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# ANALYSIS OF BIOSYNTHETIC GENE CLUSTERS OF *BACILLUS VELEZENSIS* ONU 553 IN SILICO

***The aim*** *of the work was to analyse biosynthetic gene clusters (BGC) of Bacillus velezensis ONU 553 based on bioinformatics approach.* ***Methods.*** *Identification of species was processed with tools of TYGS server; EzBioCloud was used to calculate ANI. Analysis of biosynthetic gene, bacteriocin, and antibiotic resistance gene clusters using antiSMASH, Bagel4, respectively.* ***The results.*** *It is shown that the results of identification, phylogenetic analysis and DNA-DNA hybridization (DDH) carried out in silico proved that the strain Bacillus velezensis ONU 553 belongs to the operational group B. amyloliquefaciens (OGBa). Sequences identified as possible phages and CpG-islands were found in the genome of our strain. 12 biosynthetic gene clusters (BGC) were identified using antiSMASH. One new cluster capable of synthesizing a new metabolite was identified (region 11). The presence of two clusters of bacteriocins in the genome of Bacillus velezensis ONU 553, which are assigned to uberolysin/carnocyclin and the antimicrobial peptide LCI based on the identification of the core gene, is shown.* ***Conclusions.*** *The preliminary identification of the Bacillus velezensis ONU 553 strain as a representative of the Bacillus velezensis strain of the B. amyloliquefaciens group (OGBa) was confirmed. The presence of gene clusters of secondary metabolites responsible for the synthesis of surfactins, polyene antibiotics, antimicrobial peptides, macrolide antibiotics and bacteriocins was shown. The obtained results indicate that the Bacillus velezensis ONU 553 strain is promising for use in the field of "Blue Biotechnology" for the development of new drugs with antimicrobial and antifungal activity.*

*Key words: Bacillus velezensis, biosynthetic gene clusters (BGC), bioinformatic analysis*

*Bacillus velezensis* belongs to the operational group of *B. amyloliquefaciens* (OGBa), it is found in different environments, but first of all in soils and marine bottom sediments [16].

The practical interest to the members of *Bacillus velezensis* is induced due to their ability to the major production of secondary metabolites, rapid growth of strains, as well as their significant resistance to adverse environmental conditions [2; 21; 25]. The mentioned advantages characterize *B. velezensis* as a promising producer of biologically active compounds and an object of pharmaceutical biotechnology [27].

However, the morphology and physiological properties of the strains show significant heterogeneity depending on the primary genesis of the isolate. Only certain strains have a complete set of signs and properties that define them as leaders and promising producers of industrial biotechnology. In this regard, the study of complete genomes provides a comprehensive characterization of the genes of the target clusters and reveals the genetically determined potential of the obtained strains. Within the framework of the actual research, we performed a bioinformatics study of the genome of *B. velezensis* ONU 553 in order to substantiate its synthetic potential.

**Materials and methods**

The strain *Bacillus velezensis* ONU 553 was isolated from the bottom sediments of the Black Sea and deposited in the Collection of marine and practically useful microorganisms of Odesa National University named after I. I. Mechnikov, and the genomic sequence was deposited in the GenBank (www.ncbi.nlm.nih.gov) under inventory number CP043416.

The annotation of the genome was conducted using he PATRIC server (Pathosystems Resource Integration Center) of the BV-BRC network resource (Bacterial and Viral Bioinformatics Resource Center – www.bv-brc.org) [23].

The TYGS service (Type (Strain) Genome Server – https://tygs.dsmz.de/) was used to reconstruct the phylogenetic tree based on complete genomic sequences [15]. The correctness of the topology of the tree was based on the average values of branch support and δ statistics [11]. Average nucleotide identity (OrthoANI) was calculated using the tool ANI (Average Nucleotide Identity Tool) calculator on the EzBioCloud platform (www.ezbiocloud.net) [13; 26].

Mobile genetic elements, including CpG islands and prophages of *B. velezensis* ONU 553, were identified using IslandViewer 4 (https://www.pathogenomics.sfu.ca/islandviewer/browse/) and PHASTER (PHAge Search Tool Enhanced Release – https://phaster.ca/) [1].

Bioinformatics tools antiSMASH ("antibiotics and secondary metabolite analysis shell" (https://antismash.secondarymetabolites.org/) version 7.0.0 [4] and BAGEL4 (http://bagel.molgenrug.nl/) [22]) were used to search for gene clusters (BGC) involved in the synthesis of polyketides and bacteriocins.

The MEGAX program (Molecular Evolutionary Genetics Analysis version X) [12], CLUSTAL W multiple alignment and the Neighbor-Joining method were used to reconstruct the phylogenetic tree of bacteriocins. The ITOL server (https://itol.embl.de/) was used to visualize the obtained tree.

To compare existing gene clusters in the genomes of the most closely related representatives of *Bacillus*, we used the "pheatmap" package implemented in the R 4.2.2 program.

**Results and discussion**

**General genome annotation of Bacillus velezensis ONU 553**

Genomic analysis of *Bacillus velezensis* ONU 553 using the PATRIC server (table 1) determined that the genome consisted of a circular chromosome that contains 3,934,563 base pairs (bp) and has an average content GC content of 46.69%.

Table 1

**General characteristics of the *Bacillus velezensis* ONU 553 genome**

| | | |
|---|---|---|
| Genome Statistics | Contigs | 1 |
| | Genome Length | 3934563 |
| | GC Content | 46.688286 |
| | Contig L50 | 1 |
| Genomic Features | CDS | 3953 |
| | tRNA | 86 |
| | repeat_region | 39 |
| | rRNA | 27 |
| Protein Features | Hypothetical proteins | 706 |
| | Proteins with functional assignments | 3247 |
| | Proteins with EC number assignments | 1003 |
| | Proteins with GO assignments | 838 |
| | Proteins with Pathway assignments | 744 |
| | Proteins with Subsystem assignments | 1203 |
| | Proteins with PATRIC genus-specific family (PLfam) assignments | 3688 |
| | Proteins with PATRIC cross-genus family (PGfam) assignments | 3797 |
| Specialty Genes | Virulence Factor (PATRIC_VF) | 3 |
| | Virulence Factor (Victors) | 2 |
| | Transporter (TCDB) | 193 |
| | Drug Target (DrugBank) | 47 |
| | Drug Target (TTD) | 1 |
| | Antibiotic Resistance (PATRIC) | 49 |
| | Antibiotic Resistance (CARD) | 5 |
| | Antibiotic Resistance (NDARO) | 3 |

In general, the presence presence of 3953 protein-coding DNA sequences (CDS) that were distributed along both strands was determined. Also, 86 tRNAs and 27 rRNAs, as well as 4 possible phages [20] and 11 CpG islands were detected (table 2, fig. 1).

**Taxonomic status of *Bacillus velezensis* ONU 553**

Clarification of the systematic position of *B. velezensis* ONU 553 carried out using a complex of methods, including a search using BLAST+ (version 2.9) and relevant databases (ref_prok_rep_genomes), calculations of the identity of nucleotide sequences (OrthoANI) and dDDH of the complete genome. In general, the obtained results were agreed among themselves. According to the results of phy-

Table 2

**Characterization of CpG islands in the genome of *Bacillus velezensis* ONU 553**

| Genomic Islands | Island Start | Island End | Length(bp) | Quantity CDS |
|---|---|---|---|---|
| Island 1 | 448580 | 453512 | 4932 | 11 |
| Island 2 | 509084 | 514818 | 5734 | 8 |
| Island 3 | 596350 | 611163 | 14813 | 26 |
| Island 4 | 615145 | 645952 | 30807 | 39 |
| Island 5 | 720638 | 724799 | 4161 | 6 |
| Island 6 | 1184317 | 1190581 | 6264 | 11 |
| Island 7 | 1512093 | 1516228 | 4135 | 7 |
| Island 8 | 1732796 | 1737962 | 5166 | 8 |
| Island 9 | 1841511 | 1854017 | 12506 | 23 |
| Island 10 | 3352044 | 3378255 | 26211 | 8 |
| Island 11 | 3353982 | 3375384 | 21402 | 4 |

logenetic clustering which was performed using the TYGS service, *B. velezensis* ONU 553 is a close relative of *B. amyloliquefaciens* FZB42 (CP000560.2) (fig. 2).

According to the recommended threshold values of OrthoANI [13], the obtained results confirm the relatedness of the genomes of *Bacillus velezensis* ONU 553 and *Bacillus amyloliquefaciens* FZB42 (98.87%)

**Gene clusters of secondary metabolites in the genome of *Bacillus velezensis* ONU 553**

Gene clusters associated with the biosynthesis of secondary metabolites in the genome of *B. velezensis* ONU 553 were determined by using the antiSMASH tool [14]. Among 12 clusters, four non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS), hybrid clusters of NRPS/PKS and terpenes were detected (Table 3). The generalized results of the analysis of the organization of the determined clusters of genes associated with biosynthesis are shown in figure 3.

Further analysis revealed that cluster 11 is a novel NRPS gene cluster that consists of two genes and has a total size of 59997 bp. In general, the protein products of these genes contained 18 functional domains; four condensation domains (C), five adenylation domains (A), five peptidyl carrier protein (CP) domains, one epimerization domain (E), one coenzyme A ligase (CAL) domain, and one special TIGR01720 domain with unknown function. This cluster showed no similarity to any known biosynthesis gene clusters. It was predicted that cluster 11 could biosynthesize a key structure with amino acids (Cys–Ala–X–Asn–D-Asn) (Fig. 4).

Identified secondary metabolites include surfactin (cluster 1, Table 3, Fig. 3), which is a bacterial cyclic lipopeptide and exhibits such effective characteristics as antibacterial, antiviral, antifungal, and hemolytic activity [19].

Cluster 5 encodes an atypical polyketide-nonribosomal peptide synthase, which could be potentially related to the synthesis of a new antibiotic of the bacil-

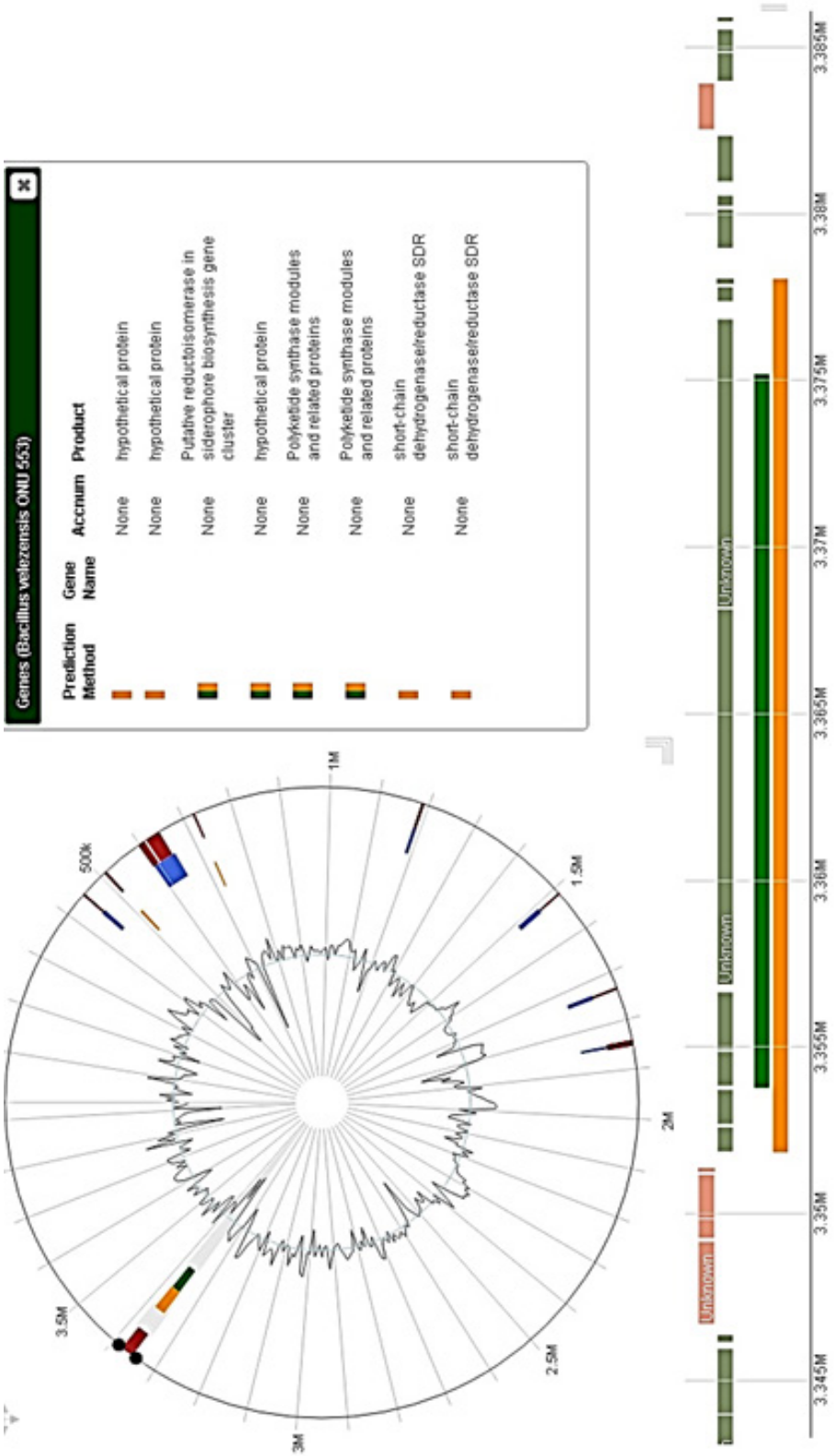


**Fig. 1. The result of searching for CpG- islands in the genome of *Bacillus velezensis* ONU 553 using the IslandViewer 4 server**

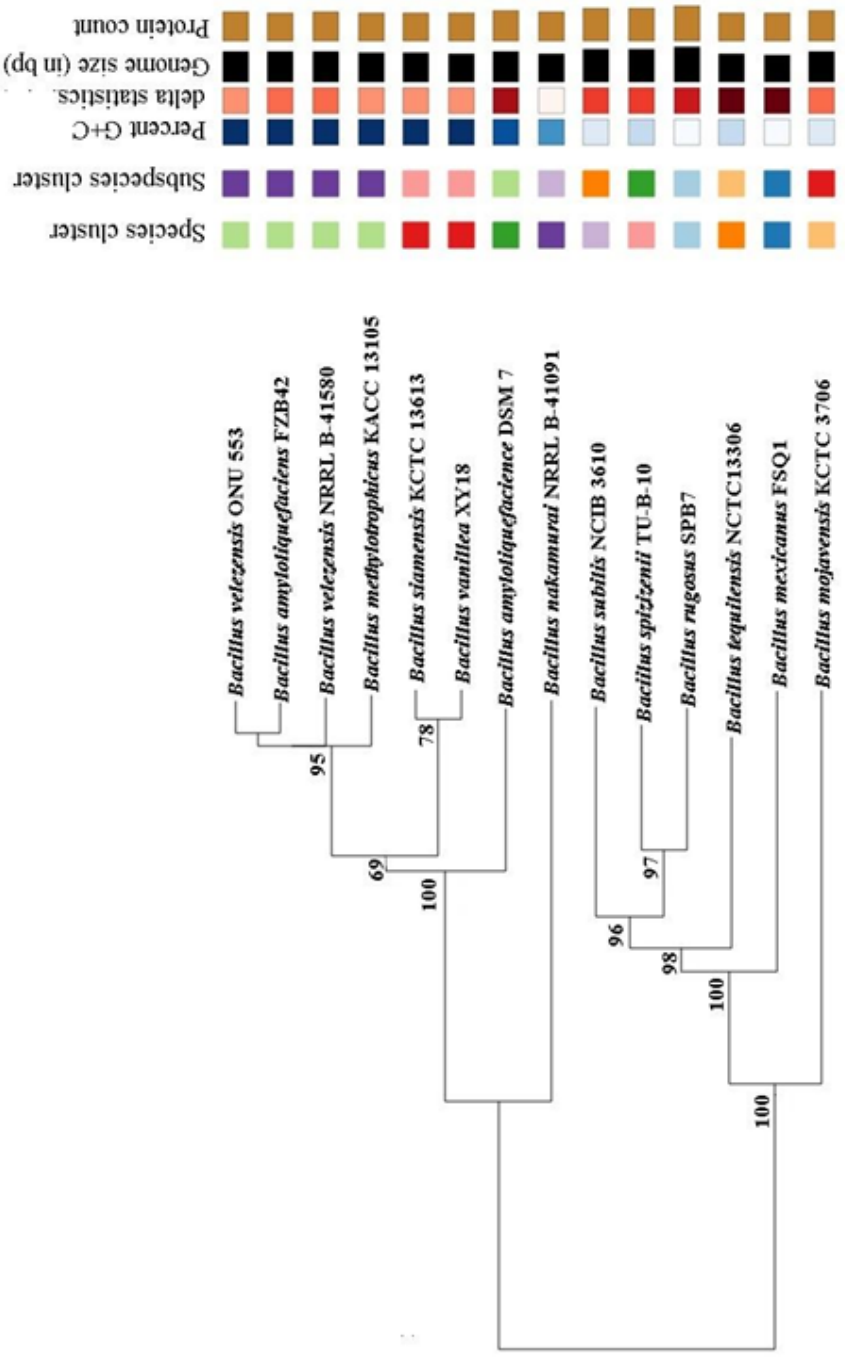


**Fig. 2. Phylogenetic position of *Bacillus velezensis* ONU 553**
Phylogenetic tree constructed using the TYGS service. GBDP (Genome BLAST Distance Phylogeny) genomic distances were estimated based on genome sequences. Branch lengths were scaled using the d5 formula for the GBDP distance. Values from 100 GBDP pseudobootstrap replicates are shown by numbers above branches

**Table 3**

**Gene clusters associated with the synthesis of secondary metabolites (BGC) in *Bacillus velezensis* ONU 553 (according to the results of analysis in antiSMASH)**

| Cluster | Type | Size (bp) | Most similar known cluster | Similarity (%) |
|---|---|---|---|---|
| 1 | NRPS (Nonribosomal peptide synthetase) | 64858 | Surfactin | 91 |
| 2 | PKS-like (Polyketide synthase) | 41244 | Butyrosin A/Butyrosin B | 7 |
| 3 | terpene (Terpenoid synthesis enzymes) | 17168 | Non identified | |
| 4 | transAT-PKS (Trans AT-polyketide synthase) | 87819 | Macrolactin | 100 |
| 5 | transAT-PKS/ NRPS (Combined nonribosomal peptide synthetase /Trans AT-polyketidesynthase) | 109203 | Bacillaene | 100 |
| 6 | NRPS (Nonribosomal peptide synthetase) | 137117 | Fengycin | 100 |
| 7 | terpene (Terpenoid synthesis enzymes) | 21883 | Non identified | |
| 8 | T3PKS (Polyketidesynthase III type) | 41100 | Non identified | |
| 9 | transAT-PKS (Trans AT-tranferase) | 106173 | Difficidin | 100 |
| 10 | NRPS (Nonribosomal peptide synthetase) | 511152 | Bacillibacin | 100 |
| 11 | NRPS (Nonribosomal peptide synthetase) | 59996 | Non identified | |
| 12 | other | 41418 | Bacilysin | 100 |

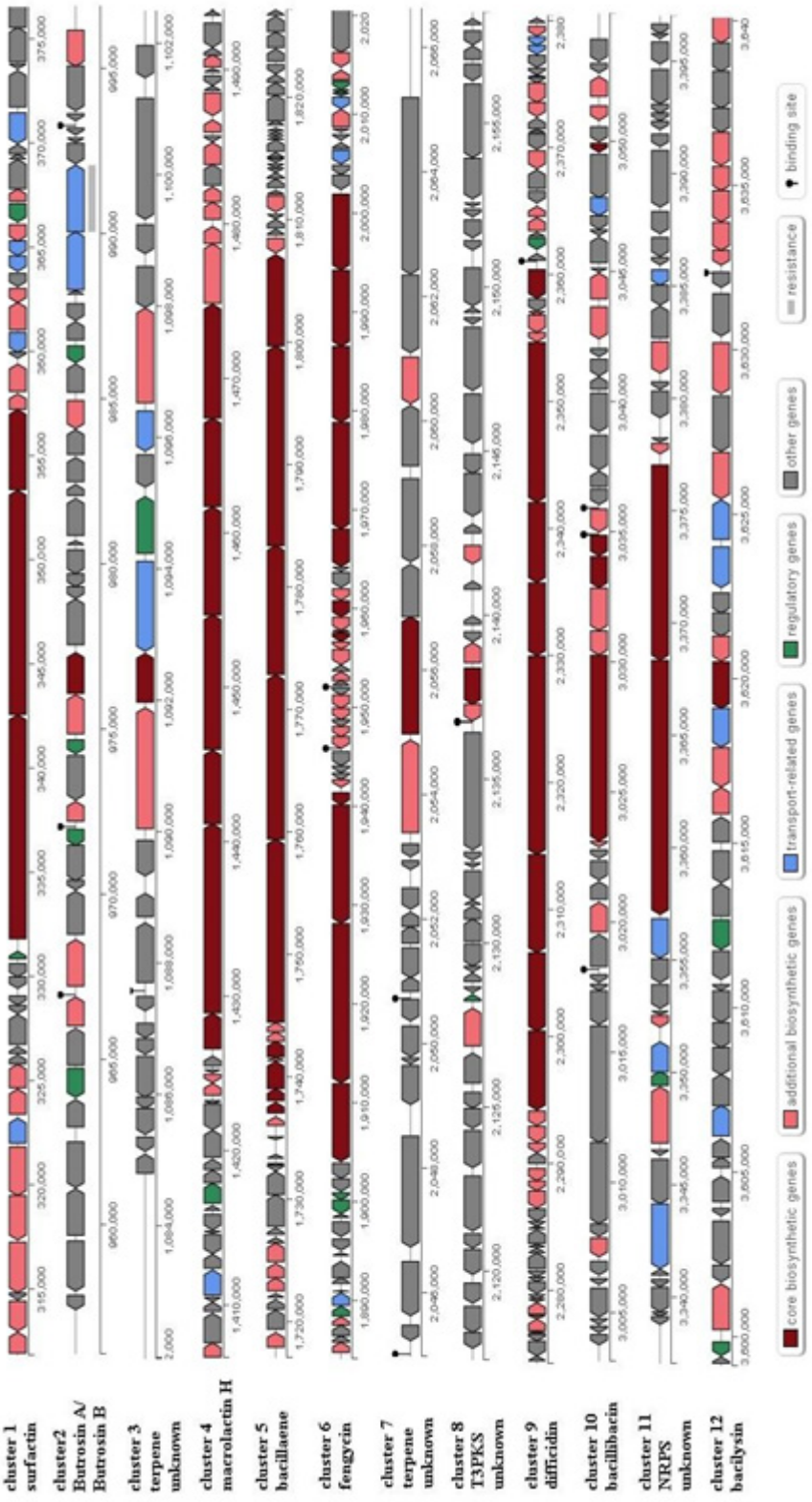


**Fig. 3. Biosynthetic gene clusters associated with the biosynthesis of secondary metabolites (BGC) of the strain *Bacillus velezensis* ONU 553 were detected using antiSMASH**

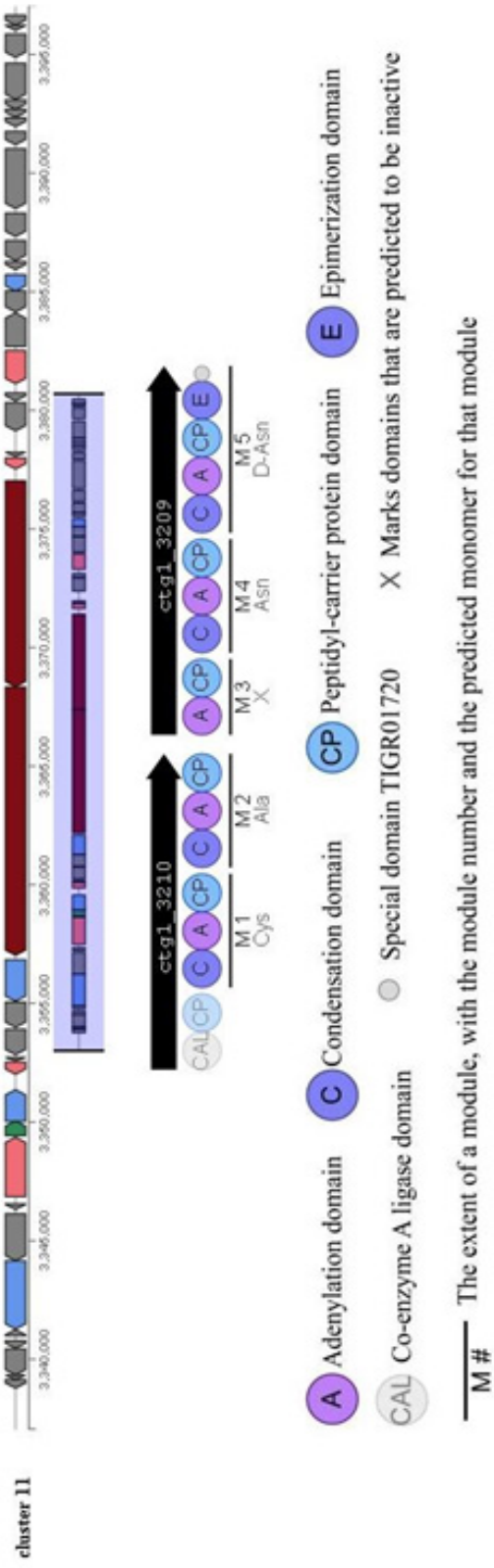


**Fig. 4. The structure of the NRPS gene cluster (region 11) identified in the genome in the genome *Bacillus velezensis* ONU 553**

laene series. The last is a polyene antibiotic first discovered in *B. subtilis* and largely uncharacterized due to its notorious instability [5]. It is known that the effect of bacillaene is to disrupt protein synthesis, the mechanism of which is still unclear. It is noteworthy that recently an almost identical cluster of genes was described for *B. amyloliquefaciens* FZB42 [24] (Table 3, Fig. 3).

The next potential product of the identified genes is the cyclic lipopeptide fengycin, which specific activity against *Fusarium graminearum, Monilinia laxa, Monilinia fructicola, Verticillium dahliae, Rhizoctonia solani and Pythium aphanidermatum* [9; 17; 19]. According to the results of bioinformatic clustering, the corresponding fengycin gene was determined in cluster 6 (table 3, fig. 3).

Another important cluster was the cluster of bacillibactin biosynthesis genes (cluster 10), which is a catecholamide siderophore that exhibits fungicidal and non-specific antibacterial effects [5].

Two transAT-PKS clusters united genes potentially encoding derivatives of macrolactin (cluster 4) and difficidin (cluster 9). The first is a large group of macrolide antibiotics which according to their chemical structure are 24-membered lactonides of the b-ring type, first found in marine strains *B. amyloliquefaciens* [10]. A potential marker product of cluster 9 – difficidin causes suppression of the expression of genes that are responsible for the virulence of cell division and the synthesis of proteins and cell walls in representatives of *Xanthomonas* [24].

Two clusters of biosynthesis of secondary metabolites, combined genes potentially associated with the production of RiPPs (ribosomally synthesized and post-translationally modified peptides) and (unmodified) bacteriocins, which were previously found in the genome of *B. velezensis* ONU 553 using the BAGEL4 web service. According to our results, the first cluster encodes bacteriocin AOI_1, homologous to amylocyclin, which is produced by *B. velezensis* strain FZB42 [24]. The sequence of the gene encoding the specified bacteriocin belongs to cluster 10. Correspondence to the structure of this cluster is confirmed by data from the MIBig resource(https://mibig.secondarymetabolites.org/repository/BGC0000616/index.html#r1c1) (fig. 5).

However, while the reconstruction of the phylogenetic tree was held based on the found bark protein homologues, we obtained results that indicate that bacteriocin AOI_1 of *B. velezensis* ONU 533 strain shows a more pronounced homology to the "uberolysin/carnocyclin" type (fig. 6).

However, when reconstructing the phylogenetic tree based on the found homologues of the cortical protein, we obtained results that indicate that bacteriocin AOI_1 of the strain *B. velezensis* ONU 533 is closer to the uberolysin/carnocyclin type (fig. 6).

It should be noted that circular bacteriocins, which make up a group of ribosomally synthesized antimicrobial peptides and are interesting as a new promising class of antibiotics [6; 7]. Circular bacteriocins are synthesized as linear precursor proteins containing a leader peptide that is excised during maturation. According to the classification of gram-positive bacteriocins, ring bacteriocins are considered unmodified class II peptides and often belong to subclasses IIc and IId [3; 8].

The second sequence of bacteriocin AOI_2 in the genome of *B. velezensis* ONU 533 was identified as a cationic antimicrobial peptide LCI whose gene is lo-

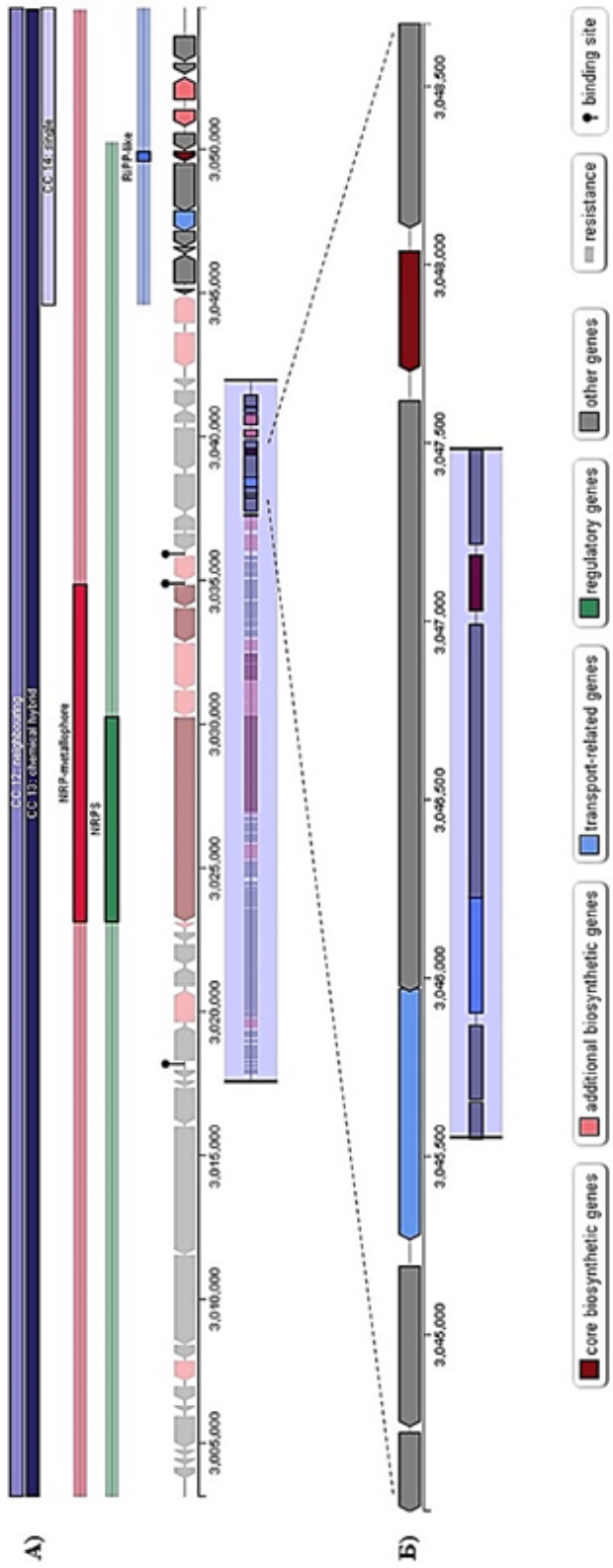


**Fig. 5. Comparative structure of cluster 11 identified using the antiSMASH version 7.0.0 server (A) and in the MIBig biological database (B) in the genome of *Bacillus velezensis* strain ONU 533**

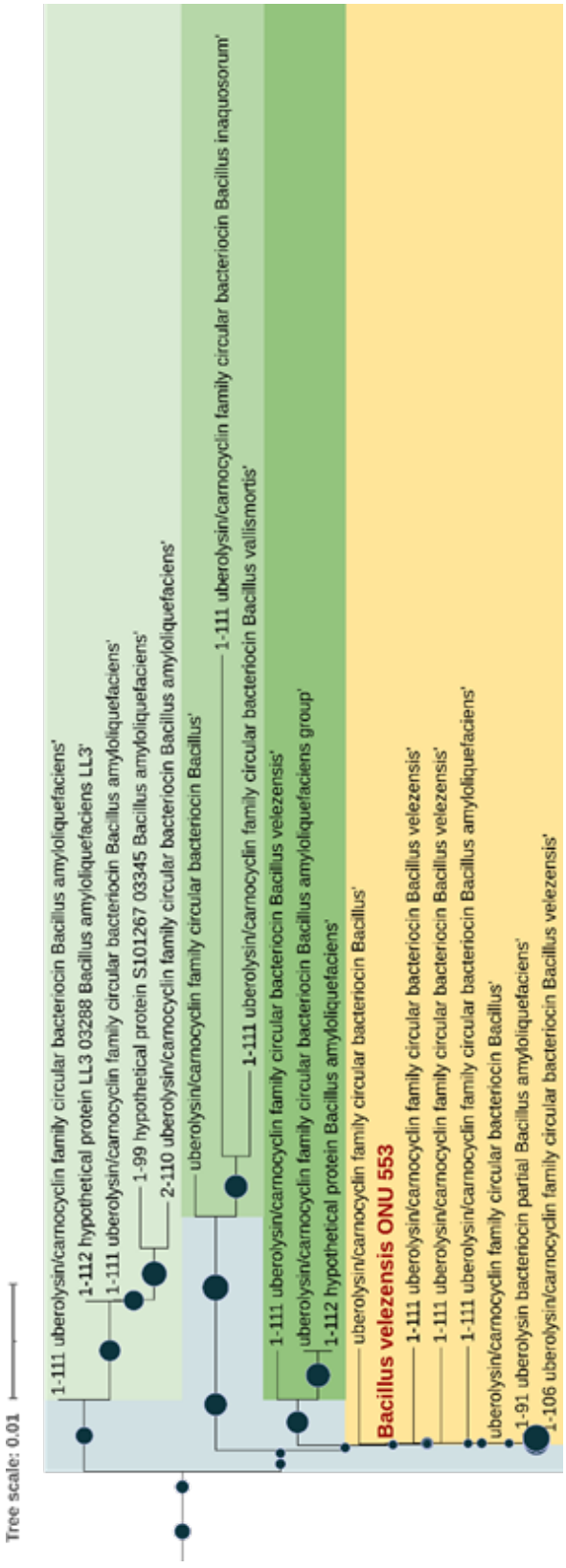


**Fig. 6. Phylogenetic tree of bacteriocin AOL_1 found in the genome of *Bacillus velezensis* strain ONU 533**

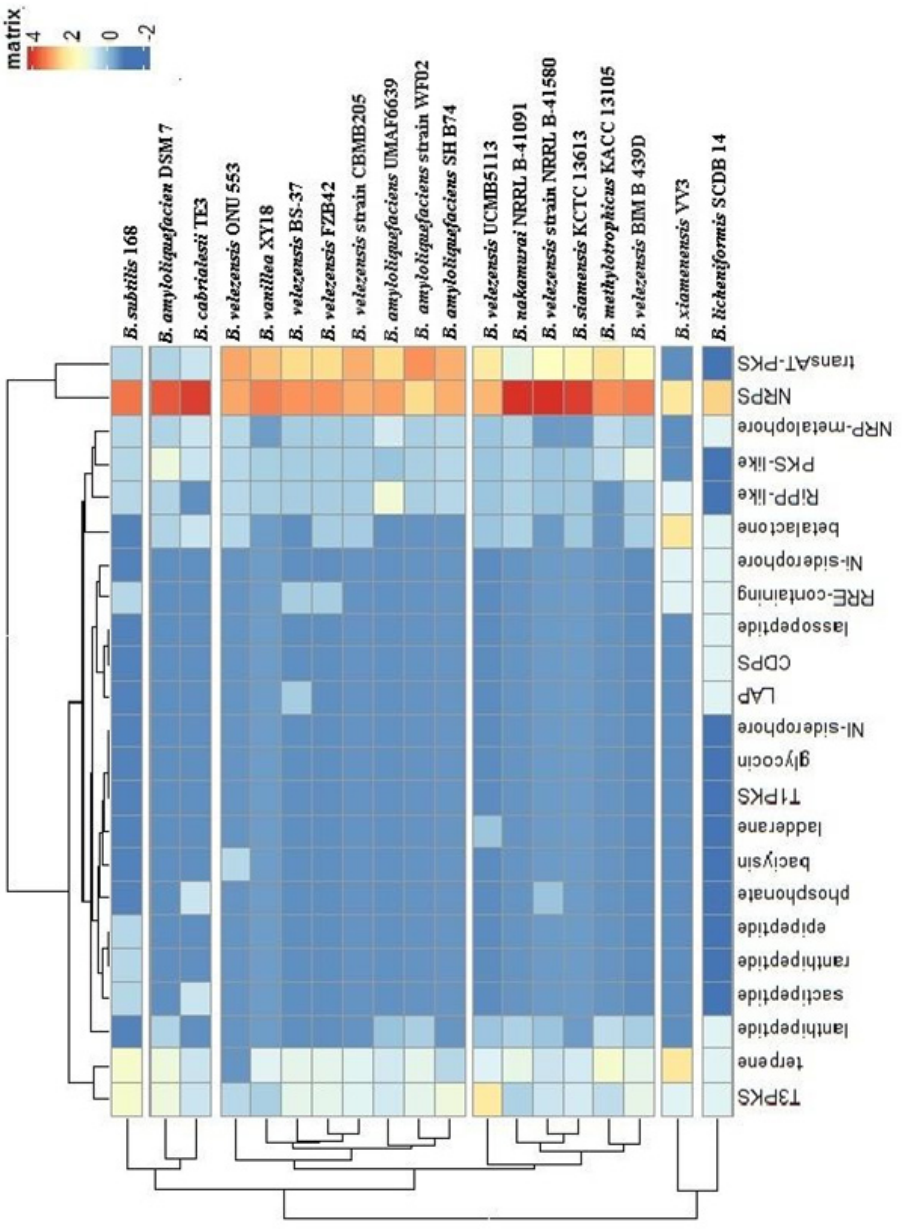


**Fig. 7. A heat map that reproduces the identity matrix between the composition of biosynthetic gene clusters (BGC) detected by AntiSMASH in the genomes of the most closely related strains to *Bacillus velezensis* ONU 553**

cated in the genome from 290078 nucleotides to 310213 nucleotides. It should be noted that the antiSMASH tool did not identify this specified bacteriocin isotype. Also, the corresponding homologues were absent in the MIBig database. However, blastp confirmed 93.48% sequence identity of bacteriocin AOI_2 with the cationic antimicrobial peptide LCI from *B. subtilis*, which exhibits potent antimicrobial activity against *Xanthomonas campestris* and *Pseudomonas solanacearum* [18].

With the aim to compare the structure and number of BGCs in *B. velezensis* ONU 553 with other representatives of the *Bacillus* family, a heat map was constructed (Fig. 7). For greater reliability of the analysis results, the test group included not only strains of the first (target) clade (Fig. 2), but also other strains that were determined to be the most closely related: *B. velezensis* strain BS-37 (NZ_CP023414.1), *B. cabrialesii* strain TE3 (NZ_CP096889.1), *B. subtilis* subsp. subtilis str. 168 (NC_000964.3), *B. xiamenensis B.* VV3 (NZ_CP017786.1), *B. licheniformis* strain SCDB 14 (NZ_CP014842.1), *B. amyloliquefaciens* UMAF6639 (NZ_CP006058.1), *B. velezensis* strain BIM B-439D (NZ_CP032144.1), *B. velezensis* UCMB5113 (NC_022081.1), *B. amyloliquefaciens* strain SH-B74 (NZ_CP030097.1), *B. amyloliquefaciens* strain WF02 (NZ_CP053376.1), *B. velezensis* strain CBMB205 (NZ_CP011937.1), *B. nakamurai* strain NRRL B-41091 (NZ_LSAZ00000000.1).

Summarizing the results of the analysis of the control group and comparing the obtained data with the results of the analysis for *B. velezensis* ONU 533, it was concluded that for the organization of biosynthesis gene clusters (BGC), the target strain belongs to a common group with *B. amyloliquefaciens* strain SH-B74, *B. velezensis* strains FZB42, BS-37 and UCMB5113, as well as *B. amyloliquefaciens* strains UMAF6639. These species are distinguished by a large number of NRPS, transAT-PKS and T3PKS (Fig. 7).

Thus, according to the results of bioinformatics analysis, the presence of gene clusters of secondary metabolites responsible for the synthesis of surfactins, polyene antibiotics, antimicrobial peptides, macrolide antibiotics and bacteriocins in the genome of *B. velezensis* ONU 553 was shown in the genome of *Bacillus velezensis* ONU 553, as well as a new cluster of secondary metabolite genes (region 11) was discovered. The obtained results indicate that *B. velezensis* strain is a promising object for further implementation in the field of "Blue Biotechnology" as a promising producer of new drugs with antimicrobial and antifungal activity.

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# АНАЛІЗ КЛАСТЕРІВ БІОСИНТЕТИЧНИХ ГЕНІВ *BACILLUS VELEZENSIS* ONU 553 IN SILICO

**Реферат**

***Метою*** *роботи був аналіз кластерів генів асоційованих з біосинтезом вторинних метаболітів (BGC) Bacillus velezensis ONU 553 з використанням біоінформатичних методів.* ***Методи.*** *Ідентифікацію виду проводили з використанням сервера TYGS; для розрахунку ANI (Average Nucleotide Identity) використовували EzBioCloud. Аналіз наявності кластерів генів, бактеріоцинів проводили за допомогою antiSMASH, Bagel4, відповідно.* ***Результати.*** *Показано, що за результатами ідентифікації, філогенетичного аналізу та ДНК-ДНК-гібридизації (DDH), проведеної in silico штам Bacillus velezensis ONU 553 відноситься до оперативної групи B. amyloliquefaciens (OGBa). В геномі дослідженного штаму виявлені послідовності, що ідентифіковані як можливі фаги та CpG-острівки. Ідентифіковано 12 кластерів біосинтетичних генів (BGC) з використанням інструменту antiSMASH. Визначено кластер нового метаболіту (регіон 11). Показана наявність двох кластерів генів бактеріоцинів в геномі Bacillus velezensis ONU 553, які на підставі гомології корового гена віднесені до uberolysin/carnocyclin та антимікробного пептиду LCI.* ***Висновки.*** *Підтверджена належність Bacillus velezensis ONU 553 до групи B. amyloliquefaciens (OGBa). Визначені кластери генів, які відповідають за синтез сурфактинів, поліенових антибіотиків, антимікробних пептидів, макролідних антибіотиків та бактеріоцинів. Отримані результати свідчать, що B. velezensis ONU 553 є перспективним для використання в галузі «Блакитної біотехнології» для розробки нових препаратів з антимікробною та антифунгіцідною активністю.*

*Ключові слова: Bacillus velezensis, кластери генів, біоінформатичний аналіз*

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