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BIOINFORMATIC ANALYSIS OF THE *Bacillus velezensis* KR-2 GENOME TO REVEAL BIOTECHNOLOGICALLY IMPORTANT PROPERTIES

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Abstract

Members of the genus *Bacillus* are actively used to create biological preparations for agriculture due to their ability to produce a wide range of biologically active molecules with antimicrobial activity, stimulating plant growth and restoring the balance of microorganisms in the digestive system of animals. This paper presents for the first time research data on the identification of a unique pathway for intracellular synthesis of the osmoprotectant glycine betaine encoded by the *BetA*, *BetB*, *BetT*, and *BetC* genes, which has not previously been found in the genus *Bacillus*. The peculiarity of the *B. velezensis* KR-2 genome associated with the synthesis of the siderophore myxochelin A, which we have identified, is probably also unique among other strains of *B. velezensis*, since it has not been previously described. The aim of the study was whole genome sequencing (WGS) and bioinformatic annotation of the *Bacillus velezensis* KR-2 genome to identify genetic determinants capable of encoding biosynthesis of various bioactive substances for agriculture. The *B. velezensis* KR-2 strain isolated from the rumen of a dairy cow (the collection of OOO BIOTROF+) was examined. Its antimicrobial activity was investigated using the differed antagonism assay. DNA was isolated according to standard procedures with the Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., USA). A DNA library for WGS was prepared using the Nextera XT kit (Illumina, Inc., USA). Nucleotide sequences were determined using a MiSeq instrument (Illumina, Inc., USA). Paired-end reads filtered by length not less than 50 to 150 bp were assembled de novo using the SPAdes-3.11.1 genomic assembler with appropriate keys. Comparative analysis of the *B. velezensis* KR-2 genome with other microorganisms was performed using the NCBI databases (https://www.ncbi.nlm.nih.gov/). For phylogenetic analysis, the 16S rRNA gene sequence was transferred to the Nucleotide BLAST web service (https://blast.ncbi.nlm.nih.gov). PROKKA 1.12 (https://bioweb.pasteur.fr/packages/pack@prokka@1.12) was used to convert contig sequences into an amino acid sequences. Functional annotation of the genome was performed using the RAST 2.0 web service (https://rast.nmpdr.org). The KEGG Pathway database (http://www.genome.jp/kegg/) was used to evaluate the pool of genes associated with biotechnologically valuable properties and build metabolic maps. WGS resulted in 16 contigs with a total length of 3936398 bp, containing 46.6 % of GC; the N50 and N75 conting size was 2109194 and N75 844068 bp, respectively. The chromosome contained 3854 coding sequences (CDS) associated with the synthesis of polypeptides. Among the 464 identified metabolic subsystems, the subsystems of amino acids and their derivatives and carbohydrates

were the most numerous, 431 and 416, respectively. The *B. velezensis* KR-2 strain has a whole range of potential properties, including the production of antimicrobial peptides, fatty acids, vitamins, siderophores, auxins, the ability to adhere, to resist toxic compounds and stress factors, to stimulate plant growth and phosphate metabolism, the motility and chemotaxis. In particular, the *B. velezensis* KR-2 genome contains genes (*BacA*, *BacB*, *BacG*, *BacF*, *BacD*) involved in the production of the dipeptide bacilysine, a non-ribosomal bacteriocin. This is consistent with the phenomenon of antagonism against *Staphylococcus aureus*, *Escherichia coli*, *Fusarium oxysporum*, and *Clostridium butyricum*. In the *B. velezensis* KR-2 genome, we have found a unique pathway for intracellular synthesis of the osmoprotectant glycine betaine (the *BetA*, *BetB*, *BetT*, *BetC* genes) not previously detected in the genus *Bacillus*. *B. velezensis* KR-2 is a putative producer of auxins, such as indole-3 ethanol (*IAR*, *TO*), indole-3-acetaldehyde (*IAD*, *AAD*, *AO*) and indole-3-acetonitrile (*N3*). Several gene clusters associated with siderophore synthesis (*DhbA*, *DhbB*, *DhbC*, *FeuA*, *FeuB*, *FeuC*, *FeuD*) were also identified in the genome of *B. velezensis* KP-2. Our findings indicate that *B. velezensis* KR-2 is a bacterial resource for agriculture. The unique biosynthesis pathway for glycine betaine (*BetA*, *BetB*, *BetT*, and *BetC*) that we have discovered are important for the *B. velezensis* KP-2 adaptation to high-osmolar stress under fluctuations in water content, for example, in dried silage and upper layers of soil.

Keywords: whole genome sequencing, *Bacillus velezensis*, bioactive substances, antimicrobial activity, bacilysine, glycine betaine, probiotics, PGPR, starter cultures

Bacteria of the genus *Bacillus* are widely used in agriculture as the basis of probiotics, plant growth stimulants, biopesticides and insecticides, starter crops for ensiling, because, having wide metabolic capabilities, they serve as an important source for the synthesis of biologically active molecules with useful properties [1, 2]. This demand is primarily associated with the presence in the genome of microorganisms of this taxonomic group of a large number of chromosomal loci that determine the synthesis of antimicrobial compounds [3]. This is one of the most important functional properties taken into account in the selection of potential biologics producers [4-6]. P. Piewngam et al. [4)] demonstrated that one of the fengycin-producing strains of the *Bacillus* genus was active against *Staphylococcus aureus* in mice. E. Lara et al. [2)] observed a decrease in the number of mold micromycetes and yeasts when introduced into the silage ecosystem of a starter culture based on the *B. subtilis* strain.

In addition, the possibility of *Bacillus* spp. to carry out metabolism along the pentose phosphate pathway makes them effective producers of vitamins, among which the most significant are cobalamin, riboflavin, folic acid and biotin [7-9]. The spectrum of valuable metabolites also includes substances with anti-inflammatory activity [10]. T.-Y. Lee et al. [10] showed that a strain of *B. subtilis* bacteria capable of synthesizing poly-γ-glutamic acid was effective in treating dermatitis in mice by suppressing the Th2-biased immune response and synthesis of IL-17A. The ability of bacillus strains to synthesize surface-associated proteins, including S-layer proteins, aminopeptidases, flagellin, and metalloproteases, provides the ability to specifically bind to mucin and fibronectin, which may play an important role in adhesion in the gastrointestinal tract and provide a probiotic effect [11]. The high capacity of the secretory systems of bacteria of the genus *Bacillus* predetermines the potential for the production of many hydrolytic extracellular substances. Among the enzymes of interest for animal husbandry are amylases (α- and β-amylases), β-glucanases [1], cellulases and xylanases [12], which are important for enhancing the degradation of complex polysaccharides during the introduction of strains into the digestive system. Another example of *Bacillus* spp. enzymes of biotechnological importance is the insecticidal chitinase metabolites produced by *B. thuringiensis* [1, 13], which act in conjunction with δ-endotoxins (Cry or Cyt) [14].

A number of bacteria of the genus *Bacillus* belong to the so-called PGPR group (plant growth promoting rhizobacteria) [15]. These microorganisms, by synthesizing phytohormones such as auxins (indole-3-acetic acid), contribute to the intensification of plant growth and beneficially affect their nutrition by solubilizing phosphates and chelating iron with siderophores [16].

Modern advances in the sequencing of the genomes of various microorganisms make it possible to discover many new gene clusters that encode new or alternative variants of already described pathways for the synthesis of bioactive molecules [4, 17]. *Escherichia coli* is traditionally used as an experimental model in molecular biology, in particular, in genomic studies [18, 19]. However, a significant body of data has now been accumulated on the genomics of many other microorganisms, including *B. velezensis*, which is recognized as an effective antibacterial agent and an important biological control agent in agricultural lands as an alternative to chemical antibiotics [20]. In many studies on the genomes of *B. velezensis* strains were carried out to establish the viability, antimicrobial and probiotic potential of this microorganism for its use in medicine [21], to identify industrially significant characteristics for the production of valuable raw materials [22], to assess the ability to synthesize antibacterial and antifungal metabolites [23] active against phytopathogens.

In the present work, in the *B. velezensis* KR-2 strain, we for the first time revealed a unique pathway of intracellular synthesis of the osmoprotectant glycine-betaine with the participation of the *BetA*, *BetB*, *BetT*, and *BetC* genes, which was not previously known in bacteria of the genus *Bacillus*. The peculiarity of the *B. velezensis* KR-2 genome associated with the synthesis of the siderophore myxochelin A, which we discovered, is probably also unique for this strain, in contrast to other studied representatives of the *B. velezensis* species, since it has not been described in the literature.

The aim of the work was a molecular analysis and bioinformatic annotation of the genome of the *Bacillus velezensis* KR-2 strain to identify genetic determinants that determine the possibility of biosynthesis of various biologically active substances important for the creation of biological products for agriculture.

Materials and methods. The *B. velezensis* KR-2 strain from the collection of OOO BIOTROF+ was isolated from the rumen of a dairy cow.

The antimicrobial activity of the strain was studied by the method of delayed antagonism (method of perpendicular strokes) according to the recommendations [24]. To do this, a suspension of the test culture (10^7 CFU/ml) was streaked along the diameter of a Petri dish on a GRM agar medium dried for 24-48 h (SRC PMB Obolensk, Russia) added with glucose (7 g/l). After 24 h of incubation at 37±1.0 °C, test strains of *Staphylococcus aureus*, *E. coli*, *Fusarium oxysporum* and *Clostridium butyricum* were added to the grown culture perpendicularly to the direction of growth. After 24 h of incubation at 37±1.0 °C, the growth inhibition of test strains was assessed by the distance to the stroke of the test culture.

DNA was isolated by standard procedures using the Genomic DNA Purification Kit (Thermo Fisher Scientific, Inc., USA) according to the attached instructions [25]. The method is based on selective detergent-mediated precipitation of DNA from a substrate using solutions for cell wall lysis and DNA precipitation, 1.2 M sodium chloride, chloroform.

A DNA library for whole genome sequencing was prepared using the Nextera XT kit (Illumina, Inc., USA). Nucleotide sequences were determined using a MiSeq NGS system (Illumina, Inc., USA) with a 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) [26]. Filtered by length not less than 50 to 150 bp pair-end sequences were assembled de novo using the SPAdes-3.11.1 genomic assembler (http://cab.spbu.ru/software/spades/) [27] with appropriate keys. Chromosomal and plasmid contigs were distinguished according to the information in the description (contigs assembled as plasmid had the corresponding mark "-plasmid"). Quality of ansavbling was

assessed using QUAST Version: 5.0.2 (A. Gurevich, 2017; http://quast.sourceforge.net/download.html).

The NCBI database (https://www.ncbi.nlm.nih.gov/genome/microbes/) was used to compare the genome of the *B. velezensis* KR-2 strain with the nucleotide sequences of other microorganisms. For phylogenetic analysis, the 16S rRNA gene sequence was transferred to the Nucleotide BLAST web service (https://blast.ncbi.nlm.nih.gov). Search settings have been set by default. The nucleotide sequences of the contigs were translated into amino acids using the PROKKA 1.12 program (https://github.com/kbaseapps/ProkkaAnnotation) [28]. Functional annotation of the genome was performed using the RAST 2.0 web service (https://rast.nmpdr.org) (29). The KEGG Pathway database (http://www.genome.jp/kegg/) was used to evaluate the pool of genes associated with biotechnologically valuable properties and build metabolic maps [30, 31]. For this, the resulting translated protein sequence was transferred to the KEGG-KAAS database server (https://www.genome.jp/kegg/kaas/). The GHOSTX and bi-directional best hit (BBH) algorithms were set as search criteria. Additionally, the UniProt database (https://www.uniprot.org/) was used.

Mathematical and statistical processing of the results was carried out using Microsoft Office Excel 2003 software packages.

Results. The strain *B. velezensis* KP-2 had a pronounced antagonistic effect (Fig. 1) against the studied test cultures of *S. aureus*, *E. coli*, *F. oxysporum*, and *C. butyricum*. The width of the zone of growth inhibition ($n = 5$) was 13.0±0.75, 5.0±0.30, 19.0±0.75, and 14.0±0.70 mm, respectively. This suggests the presence of antimicrobial substances diffusing into the agar in the culture liquid of *B. velezensis* KP-2.

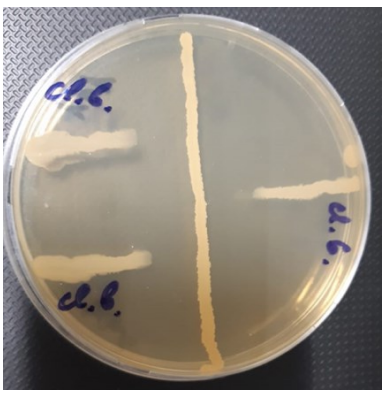

Fig. 1. Antagonistic effect of *Bacillus velezensis* KR-2 on the test culture *Clostridium butyricum*. Suspension of *B. velezensis* KR-2 was plated along the diameter of a Petri dish. The test strain *C. butyricum* was plated by streaking in the perpendicular direction. Closer to the central part, a zone of no growth of *C. butyricum* is visualized.

The data obtained are of great practical importance, since *S. aureus* is dangerous for farm animals, as it can cause diseases in cattle, primarily mastitis [32]. *C. butyricum* is able to act as the main initiator of clostridial fermentation in silage fermentation, resulting in loss of feed quality [33]. Therefore, *B. velezensis* KP-2 is promising in the development of biocontrol agents to suppress pathogenic microbiota, in particular, when introduced into the silage and into the digestive system of farm animals.

In this regard, we performed whole genome sequencing and genome annotation of the *B. velezensis* KP-2 strain using the RAST 2.0 web service. When performing BLAST analysis in the NCBI database, contig NZ_JAILSD010000003.1 was determined as the 16S C region of *Bacillus velezensis* (strain FZB42). The match was 99.81% (1547/1550 bp, 3 mismatches). The genomic sequence was deposited with the BioProjects Collection (NCBI, https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA756418.

For uploading to the server, 16 contigs were used with a total length of 3936398 bp, a share of GC pairs of 46.6%, and N50 assembly quality indicators of 2109194 bp and N75 844068 bp. We did not find any inconsistencies and inconsistencies in the resulting assembly.

The chromosome included 3854 coding sequences (CDS) associated with

the synthesis of polypeptides, with 93 genes for tRNA and 9 genes for rRNA. Plasmid DNA (8162 bp) contained 52.1% GC pairs. The largest of the annotated proteins in length consisted of 5434 amino acid residues, the smallest of 37 amino acid residues (Fig. 2). The dominant number of proteins had a length of 37 to 500 amino acid residues.

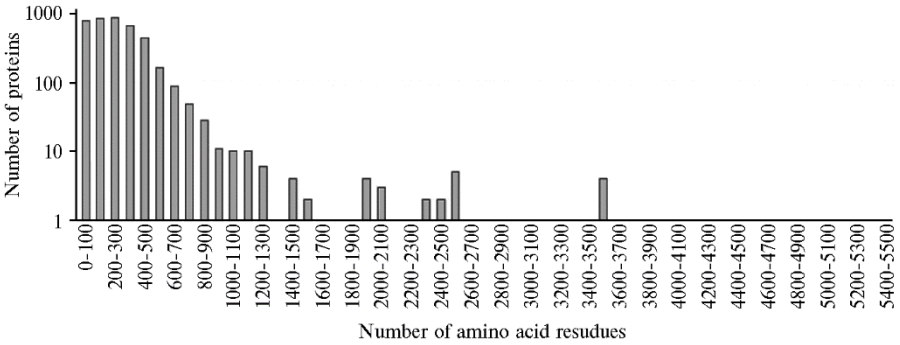

Fig. 2. The length profile of the annotated proteins of the *Bacillus velezensis* KR-2 strain based on whole genome sequencing data and genome annotation (the RAST 2.0 web tool, https://rast.nmpdr.org).

Comparison of the *B. velezensis* KP-2 genome with the nucleotide sequences of other microorganisms in the NCBI Microbial Genomes database revealed a high degree of similarity with the genome of *Bacillus velezensis* (strain FZB42), as well as with the genomes of other members of the *Bacillus* genus (*B. subtilis* QB928, *B. subtilis* subsp. *subtilis* str. AUSI98, *B. subtilis* subsp. *subtilis* str. 168). In addition, the *B. velezensis* KR-2 strain was closely related to the *B. amyloliquefaciens* and *B. atrophaeus* clusters.

B. amyloliquefaciens strains are of interest for their ability to stimulate the growth of host plants through the production of auxins, suppress soil pathogens by synthesizing antibacterial and antifungal metabolites, and induce plant resistance to adverse environmental factors [34]. Among *B. atrophaeus* strains, industrially important ones are also often found, including active producers of antimicrobial substances used as biological protection agents [35]. Previously, A. Niazi et al. [34] reported about high genetic identity of *B. amyloliquefaciens* UCMB5033 to *B. atrophaeus* and *B. subtilis* revealed by whole genome sequencing.

Based on the analysis of the *B. velezensis* KP-2 genome using the RAST web service, we identified 464 metabolic subsystems for the groups of proteins that together ensure the implementation of a certain biological process (Fig. 3). The most represented subsystems were those for the metabolism of amino acids and their derivatives (431 subsystems) and the metabolism of carbohydrates (416 subsystems).

In the *B. velezensis* KR-2 strain, we found a set of potential properties, including the synthesis of antimicrobial peptides, fatty acids, vitamins, siderophores, auxins, the ability to adhere, motility, and chemotaxis, resistance to toxic compounds, the ability to withstand stress factors, stimulate plant growth, and participate in the metabolism of phosphates (see Fig. 3).

In the genome of *B. velezensis* KR-2, we have identified genes (*BacA*, *BacB*, *BacG*, *BacF*, *BacD*) involved in the synthesis of bacilizin. Bacilizin is a nonribosomal synthesized antimicrobial dipeptide that was discovered in one of the strains of *B. subtilis* as early as 1946 as a substance that causes partial lysis of growing cultures of *Staphylococcus aureus* [36]. Later, M. Kenig and E. Abraham [37] noted a high activity of bacilizin (10^{-3} mg · ml^{-1}) against *E. coli*. The data on the possibility of synthesizing this peptide are consistent with the observed

phenomenon of *B. velezensis* KR-2 antagonism against *S. aureus* and *E. coli*. It is known [38] that bacilizin (L-alanine-[2,3-epoxycyclohexano-4]-L-alanine) consists of an L-alanine residue at the N-end and a non-protein amino acid L-anticapsin at the C-end. Despite its relatively simple chemical structure, bacilizin is active against a wide range of bacteria, yeasts, and micromycetes [37].

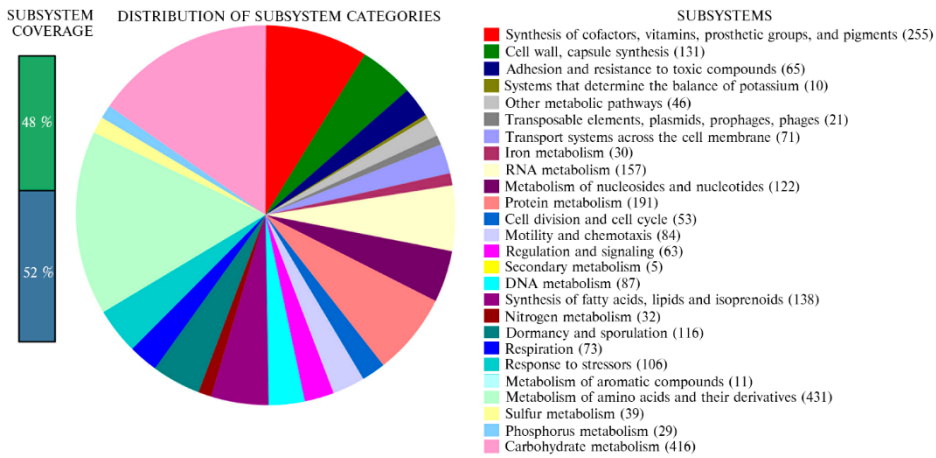

Fig. 3. Distribution of cell metabolism subsystems in the *Bacillus velezensis* KR-2 strain based on functional annotation (RAST, https://rast.nmpdr.org). The pie chart represents the percentage of proteins for each subsystem category. The categories of subsystems are listed in the legend from top to bottom according to the direction of movement along the pie chart in a clockwise direction. The numbers in parentheses are the number of metabolic pathways in the corresponding category of the subsystem. Subsystem coverage is the ratio of known proteins that can be placed into existing subsystems (green) and unknown proteins that cannot be placed into any existing subsystem (blue).

It is interesting that by the annotation in the PROKKA 1.12 program and the UniProt database, in *B. velezensis* KP-2, the *ComA* and *ComP* genes were identified which are responsible for the "quorum sensing" in bacterial populations, that is, the ability to coordinate individual behavior for secretion of molecular signals [38]. It is suggested that a quorum-sensitive pathway involving these genes regulates the bacilizin production in the genus *Bacillus* [38].

The described systems for the synthesis of antimicrobial peptides are not new for bacteria of the genus *Bacillus* and are quite widespread among them [39]. Thus, earlier, using the example of *B. subtilis* BAB-1, it was found that about 5.2% of the strain genome is associated with the synthesis of antimicrobial products, including antibiotics produced by nonribosomal peptide synthetases and polyketide synthases, lantibiotics, and bacillibactin. C. Luo et al. [17] performed whole genome sequencing of the *B. subtilis* 916 strain and found four clusters of genes (*srf*, *bmy*, *fen*, and *loc*) associated with the synthesis of lipopeptides surfactins, bacillomycin, fengycin, and lokillomycins which are active against moulds. Previously [21], whole genome sequencing of the *B. velezensis* KMU01 strain showed that its genome contained the lantibiotic mersacidin operon, including the genes for premersacidin (IM712_RS05205), modification protein (IM712_RS05195), and bacteriocin export protein (IM712_RS05185).

In addition, we found that the *B. velezensis* KR-2 strain is capable of synthesizing and accumulating osmoprotectors (see Fig. 3). One of the most important osmoprotectants is glycine-betaine which is present in the environment, for example, is synthesized by plants [40]. *B. velezensis* KP-2 has the potential to accumulate glycine-betaine directly from the environment through three osmotically regulated uptake systems controlled by the *OpuD*, *OpuAA*, and *OpuAB* genes. The relationship of these genes with the accumulation of glycine-betaine, as well

as their presence in the genome of *Bacillus* spp. has been described in other studies [41]. Earlier, C. von Blohn et al. [42] sequenced a 2781 bp DNA fragment of the *B. subtilis* pORT4 plasmid. This region was associated with the synthesis of the OpuE protein which is important for proline uptake in high osmolarity media. Proline serves as an osmoprotectant in *B. subtilis*. The proline uptake system controlled by *OpuE* works independently of the known transport systems for the osmoprotectant glycine-betaine. S. Heo et al. [21] showed that the genome of the strain *B. velezensis* KMU01 contained two osmoprotective uptake systems for glycine betaine and proline betaine (the *OpuA* and *OpuD* genes, respectively).

It turned out that, in addition to the direct intake of glycine betaine from the environment, *B. velezensis* KR-2 has the potential to accumulate this osmoprotectant through its intracellular synthesis, which requires the presence of precursors (choline or glycine betaine aldehyde) in the environment [43]. The *BetA* gene identified in the genome of *B. velezensis* KP-2 is associated with the synthesis of flavin adenine dinucleotide-dependent choline dehydrogenase (EC 1.1.99.1) which oxidizes choline to glycine betaine aldehyde. The *BetB* gene is associated with the production of betaine aldehyde dehydrogenase (EC 1.2.1.8) which converts glycine betaine aldehyde into osmoprotective glycine betaine, while having a high substrate specificity. The use of choline (the precursor molecule) is due to the high affinity of the BetT transporter for it [43].The *betIBA* operon was under the transcriptional control of the *AnoR* quorum regulator. Previously, a similar pathway for the synthesis of glycine-betaine involving the genes *BetA*, *BetB*, *BetT*, *BetC* was found in *E. coli* [43] and *Acinetabacter nosocomialis* [44], but was not demonstrated for bacteria of the genus *Bacillus*, therefore, it is a unique characteristic of the strain studied by us.

Traditionally, the conditions of increased osmotic pressure in the media are considered as limiting for the development of microorganisms, since high salinity is associated with a decrease in water activity [45]. The pathways we discovered not only for absorption but also for the synthesis of glycine-betaine are important from the point of view of cellular adaptation of the *B. velezensis* strain to high-osmolar stress [45] which is created in environments subject to frequent fluctuations in water content, for example, in dried plant silage and upper layers of the soil [46]. It is believed that the synthesis and accumulation of osmoprotectants is the most flexible response of microorganisms to limited water availability [47].

In addition, *B. velezensis* KR-2 showed a potential for the synthesis of indole derivatives, such as indole-3-ethanol, with the production of which the *IAR*, *TO* genes, indole-3-acetaldehyde (*IAD*, *AAD*, *AO* genes) and indole-3-acetonitrile (*N3* gene) are associated. The possibility of synthesizing tryptophan, an important precursor of auxin, the indolyl-3-acetic acid, has also been found [48], which is determined by the *PRAI*, *IGS*, *TSa*, *TSb*, and *APRT* genes. It is known that auxins have a positive effect on the growth rate, the time of flowering and fruiting of plants, the photosynthesis and production of various metabolites, the resistance to outer stressors, and regulate gene expression [49]. Therefore, the synthesis of auxins is considered as an important advantage for the associative interaction of PGPR bacteria with plants [50]. Biochemical studies have shown that strains of the genus *Bacillus* can produce auxins, in particular indole-3-acetic acid [51]. Whole genome sequencing of *B. subtilis* EA-CB0575 predicted the potential for the synthesis of some auxins (metabolism of indole via tryptophan, as well as the possibility of production of indole acetate and indoacetamide) [52]. Analysis of the genome of *B. velezensis* BS89 revealed the presence of gene clusters responsible for the synthesis of indole-3-acetic acid [53].

With the functional annotation of the *B. velezensis* KR-2 genome, we also

identified potential pathways for the synthesis of vitamins, including biotin, thiamine, riboflavin, and menaquinone. In particular, we showed the presence of genes associated with the synthesis of biotin, the *BPL*, *BR*, BioF, *BioA*, *BioD*, *BioB*, *BioW*, *BioC*, *BioN*, *BioG*, *BioK*, *BioZ*. The ability to synthesize vitamins was previously discovered in many strains of the genus *Bacillus* [41, 54]. Thus, whole genome sequencing of *B. subtilis* UBBS-14 [55] isolated from fermented food products revealed genes associated with the biosynthesis of biotin, riboflavin, vitamin K, cobalamin, vitamin B_6, and folic acid. Vitamins play an important role in many metabolic processes in animals, affecting productivity [56]. It has been reported that these substances can increase plant stress tolerance as well as disease resistance when infected with plant pathogens [57].

In the *B. velezensis* KR-2 genome, we identified several gene clusters (*DhbA*, *DhbB*, *DhbC*, *DhbE*, *DhbF*, *YuiI*, *FeuABCD*) associated with siderophore synthesis and phosphorus assimilation (*PstS*, *PstC*, *PstA*, *PstS hal*, P*stS C*, *PhoP*, *PhoR*, *PhoB*). Almost all genes necessary for the process of iron binding with the participation of bacillibactin (*DhbA*, *DhbB*, *DhbC*, *DhbE*, *DhbF*) were found. A cluster of the *DhbA*, *DhbB*, *DhbC* genes associated with the production of 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28), isochorismatase (EC 3.3.2.1), and isochorismate synthase (EC 5.4.4.2) enzymes, which are responsible for synthesis of the bacillibactin precursor. The *FeuA*, *FeuB*, *FeuC*, and *FeuD* gene cluster is associated with the synthesis of substrate-binding proteins of the iron transport system. In the genome of *B. velezensis* KP-2, genes involved in the synthesis of other siderophores, the enterochelin (*EntE*, *EntB*) and myxochelin A (*MxcE*, *MxcF*), were also found. Probably, bacteria of the genus *Bacillus* can synthesize several siderophores acting in synergy to increase their competitiveness, which was previously shown for other microorganisms [58].

Bacterial siderophores are of great interest for agricultural applications [59]. The fact is that many bacteria, primarily pathogenic ones, require metal ions, in particular iron cations, which are involved in electron transfer and serve as cofactors for enzymes that control DNA and RNA synthesis to ensure the vital activity of many bacteria [60, 61]. Therefore, during evolution, microorganisms have formed siderophores, the specific molecular structures (low molecular weight chelating agents) that provide assimilation of iron ions in a bound state [62]. The use of probiotic preparations based on beneficial microorganisms that produce siderophores, which reduce the concentration of iron ions available to pathogens, have a positive effect on animal health [63]. Through the production of siderophores and successful competition for iron ions present in the soil, PGPR rhizobacteria can inhibit pathogenic microorganisms [64]. Previously, whole genome sequencing of *B. subtilis* EA-CB0575 [52] revealed the potential for the production of siderophores such as bacillibactin, enterochelins, and vibriobacins.

Summing up, it should be noted that the potentially useful properties of *B. velezensis* KP-2 revealed via whole genome sequencing make it possible to recognize this strain as a bacterial resource promising for use in agriculture. Due to the potential for synthesis of a complex of metabolites, the strain can adapt to a specific environment in the host's digestive system, in the rhizosphere or in feed during fermentation, successfully compete with other members of the autochthonous microbiota. Additionally, *B. velezensis* KP-2 exhibit functions that mediate the positive effect of the strain on microbiological processes during introduction into various environments.

Thus, as a result of whole genome sequencing of the *Bacillus velezensis* KR-2 strain isolated by us from the rumen of a dairy cow, 16 contigs are obtained with a total length of 3936398 bp, containing 46.6% GC pairs, with quality N50 2109194 bp and N75 844068 bp. The chromosome includes 3854 coding sequences

(CDS) associated with the synthesis of polypeptides. We have found 464 metabolic subsystems the most represented of which are the subsystems of the metabolism of amino acids and their derivatives (431 subsystems) and the metabolism of carbohydrates (416 subsystems). The functional annotation revealed a complex of potential properties, including the synthesis of antimicrobial peptides, fatty acids, vitamins, siderophores, auxins, the ability to adhere, motility and chemotaxis, resistance to toxic compounds, the ability to withstand stress factors, stimulate plant growth and participate in phosphate metabolism. In particular, in the genome of the *B. velezensis* KR-2 strain, genes involved in the production of bacillicin bacillisin (*BacA*, *BacB*, *BacG*, *BacF*, *BacD*) are found. A unique pathway for intracellular synthesis of the osmoprotectant glycine-betaine with the participation of the *BetA*, *BetB*, *BetT*, and *BetC* genes is also identified. The potential for the synthesis of auxins such as indole-3-ethanol (*IAR*, *TO* genes), indole-3-acetaldehyde (*IAD*, *AAD*, *AO*) and indole-3-acetonitrile (*N3*) is shown. In addition, in the *B. velezensis* KP-2 genome, several clusters of genes associated with siderophore synthesis (*DhbA*, *DhbB*, *DhbC*, *FeuA*, *FeuB*, *FeuC*, *FeuD*) are identified. The peculiarity of the *B. velezensis* KP-2 genome associated with the synthesis of the siderophore myxochelin A which we have identified, is probably unique, since this was not found in other strains of the species *B. velezensis*. Glycine-betaine synthesis pathways involving the *BetA*, *BetB*, *BetT*, and *BetC* genes identified by us are important for the cellular adaptation of the *B. velezensis* KP-2 strain to high-osmolar stress occurred in environments subject to frequent fluctuations in water content, for example, in dried plant mass of silage and in the upper layers of the soil. The ability to synthesize bacteriocins are annotated in the *B. velezensis* KR-2 strain, which is consistent with the antagonism against *Staphylococcus aureus*, *Escherichia coli*, *Fusarium oxysporum*, and *Clostridium butyricum* observed in vitro. In the future, using various methods (liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy), we plan to compare the obtained molecular characteristics with the empirically observed biochemical and physiological patterns of beneficial metabolite production in *B. velezensis* KR-2. This will provide new fundamental knowledge about the genetic control and regulation of the synthesis of bioactive substances in bacilli and the use of *B. velezensis* KR-2 in agriculture.

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