld M., Michel A., Mösslacher G., Naskova J., Nelson S., Podpečan O., Raemy A., Ryan E., Salat O., Zangerl P., Steiner A., Graber H.U. Bovine *Staphylococcus aureus*: Subtyping, evolution, and zoonotic transfer. *Journal of Dairy Science*, 2016, 99(1): 515-528 (doi: 10.3168/jds.2015-9589).
27. Loken K.I., Thompson E.S., Hoyt H.H., Ball R.A. Infection of the bovine udder with *Candida tropicalis*. *Journal of the American Veterinary Medical Association*, 1959, 134(9): 401-403.
28. Wohlgemuth K., Knudtson W. Bovine abortion associated with *Candida tropicalis*. *Journal of the American Veterinary Medical Association*, 1973, 162(6): 460-461.
29. Porwal S., Lal S., Cheema S., Kalia V.C. Phylogeny in aid of the present and novel microbial lineages: diversity in *Bacillus*. *PLoS ONE*, 2009, 4(2): e4438 (doi: 10.1371/journal.pone.0004438).
30. Ondov B.D., Treangen T.J., Melsted P., Mallonee A.B., Bergman N.H., Koren S., Phillippy A.M. Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biology*, 2016, 17: 132 (doi: 10.1186/s13059-016-0997-x).
31. Wattam A.R., Davis J.J., Assaf R., Boisvert S., Brettin T., Bun C., Conrad N., Dietrich E.M., Disz T., Gabbard J.L., Gerdes S., Henry C.S., Kenyon R.W., Machi D., Mao C., Nordberg E.K., Olsen G.J., Murphy-Olson D.E., Olson R., Overbeek R., Parrello B., Pusch G.D., Shukla M., Vonstein V., Warren A., Xia F., Yoo H., Stevens R.L. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Research*, 2017, 45(D1): D535-D542 (doi: 10.1093/nar/gkw1017).
32. Frye T.M., Williams S.N., Graham T.W. Vitamin deficiencies in cattle. *Veterinary Clinics of North America: Food Animal Practice*, 1991, 7(1): 217-275 (doi: 10.1016/s0749-0720(15)30817-3).
33. Szafranska A.E., Hitchman T.S., Cox R.J., Crosby J., Simpson T.J. Kinetic and mechanistic analysis of the malonyl CoA:ACP transacylase from *Streptomyces coelicolor* indicates a single catalytically competent serine nucleophile at the active site. *Biochemistry*, 2002, 41(5): 1421-1427 (doi: 10.1021/bi012001p).
34. Cronan J.E., Thomas J. Bacterial fatty acid synthesis and its relationships with polyketide synthetic pathways. *Methods in Enzymology*, 2009, 459: 395-433 (doi: 10.1016/S0076-6879(09)04617-5).
35. Wang H., Cronan J.E. Functional replacement of the FabA and FabB proteins of *Escherichia coli* fatty acid synthesis by *Enterococcus faecalis* FabZ and FabF homologues. *Journal of Biological Chemistry*, 2004, 279(33): 34489-34495 (doi: 10.1074/jbc.M403874200).
36. Yu X., Liu T., Zhu F., Khosla C. In vitro reconstitution and steady-state analysis of the fatty acid synthase from *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 2011, 108(46): 18643-18648 (doi: 10.1073/pnas.1110852108).
37. Johnson S.L., Daligault H.E., Davenport K.W., Jaissle J., Frey K.G., Ladner J. T., Broomall S.M., Bishop-Lilly K.A., Bruce D.C., Gibbons H.S., Coyne S.R., Lo C.-C., Meincke L., Munk A.C., Koroleva G.I., Rosenzweig C.N., Palacios G.F., Redden C.L., Minogue T.D., Chain. P.S. Complete genome sequences for 35 biothreat assay-relevant *Bacillus* species. *Genome Announcements*, 2015, 3(2): e00151-15 (doi: 10.1128/genomeA.00151-15).
38. Kovanda L., Zhang W., Wei X., Luo J., Wu X., Atwill E.R., Vaessen S., Li X., Liu Y. In vitro antimicrobial activities of organic acids and their derivatives on several species of gram-negative and gram-positive bacteria. *Molecules*, 2019, 24(20): 3770 (doi: 10.3390/molecules24203770).
39. Huang C.B., Alimova Y., Myers T.M., Ebersole J.L. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of Oral Biology*, 2011, 56(7): 650-654 (doi: 10.1016/j.archoralbio.2011.01.011).
40. Prabhadevi V., Sahaya S.S., Johnson M., Venkatramani B., Janakiraman N. Phytochemical studies on *Allamanda cathartica* L. using GC-MS. *Asian Pacific Journal of Tropical Biomedicine*, 2012, 2(2): S550-S554 (doi: 10.1016/S2221-1691(12)60272-X).
41. Rahman M.M., Ahmad S.H., Mohamed M.T., Ab Rahman M.Z. Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. *Scientific World Journal*, 2014, 2014: 635240 (doi: 10.1155/2014/635240).
42. Umezawa S., Shibahara S., Omoto S., Takeuchi T., Umezawa H. Studies on the biosynthesis of 3-amino-3-deoxy-D-glucose. *The Journal of Antibiotics*, 1968, 21(8): 485-491 (doi: 10.7164/antibiotics.21.485).
43. Milner J.L., Silo-Suh L., Lee J.C., He H., Clardy J., Handelsman J. Production of kanosamine by *Bacillus cereus* UW85. *Applied and Environmental Microbiology*, 1996, 62(8): 3061-3065.
44. Kevany B.M., Rasko D.A., Thomas M.G. Characterization of the complete zwittermicin A biosynthesis gene cluster from *Bacillus cereus*. *Applied and Environmental Microbiology*, 2009, 75(4): 1144-1155 (doi: 10.1128/AEM.02518-08).
45. Vetter N.D., Langill D.M., Anjum S., Boisvert-Martel J., Jagdhane R.C., Omene E., Zheng H., van Straaten K.E., Asiamah I., Krol E.S., Sanders D.A., Palmer D.R. A previously unrecognized kanosamine biosynthesis pathway in *Bacillus subtilis*. *Journal of the American Chemical Society*, 2013, 135(16): 5970-5973 (doi: 10.1021/ja4010255).

46. Arakawa K., Müller R., Mahmud T., Yu T.W., Floss H.G. Characterization of the early stage aminoshikimate pathway in the formation of 3-amino-5-hydroxybenzoic acid: the RifN protein specifically converts kanosamine into kanosamine 6-phosphate. *Journal of the American Chemical Society*, 2002, 124(36): 10644-10645 (doi: 10.1021/ja0206339).
47. Kim W.-G., Song N.-K., Yoo I.-D. Trienomycin G, a new inhibitor of nitric oxide production in microglia cells, from *Streptomyces* sp. 91614. *The Journal of Antibiotics*, 2002, 55(2): 204-207 (doi: 10.7164/antibiotics.55.204).
48. Kang Q., Shen Y., Bai L. Biosynthesis of 3,5-AHBA-derived natural products. *Natural Product Reports*, 2012, 29: 243-263 (doi: 10.1039/C2NP00019A).
49. Wrona I.E., Agouridas V., Panek J.S. Design and synthesis of ansamycin antibiotics. *Comptes Rendus Chimie*, 2008, 11: 1483-1522 (doi: 10.1016/j.crci.2008.07.003).
50. Yu T.W., Bai L., Clade D., Hoffmann D., Toelzer S., Trinh K.Q., Xu J., Moss S.J., Leistner E., Floss H.G. The biosynthetic gene cluster of the maytansinoid antitumor agent ansamitocin from *Actinosynnema pretiosum*. *Proceedings of the National Academy of Sciences*, 2002, 99(12): 7968-7973 (doi: 10.1073/pnas.092697199).
51. Khatri I., Sharma G., Subramanian S. Composite genome sequence of *Bacillus clausii*, a probiotic commercially available as Enterogermina®, and insights into its probiotic properties. *BMC Microbiology*, 2019, 19(1): 307 (doi: 10.1186/s12866-019-1680-7).
52. Al-Thubiani A.S.A., Maher Y.A., Fathi A., Abourehab M.A.S., Alarjah M., Khan M.S.A., Al-Ghamdi S.B. Identification and characterization of a novel antimicrobial peptide compound produced by *Bacillus megaterium* strain isolated from oral microflora. *Saudi Pharmaceutical Journa*l, 2018, 26(8): 1089-1097 (doi: 10.1016/j.jsps.2018.05.019).
53. Malanicheva I.A., Kozlov D.G., Sumarukova I.G., Efremenkova O.V., Zenkova V.A., Katrukha G.S., Reznikova M.I., Tarasova O.D., Sineokiĭ S.P., Él'-Registan G.I. Antimicrobial activity of *Bacillus megaterium* strains. *Microbiology*, 2012, 81: 178-185 (doi: 10.1134/S0026261712020063).