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Genetic Characteristics and Enzymatic Activities of *Bacillus velezensis* KS04AU as a Stable Biocontrol Agent against Phytopathogens

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Abstract: *Bacillus velezensis* has a broad application in the agricultural and industrial sectors for its biocontrol properties and its potential active secondary metabolites. The defined phenotypic characteristics of a strain vary according to its ecosystem. We report the complete genomic analysis of *B. velezensis* KS04AU compared to four strains of *B. velezensis* (SRCM102752, ONU-553, FZB42, and JS25R) and two closely related *Bacillus amyloliquefaciens* (LL3 and IT-45). A total of 4771 protein coding genes comprises the KS04AU genome, in comparison with 3334 genes core genes found in the six other strains and the remaining 1437 shell genes. Average nucleotide identity of the target strain to the six other strains showed 99.65% to *B. velezensis* ONU-553, sharing 60 orthologous genes. Secondary metabolite gene cluster analysis of all strains showed that KS04AU has a mersacidin cluster gene, which is absent in the genome of the other strains. PHASTER analysis also showed KS04AU harboring two phages (*Aeribacllus* AP45 NC_048651 and *Paenibacillus*_Tripp NC_028930), which were also unique in comparison with the other strains. Analysis on anti-microbial resistance genes showed no difference in the genome of KS04AU to any of the other genomes, with the exception of *B. amyloliquefaciens* IT-45 which had one unique small multidrug-resistance antibiotic efflux-pump gene (*qacJ*). The CRISPR-Cas systems in the strains were also compared showing one CRISPR gene found only in KS04AU. Hydrolytic activity, antagonistic activity against phytopathogens (*Fusarium oxysporum*, *Fusarium graminearum*, *Alternaria alternata* and *Pseudomonas syringae*) and biocontrol against tomato foot and root rot experiments were carried out. *B. velezensis* KS04AU inhibits the growth of all phytopathogens tested, produces hydrolytic activity, and reduces *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*Forl)* ZUM2407 lesions up to 46.02 ± 0.12%. The obtained results confirm *B. velezensis* KS04AU as a potential biocontrol strain for plant protection.

Keywords: bacillus velezensis; full genome; secondary metabolites; phages; hydrolytic activity; phytopathogens; biocontrol

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1. Introduction

Bacteria strains from the genus *Bacillus* are aerobic or facultative anaerobic endospore-forming bacteria, known to produce secondary metabolites that are antagonistic to most phytopathogens [1]. As an endospore-forming rhizobacteria and possessing the ability to grow under extreme abiotic conditions, strains of *B. velezensis* can be stored for a long period and are suitable for application in any type of soil [2,3]. Its antimicrobial, plant-growth-promoting ability including the ability to induce plant resistance (ISR); their probiotic

ability in animals were also reported in several scientific manuscripts [4–6]. The main mechanism of *B. velezensis* as a biocontrol agent in wheat blight is due to its secondary metabolites that include polyketides and lipopeptide surfactins [7].

Recently, the systematic classification of *B. velezensis* by association or relation with one organism varied since its isolation from the River Vélez [3]. The origin of *B. velezensis* was said to be a latter heterotypic species of *Bacillus amyloliquefaciens* but genomic comparison of *B. velezensis* strain NRRL B-41580^{T}, based on DNA–DNA relatedness calculation, showed it to be synonymous to *Bacillus methylotrophicus* [8]. The researchers suggested *B. methylotrophicus* KACC 13015, *B. amyloliquefaciens* subsp. *plantarum* FZB42 (recently *B. velezensis* FZB42), and *B. oryzicola* KACC 18228 to be reclassified since the *B. velezensis* strain NRRL B-41580 was published earlier [9]. These problems are frequently met in GenBank sequence database during identification of bacteria species based on 16S rRNA, which might differ from the full genome sequence.

The other importance of characterizing strains of the same species is based on their variations due to abiotic stress and biotic population bringing about mutations in the strains. A study by Kaltz and co-workers statistically proved that evolutionary factors (mutation rate, selection) and ecological factors (abiotic, biotic) affected the variance of population density obeying Taylor's law [10]. An in-depth work on the phylogenomic interrelations, agricultural, industrial, and environmental applications of *B. velezensis* was well documented [11]. In their study, although the similarity of 17 *B. velezensis* was ≥98%, the NWUMFkBS10.5 strain had distinctive genes also found in several other unique strains.

In our work, a rhizobacterium strain of *Senna occidentalis*, a native weed plant to Africa, was identified as *B. velezensis* by 16S rRNA sequence; this was followed by a whole genome sequence analysis and comparison. The complete genome comparison was performed against five *B. velezensis* strains and two *B. amyloliquefaciens*, including genes responsible for secondary metabolites clusters, prophage regions, CRISPR–Cas system, antimicrobial resistance (ARM) genes, and insertion sequence (IS) elements. The strain was further characterized by enzymatic assay and antagonist activity against *Fusarium graminearum* and *Alternaria alternata*.

2. Materials and Methods

2.1. Genome Sequencing, Assembly, Genome Annotation and Gene Prediction

Total DNA was isolated from an overnight culture of *B. velezensis* KS04AU grown on Luria-Bertani (LB) broth (Tryptone, 10 g; Yeast Extract, 5 g; NaCl, 10 g) using TRIzol reagent (Thermo Fisher, Waltham, MA, USA) according to the manufacturer's protocol. The whole genome was sequenced by the Genotek company (Moscow, Russia) on the Illumina HiSeq 2500 with 2 × 125 bp paired-end reads. Sequencing quality was analyzed using FastQC (v. 0.11.2) [12]. To remove adapters and low-quality reads, sequencing reads were trimmed using Trimmomatic v. 0.36 [13]. The genome was de novo assembled using the Unicycler v. 0.5.0 [14]. The quality of the assembled genomes was evaluated using QUAST [15]. ANI (average nucleotide identity) was used to select the close-related reference strain by measuring nucleotide-level similarity between the coding regions of genomes. For this purpose, the 16S rRNA gene of *Bacillus velezensis* KS04AU (GenBank: MW350014.1) was blasted on NCBI BLAST. The first seven closest-related genome species of KS04AU with their respective accession numbers (Table 1) downloaded from NCBI genome database were used for the Pairwise Analysis of ANIb (average nucleotide identity based on BLAST) analysis. We included only full chromosome-level assembly genomes to minimize mismatch data which can induce uncompleted genomes. Pairwise ANIb was performed using the Web server program tool JSpeciesWS (https://jspecies.ribohost.com/jspeciesws/#home, accessed on 22 June 2022), where ANI values >95% were considered the threshold for species delimitation [16]. Mauve Contig Mover [17] was later used to reorder contigs based on comparison with the complete reference genome. Gaps within the scaffolds were filled and closed using GAPPadder, v. 1.10 [18].

Table 1. Genomes used in this study.

Microbial Strain	NCBI Reference Sequence
B. velezensis SRCM102752	NZ_CP028961.1
B. velezensis FZB42	NC_009725.2
B. velezensis JS25R	NZ_CP009679.1
B. velezensis KS04AU	NZ_CP092750.1
B. velezensis ONU-553	NZ_CP043416.1
B. amyloliquefaciens IT-45	NC_020272.1
B. amyloliquefaciens LL3	NC_017190.1

2.2. Genome Annotation, Gene Prediction and Comparative Genomic Analysis

The complete genome sequence analysis of strain KS04AU was performed to identify genes and sequence motifs of interest based on different databases. Genome annotation of *B. velezensis* strain KS04AU was carried out using Rapid prokaryotic genome annotation (Prokka) [19] and the Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) provided by NCBI [20]. GeneMarkS was used to predict the open reading frame [21]. The potential antibiotic resistance genes were identified based on a homology search using the Comprehensive Antibiotic Resistance Database (CARD) [22]. The Kyoto encyclopedia of genes and genomes (KEGG) database was also used to examine the high-level functionality of *B. velezensis* KS04AU. The presence of prophage was performed using the PHAge SearchTool (PHAST) according to Zhou et al. [23]. Secondary metabolite biosynthetic gene clusters (BGC) were analyzed using AntiSMASH 6.1.1 [24]. Circular genome of KS04AU was plotted using DNAplotter [25].

In order to compare the genomic diversity, relationships and biochemical diversity between *B. velezensis* KS04AU and its closely related species, we first constructed a phylogenetic tree using MEGA-11 analysis software [26] by the Maximum Likelihood (ML) method using the JTT matrix [27], to determine the distance between genomes. Pangenome analysis was performed using Roary (which uses the annotated assemblies produced by Prokka), while the identified genes were classified into core, shell, and cloud genes, in which core gene families refer to gene families that are more than 95% identical in 7 genomes; shell gene families refer to genes that are more than 95% identical in more than one genomic gene family, and cloud gene families refer to gene families only present in one genome of aligned genomes. For fully genomic comparison, several complementary approaches were also used. OrthoVenn2 [28] was used for whole genome comparison and annotation of orthologous clusters. The web tool CRISPR Finder was used to identify and compare the CRISPR–Cas systems in *Bacillus* genomes [29,30]. AntiSMASH was used for the secondary metabolite comparison gene cluster. ISfinder was used to explore bacterial insertion sequences among strains [31]. The PHAge SearchTool (PHAST) [23] was used for the genomic comparison of prophages. The potential antibiotic resistance genes were identified based on a homology search using the Comprehensive Antibiotic Resistance Database (CARD) [22]. RAST and SEED [32] servers were used to compare the subsystem distribution statistics among selected bacterial strains. The BLAST Ring Image Generator (BRIG) was used to visualize the comparison of the whole genome sequence.

2.3. Antagonistic and Hydrolytic Activities

2.3.1. Bacterial Suspension Preparation

The bacterial suspensions were obtained by centrifuging the bacterial cultures at 8000 rpm at 4 °C for 15 min of the strains (*B. velezensis* KS04AU, *P. putida* PLC1760, and *B. mojavensis* PS17) grown overnight at 190 rpm in LB broth. Furthermore, the pellets were rinsed three times with phosphate buffered saline (PBS) (VWR, Radnor, PA, USA) and diluted to an optical density of 0.1 at 595 nm.

2.3.2. Hydrolytic Activities

The ability of *B. velezensis* KS04AU to produce hydrolytic enzymes, such as amylase, protease, cellulase, lipase, and chitinase was tested by pipetting 10 µL of cell suspension into basal medium amended with 1% of starch, milk powder, sodium carboxymethyl cellulose (Na-CMC), tween-80, and colloidal chitin, respectively. After incubating at 30 °C for 4 days, for cellulase and chitinase activity, plates were stained with 0.2% Congo red solution for 15 min and destained with 1 N NaCl [33]. For amylase activity, plates were stained with Gram's iodine. Phytase activity was tested on phytate agar medium according to [34]. The appearance of hydrolysis or the formation of halo zones around colonies was considered as an enzymatic activity.

2.3.3. Antagonistic Activity

The ability of strain KS04AU to inhibit the growth of phytopathogenic fungi (*Forl* ZUM2407, *F. graminearum*, and *A. alternate*) and bacterium (*Pseudomonas syringae*) was tested using the confrontation assay in solid media in a Petri dish. For this purpose, phytopathogenic fungi were inoculated in the center of the plate and allowed to grow for one day. The bacterial strains were then co-inoculated on the same agar plate at a distance of 2.5 cm from the fungal strains. *P. putida* PLC1760 and *B mojavensis* PS17 were used as positive and negative controls, respectively. Antagonistic activity of KS04AU against *P. syringae* was assayed by spreading100 µL of overnight *P. syringae* culture with an optical density (595 nm) 0.5 on to LB agar medium using a plate spreader. Further, 5 µL of bacterial suspensions KS04AU, PS17 and PCL1760 were inoculated into the same plates. Plates were incubated at 28 °C for 3 days. The formation of halo zones around colonies was considered as antagonistic activity.

2.4. Biocontrol Ability of B. velezensis KS04AU to Suppress Tomato Foot and Root Rot

The biocontrol ability of *B. velezensis* KS04AU to suppress tomato foot and root rot caused by *Forl* ZUM2407 was performed under controlled laboratory conditions, in a pot (41 cm × 14 cm × 8 cm) containing a mineral wool presoaked with the mixture of plant nutrient solution (PNS[(PNS: 1.25 mM $Ca(NO_3)_2$; 1.25 mM KNO_3; 0.50 mM $MgSO_4$; 0.25 mM KH_2PO_4 and trace elements (0.75 mg/L KI; 3.00 mg/L H_3BO_3; 10.0 mg/L $MnSO_4.H_2O$; 2.0 mg/L $ZnSO_4.5H_2O$; 0.25 mg/L $Na_2MoO_4.2H_2O$; 0.025 mg/L $CuSO_4.5H_2O$; 0.025 mg/L $CoCl_2.6H_2O$)] with spores of *Forl* ZUM2407 to a final concentration of 1×10^3 spores/mL. The bacterial suspensions were prepared as described above with a little modification. In this case, bacterial suspensions were obtained by centrifuging overnight bacterial cultures of *B. velezensis* KS04AU and *P. putida* PLC1760 at 8000 rpm at 4 °C for 15 min. After rinsing three times with PBS, the obtained aliquots were diluted in 1% Na-CMC to an optical density of 0.3 at 595 nm. The tomato seeds were inoculated with the bacterial suspensions for 15 min, then dried in laminar hoods. Pots were incubated for up to 21 days after sowing, with a 16–8 h day–night light cycle, constant humidity and temperatures not exceeding 70% and 26 °C, respectively. In total, 65 plant seeds in each group were maintained for statistical viability of the experiment. The biocontrol ability of *B. velezensis* KS04AU in planta against *Forl* ZUM2407 was determined using a visual scoring of the intensity of disease development (DI) as follows:

$$DI = (n0 \times 0 + n1 \times 1 + n2 \times 2 + n3 \times 3 + n4 \times 4)/(n0 + n1 + n2 + n3 + n4)$$

in which n0, n1, n2, n3 and n4 are the number of plants with the indexes 0, 1, 2, 3 and 4, respectively.

Statistical analysis was performed using the statistical program package originLab pro SR1 b9.5.1.195 (OriginLab Corp., Northampton, Northampton, MA, USA). The significant difference between groups was analyzed using one-way ANOVA and post hoc Tukey's honestly significant difference test at $p < 0.05$.

3. Result

3.1. Genome Sequencing, Assembly and Comparison

The sequenced complete genome of *B. velezensis* KS04AU comprised a circular chromosome of 4,063,541 bp in length, with an average G + C content of 46.5%; it did not contain any plasmids (Table 2; Figure 1). The whole genome of KS04AU was predicted to contain a total of 4028 genes with 3941 potential CDS, 3860 of which being CDS-encoding (CDS with proteins). KS04AU contains 87 genes (RNA), 79 of which are transfer ribonucleic acid (tRNA), 5 are non-coding RNA (ncRNA), and 81 pseudogenes. The obtained genome assembly was submitted to the NCBI genome Refseq database under accession number: CP092750.1.

Table 2. Comparative genomic features of *B. velezensis* KS04AU with closest-related strain.

Features	KS04AU	SRCM102752	ONU-553	FZB42	JS25R	LL3	IT-45
Genome (bp)	4,063,541	3,971,509	3,934,563	3,918,596	4,006,002	3,995,227	3,928,857
G + C (%)	46.5	46.40	46.70	46.50	46.39	45.69	46.59
Genes (total)	4028	3950	3889	3870	3933	4151	3927
Total CDS	3941	3832	3771	3749	3826	4052	3797
CDS coding	3860	3761	3706	3676	3768	3943	3733
Genes (RNA)	87	118	118	121	107	99	130
tRNA	79	86	86	88	81	72	95
ncRNA	5	5	5	4	5	5	5
Pseudo Genes	81	71	65	73	58	109	64

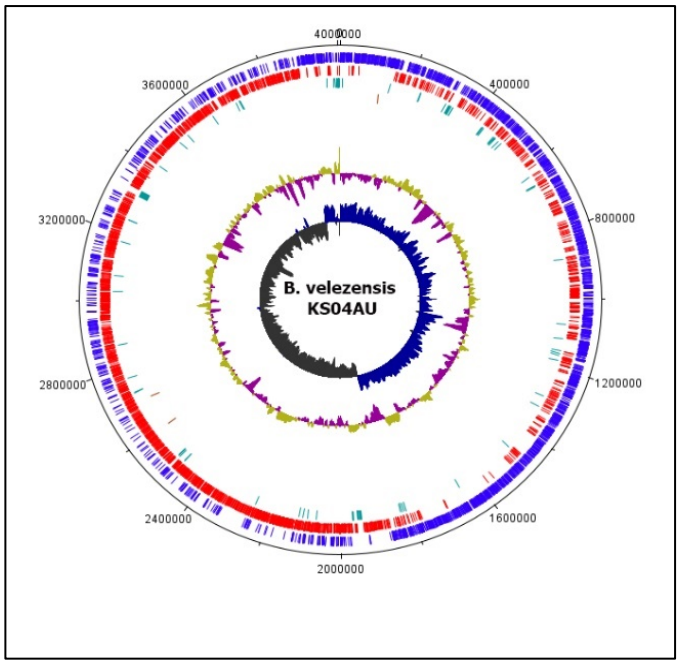

Figure 1. Draft genome of *B. velezensis* KS04AU. Blue circle—CDS on the plus strand, red circle—CDS on the minus strand, black circle is GC-skew plot (dark blue portion—GC-skew positive, olive-purple portion—GC-skew negative).

The Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) of the KS04AU genome revealed that 95.82% of CDS are assigned putative biological functions, while 4.18% are genes of hypothetical proteins with unknown functions. This compares with its related genomes SCRM102752, JS25R, FZB42, ONU553, LL3 and IT-45, which have, respectively, 95.29%, 95.80%, 94.98%, 98.27%, 94.98%, and 95.05% CDS associated with putative biological functions (Table 2). Among these strains, KS04AU was predicted to

have the largest genome size (Table 2), while their percentages of the total C+ G content in the total genomic nucleotides were approximately equal. Pseudogenes are more present in LL3 and less present in JS25R. Each genome contains more than 79 tRNA, except strain LL3 which carries 72 tRNA. Genes (RNA) are less predicted in KS04AU, compared with other genomes. Among the content of ncRNA (noncoding RNAs), genome FZB42 was predicted to have a smaller number compared with other genomic assemblies (Table 2).

The phylogenetic tree relationship based on the 16S rRNA of *B. velezensis* KS04AU with other species is represented in Figure 2. The result shows that the 16S rRNA of strain KS04AU is 98.84% (maximal score) identical to *B. amyloliquefaciens* (GenBank accession no. KU161297.1) and 98.90% (percent identity) to *B. subtilis* (GenBank accession no. EU489517.1) when blasted on NCBI Blast.

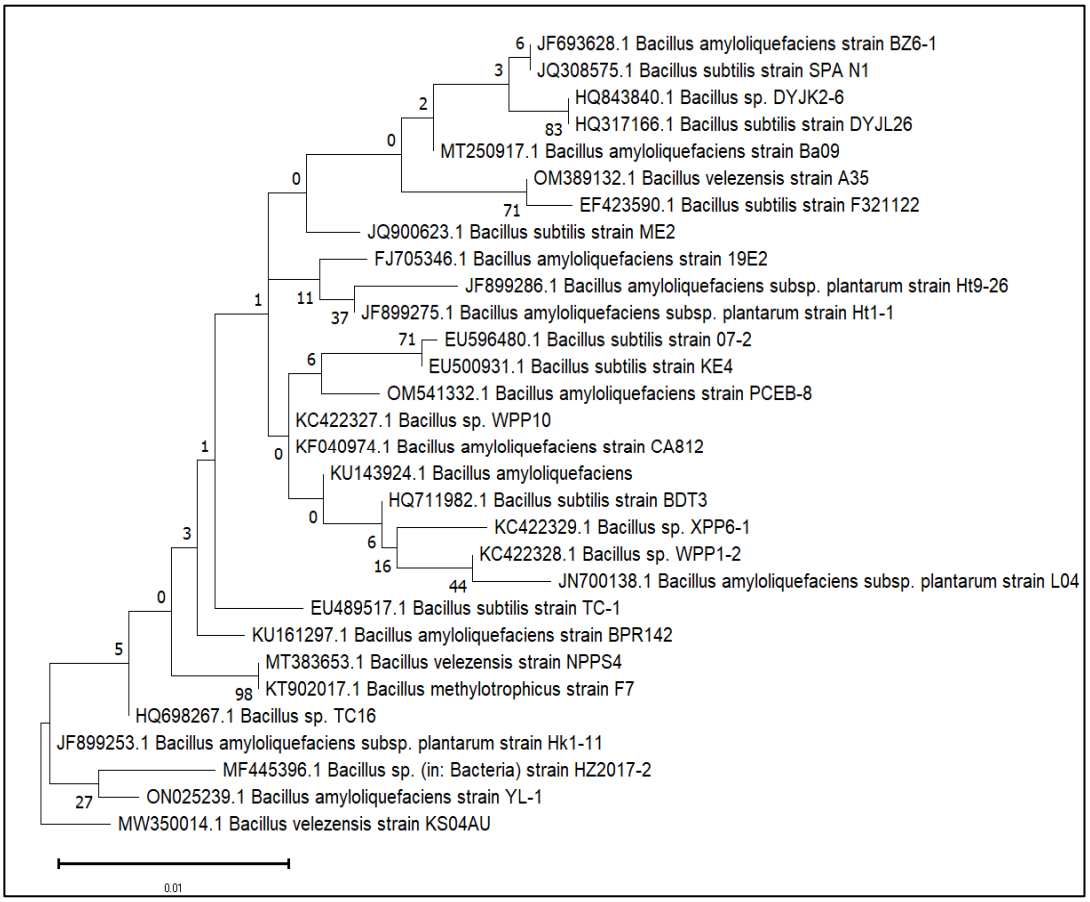

Figure 2. The phylogenetic analysis based on the 16S rRNA genes of strain KS04AU based on NCBI blast System. The tree was generated by MEGA 11 using the neighbor-joining method.

ANI nucleotide analysis revealed that the genome KS04AU is closely related to *B. velezensis* ONU-553 (Table 3; Figures 3 and 4), with an average nucleotide identity of 99.65% and an average aligned nucleotide of 97.83%, compared to *B. velezensis* strains JS25R, FZB42, ONU-553, SRCM102752, and *B. amyloliquefaciens* strains LL3 and IT-45, whose average nucleotide identity and average aligned nucleotide were less than 99.0% (Table 3). In addition, the results showed that *B. amyloliquefaciens* LL3 is not closely related to the other six strains, since its similarity with other strains was less than 94%.

Table 3. Genomic comparative analysis of *B. velezensis* KS04AU with 6 closest related *Bacillus* species based on ANI (average nucleotide identity).

	***B. velezensis* KS04AU**	***B. velezensis* JS25R**	***B. velezensis* FZB42**	***B. velezensis* ONU-553**	***B. velezensis* SRCM102752**	***B. amyloliquefaciens* LL3**	***B. amyloliquefaciens* IT-45**
B. velezensis KS04AU	—	**98.19** *(91.60)*	**98.66** *(91.09)*	**99.53** *(95.32)*	**98.31** *(90.54)*	**93.29** *(86.11)*	**97.38** *(90.99)*
B. velezensis JS25R	**98.20** *(92.47)*	—	**98.19** *(92.03)*	**98.20** *(92.58)*	**97.88** *(91.14)*	**93.34** *(86.41)*	**97.47** *(92.00)*
B. velezensis FZB42	**98.76** *(93.84)*	**98.26** *(94.17)*	—	**98.77** *(94.59)*	**98.62** *(93.57)*	**93.30** *(87.70)*	**97.51** *(93.32)*
B. velezensis ONU-553	**99.65** *(97.83)*	**98.31** *(94.21)*	**98.78** *(94.25)*	—	**98.44** *(93.53)*	**93.40** *(88.43)*	**97.53** *(94.09)*
B. velezensis SRCM102752	**98.34** *(92.08)*	**97.80** *(92.01)*	**98.56** *(92.41)*	**98.39** *(92.62)*	—	**93.32** *(87.67)*	**97.18** *(91.83)*
B. amyloliquefaciens LL3	**93.78** *(86.01)*	**93.77** *(85.59)*	**93.74** *(84.70)*	**93.82** *(86.01)*	**93.80** *(86.04)*	—	**93.65** *(85.92)*
B. amyloliquefaciens IT-45.	**97.59** *(93.22)*	**97.67** *(93.34)*	**97.60** *(92.86)*	**97.62** *(93.94)*	**97.40** *(92.58)*	**93.31** *(88.40)*	—

N.B. Data in bold—average nucleotide based on blast (ANib); data in italics—average aligned nucleotide.

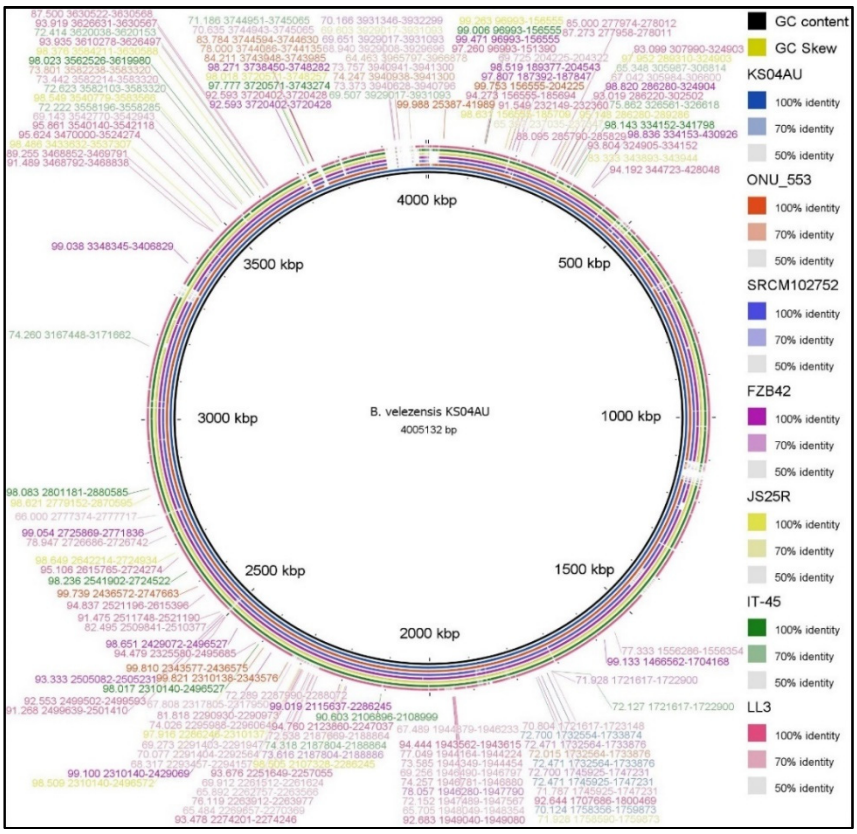

Figure 3. Blast Ring Image Generator (BRIG) plot showing a whole genome comparison. The figure shows BLAST comparisons of *B. velezensis* strains KS04AU, ONU-553, FZB42, JS25R, SRCM102752 and *B. amyloliquefaciens* strains LL3 and IT-45.

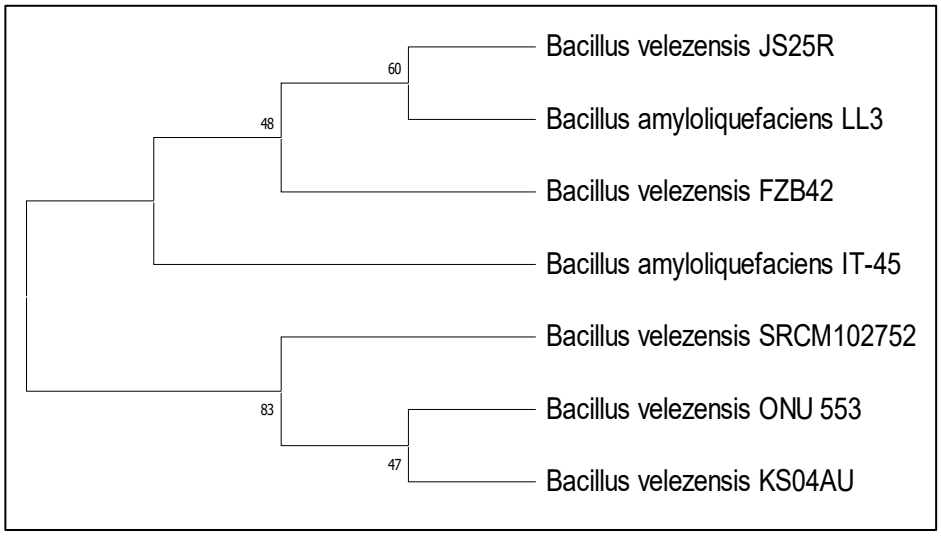

Figure 4. Neighbor-joining phylogenetic tree constructed from 16S rRNA extracted from genomes using the ContEst16S tool. The tree was generated by MEGA 11 using the neighbor-joining method.

RAST, the analysis of subsystem distribution followed by comparison among genomes, revealed in the *B. velezensis* KS04AU genome the presence of 44 genes associated with the control of bacterial mobility and Chemotaxis; 81 genes associated with the cell wall and capsule; 36 genes responsible for virulence, diseases and defense; and 215 for carbohydrates (Figure 5). The KS04AU strain also encodes numerous pathways that are related with the utilization of plant-derived molecules, the production of enzymes, and plant-growth substances. Genes responsible for xenobiotic degradation, such as membrane transport and signal transduction, terpenoids and polyketide metabolism, carbohydrate, lipid, and amino acid metabolic functional genes, translation and metabolism of cofactors such as Fe, P, vitamins, and cell motility were also found in *B. velezensis* KS04AU.

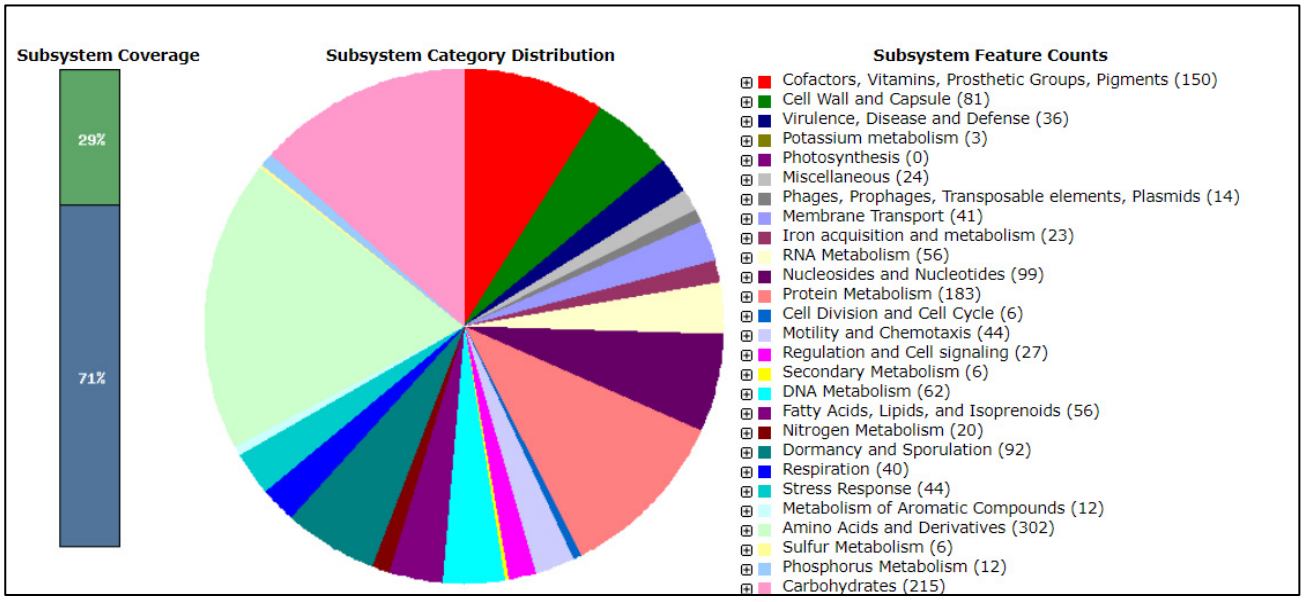

Figure 5. Subsystem Information for *B. velezensis* KS04AU. In subsystem coverage, 29% is indicated in subsystem coverage with a total of 1180 genes (1125 non-hypotheticals and 55 hypotheticals) and 71% is not indicated in subsystem coverage with a total of 2961 genes (1508 non-hypotheticals and 1453 hypotheticals).

Identical subsystem features show the presence of all genes, with the exception of FZB42, without phages, prophages, transposable elements, and plasmid genes. However, a difference was observed in the number of genes contained in each subsystem (Figure 6). For example, compared with KS04AU, strain ONU-553 and SCRM102752 have 77 genes involved, respectively, in cell and capsules; 43 and 42 genes associated with control of bacterial mobility and Chemotaxis; 36 and 39 in genes in virulence, diseases and defense; and 215 and 212, respectively, involved in carbohydrates (Table S1).

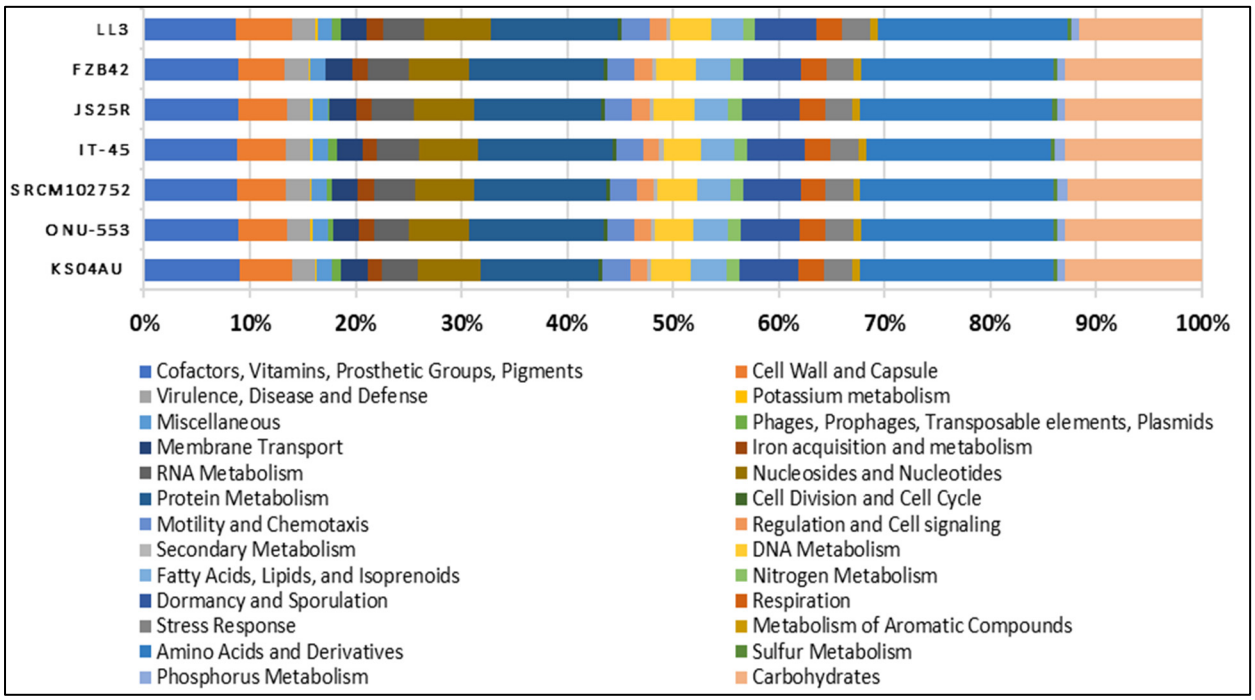

Figure 6. Comparison of the functional subsystem category in genomes of *B. velezensis*, SCRM102752 KS04AU, FZB42, ONU553, KS04AU, and *B. amyloliquefaciens* LL3 and IT-45 based on SEED servers. Functional classification is based on annotated and assigned roles of genes using RASTtk. Each color bar shows genes involved in a specific subsystem category.

The PHASTER analysis of the *B. velezensis* KS04AU genome revealed the presence of four phages in its genome (Table 4 and Figure S1). Two phages were scored as intact (score > 90), one as incomplete (score < 90), and one as questionable (score = 70–90). These two intact prophages (2 and 4) were predicted as phage *Aeribacllus* AP45 NC_048651 and phage *Paenibacillus*_Tripp NC_028930, respectively. The incomplete phage was predicted to be *Bacillus* spp. NC_004166 and the questionable phage *Brevibacillus Jimmer* NC_041976.

The prophage comparative analysis is presented in Table 5. As can be observed, an incomplete phage (*Brevibacillus Jimmer* NC_041976) was found in the genomes of KS04AU, ONU-553 and SCRM102752. Among prophages (PHAGE_Bacill_phi105_NC_004167, PHAGE_Brevib_Osiris_NC_028969, and PHAGE_Thermu_OH2_NC_021784) present in LL3, only PHAGE_Brevib_Osiris_NC_028969 was predicted in the JS25R strain, and absent in strains SCRM102752 KS04AU, FZB42, ONU553, KS04AU, and IT-45. PHAGE_Bacill_SPP1_-NC_004166 is present only in the KS04AU and ONU 553 strains. PHAGE_Aeriba_AP45_NC-_048651 and PHAGE_Paenib_Tripp_NC_028930 are present only in KS04AU.

Table 4. Prophage regions found in *Bacillus velezensis* KS04AU genome.

Region	Region Length	Completeness	Phage Hit Protein	Hypothetical Protein	Specific Keyword	Region Position	Possible Phage	G + C Percentage
1	18.1 Kb	Incomplete (10)	13	5	NA	3336–21,513	PHAGE_Bacill-_SPP1_NC_004166	44.55%
2	49.1 Kb	intact (120)	41	31	integrase, terminase, tail	1,107,820–1,157,010	PHAGE_Aeriba-_AP45_NC_048651	41.77%
3	31.3 Kb	questionable	29	16	tail, plate, capsid	1,203,112–1,234,419	PHAGE_Brevib-_Jimmer2_NC_041976	46.98%
4	97.5 Kb	intact	61	41	integrase, tail, terminase, capsid	3,892,492–3,990,010	PHAGE_Paenib-_Tripp_NC_028930	47.43%

Table 5. Comparison of prophages found in selected genomes.

Phage	Presence (+) or Absence (−) in Related Strains						
	KS04AU	SRCM102752	ONU 553	FZB42	JS25R	LL3	IT-45
PHAGE_Aeriba-_AP45_NC_048651	+	−	−	−	−	−	−
PHAGE_Brevib-_Jimmer2_NC_041976	+	+	+	−	−	−	−
PHAGE_Paenib-_Tripp_NC_028930	+	−	−	−	−	−	−
PHAGE_Bacill-_SPP1_NC_004166	+	−	+	−	−	−	−
PHAGE_Thermu-_OH2_NC_021784	−	−	−	−	−	+	−
PHAGE_Thermu-_TMA_NC_015937	−	−	−	−	+	−	−
PHAGE_Brevib-_Osiris_NC_028969	−	−	−	−	+	+	−
PHAGE_Bacill-_phi105_NC_004167	−	−	−	−	−	+	−

The prediction of gene clusters involved in synthesizing polyketides and bacteriocins using antiSMASH showed that strain KS04AU possesses 13 gene clusters (Table 6). A comparison to the majority of known gene clusters revealed that these three gene clusters are involved in NRPS (Non-Ribosomal Peptide Synthetase), three transATPKS (trans-Acyl Transferase Polyketide Synthetase), two terpenes, one lantipeptide, two T3PKS, one other KS, and one lantipeptide class-II. Eight clusters were clearly identified as being involved in the synthesis of surfactin, macrolactin, bacillaene, fengycin, difficidin, bacilysin, bacillibactin (siderophore), and mersacidin. This analysis revealed the presence of gene clusters in *B. velenzensis* KS04AU responsible for the biosynthesis of antimicrobial compounds, regulation, and transport of mineral elements. However, four biosynthetic gene clusters (two terpene, one T3PKS, and one NRPS) failed to match pathways for most known secondary metabolites (Table 6).

Table 6. Secondary metabolite biosynthetic gene clusters into genomic regions of *B. velezensis* KS04AU.

Genomic Region	Type	From	To	Most Similar Known Cluster		Similarity
Region 1	NRPS	297,001	359,149	surfactin	NRP: Lipopeptide	95%
Region 2	PKS-like	881,875	923,119	butirosin A/butirosin B	Saccharide	7%
Region 3	terpene	1,009,298	1,026,466			
Region 4	transAT-PKS	1,379,829	1,467,645	macrolactin H	Polyketide	100%
Region 5	transAT-PKS, T3PKS, NRPS	1,689,828	1,790,022	bacillaene	Polyketide + NRP	100%
Region 6	NRPS, transAT-PKS, betalactone	1,856,677	1,988,381	fengycin	NRP	100%
Region 7	terpene	2,011,406	2,033,289			
Region 8	T3PKS	2,083,724	2,124,824			
Region 9	transAT-PKS	2,252,798	2,344,192	difficidin	Polyketide + NRP	100%
Region 10	NRPS, RiPP-like	2,955,287	3,005,799	bacillibactin	NRP	100%
Region 11	NRPS	3,284,182	3,330,146			
Region 12	other	3,550,785	3,592,203	bacilysin	Other	100%
Region 13	lanthipeptide-class-ii	3,740,316	3,763,504	mersacidin	RiPP: Lanthipeptide	100%

Comparative analysis shows that twelve regions are present in *B. Velezensis* SRCM10-2752, *B. amyloliquefaciens* IT-45, and *Bacillus velezensis* ONU-553; ten regions in *B. amyloliquefaciens* LL3; thirteen regions in *B. velezensis* FZB42, *B. velezensis* JS25R, and *B velezensis* KS04AU; four regions with non-encoding synthesis metabolites (two regions of terpene, one T3PKS, and NRPS) are present in *B. velezensis* KS04AU and *B. velezensis* FZB42; four regions (two terpenes, T3PKS and lanthipeptide-class-II) in *B.amyloliquefaciens* LL3 and IT-45; and three regions (2 terpene regions and T3PKS) in genomic strains ONU-553, SRCM102752, and JS25R. As shown in (Table 7), the cluster precursor peptide recognition element (RRE) containing LAP responsible for the synthesis of plantazoticin present in *B. velezensis* FZB42 was absent in KS04AU, SRCM102752, ONU553, JS25R, LL3 and IT-45. The NRPS, transAT-PKS gene cluster responsible for the synthesis of rhizoctocin A present in the SCRM102752 genome was absent in KS04AU, FZB42, ONU553, JS25R, LL3 and IT-45. The gene cluster responsible for synthesis kijanimicin (with 4% of similarity) present in genome SCRM102752 is absent in the other genomes. The class II cluster lanthopeptide responsible of synthesis of meracidin was present only in the genome KS04AU. The transAT-PKS, T3PKS, NRPS gene clusters responsible of synthesis of macrolactin H are absent only in genome strains SCRM102752 and LL3. Gene clusters transAT-PKS and NRPS responsible of synthesis of fengycin and difficidin are not present in the two genomes of *Bacillus amyloliquefaciens.*

Table 7. Comparative analysis of secondary metabolite clusters of *B. velezensis* KS04AU with closest-related strains.

		Presence (+) or Absence (−) of Secondary Metabolite Clusters in Related Strains						
Synthetase	**Metabolites**	**KS04AU**	**SRCM102752**	**ONU-553**	**FZB42**	**JS25R**	**LL3**	**IT-45**
PKS-like	surfactin	+	+	+	+	+	+	+
terpene	−	+	+	+	+	+	+	+
transAT-PKS	butirosin A/butirosin B	+	+	+	+	+	+	+
transAT-PKS, T3PKS, NRPS	macrolactin H	+	−	+	+	+	−	+
NRPS, transAT-PKS, betalactone	bacillaene	+	+	+	+	+	+	+
terpene	−	+	+	+	+	+	+	+
T3PKS	−	+	+	+	+	+		
transAT-PKS	fengycin	+	+	+	+	+		
NRPS, RiPP-like	−	+	+	+	+	+	+	+
NRPS	difficidin	+	+	+	+	+		
NRPS, RiPP-like	bacillibactin	+	+	+	+	+	+	+
NRPS		+	+	+	+	+		
other	bacilysin	+	+	+	+	+	+	+
lanthipeptide-class-ii	mersacidin	+	−	−	−	−	−	−
cyclic-lactone-autoinducer, lanthipeptide-class-II	kijanimicin	−	+	−	−	−	−	−
NRPS, transAT-PKS	rhizocticin A	−	+	−	−	−	−	−
RRE-containing, LAP	plantazolicin	−	−	−	+	−	−	−

The results obtained by comparison to the ARM gene family (Table 8) show the similarity of the AMR genes. All genomes have the anti-microbe resistance gene family *Cfr* gene of 23 S ribosomal RNA methyl transferase (*clbA*), which provides the resistance to antibiotic binding to the ribosomal peptidyl transferase center on the ribosome, reflecting to many drug classes; *tet (45)* major facilitator superfamily (MFS) antibiotic efflux pump which provides resistance to tetracycline antibiotic; and three small multidrug resistance (SMR) (two gacJ and one gacG) providing bacterial resistance to disinfecting and antiseptic agents.

Table 8. Comparison of *B. velezensis* KS04AU ARM genes with closest-related strains.

ARO Term ARM	**Gene Family**	**Drug Class**	**Resistance Mechanism**	**Presence (+) or Absence (−)**						
				KS04AU	**SRCM102752**	**ONU 553**	**FZB42**	**JS25R**	**LL3**	**IT-45**
clbA	Cfr 23S ribosomal RNA methyltransferase	Incosamide antibiotic, streptogramin antibiotic, streptogramin A antibiotic, oxazolidinone antibiotic, phenicol antibiotic, pleuromutilin antibiotic	Antibiotic target alteration	+	+	+	+	+	+	+
tet (45)	Major facilitator superfamily (MFS) antibiotic efflux pump	Tetracycline antibiotic	Antibiotic efflux	+	+	+	+	+	+	+
qacJ	small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agents and antiseptics	Antibiotic efflux	+	+	+	+	+	+	+
qacG	small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agents and antiseptics	Antibiotic efflux	+	+	+	+	+	+	+
qacJ	small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agents and antiseptics	Antibiotic efflux	+	+	+	+	+	+	+

Table 8. *Cont.*

ARO Term ARM	Gene Family	Drug Class	Resistance Mechanism	Presence (+) or Absence (−)						
				KS04AU	SRCM102752	ONU 553	FZB42	JS25R	LL3	IT-45
qacJ	small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agents and antiseptics	Antibiotic efflux	−	−	−	−	−	−	+
BcI	class A Bacillus cereus Bc beta-lactamase	cephalosporin, penem	antibiotic inactivation	+	+	+	+	+	+	+

The comparison of the CRISPR/Cas systems is shown in Table 9. Results revealed that among these strains, CRISPR/Cas Systems are only absent in the LL3 and FZB42 strains. ONU-553, JS25R, and SRCM102752 each carry a single CRISPR element with a different Cas gene number and direction (positive and negative-sense). The Cas genes present in ONU-553 and SRCM102752 have the same direction and repeating consensus, while JS25R carries a unique repeating consensus and 13 Cas genes (where six have a positive sense and seven have a negative sense). KS04AU and IT-45 contain two CRISPR elements with 12 and 13 Cas genes, respectively. The Cas genes present in these genomes are located in different directions. The repeated consensus present in KS04AU is identical to the consensus present in SRCM102752 and ONU 553. The CRISPR/Cas systems present in all genomes are located in different positions (Table 9). Furthermore, a supplement element CAS-TypeID present in strains IT-45 and JS25R, which are located at these positions 1779683–1781929 and 2171815–217406, respectively, is absent in genomes KS04AU, FZB42, ONU-553, SRCM102752, and LL3.

Table 9. Comparison of the CRISPR elements in *Bacillus velezensis* and *amyloliquefaciens* strains.

Strain	Number of CRISPR/CAS	Element	Start	End	Spacer/Gene	Repeat Consensus/Cas Genes	Direction
KS04AU	**2**	**Cas**	66,995	3,697,966	12	Cas3_TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI	(–)—8 Cas genes (+)—4 Cas genes
		CRISPR	665,256	665,363	1	CGGAGGATATCCGGGATACGGTTT	ND
		CRISPR	712,560	712,654	1	TTCACCGGGGCAACGGGGCTGAC	ND
SRCM102752	**1**	**CAS**	61,088	3,747,587	12	Cas3_TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI	(–)—8 Cas genes (+)—4 Cas genes
		CRISPR	780,220	780,314	1	TTCACCGGGGCAACGGGGCTGAC	ND
ONU 553	**1**	**CAS**	61,088	3,747,587	12	Cas3_TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI	(–)—8 Cas genes (+)—4 Cas genes
		CRISPR				TTCACCGGGGCAACGGGGCTGAC	ND
FZB42	**0**						
JS25R	**1**	**CAS**	61,500	3,812,920	13	Cas3_TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI	(–)—7 Cas genes (+)—6 Cas genes
		CRISPR	447,873	447,955	1	AAGAAATCGGCCAAAAAGGCGGA	ND
		CAS-TypeID	2,171,815	2,174,061	1	cas3_TypeID	-
LL3	**0**						
IT-45	**2**	**CAS**			13	Cas3_TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ TypeI, Cas3_ Type	(–)—6 Cas genes (+)—7 Cas genes
		CRISPR	2,680,276	2,680,402	**1**	TGCTCGCAATCTCGTCCGCTTTT-CCCATGAATGAGGTCGTGAACTT	ND
		CRISPR	3,044,191	3,044,320	**1**	AACAGGCTTTCAGCGGGGAATC-CGGCGGACAGCAGCA	ND
		CAS-TypeID	1,779,683	1,781,929	**1**	cas3_TypeID	

To compare mobile genetic elements in genomes, we used the results obtained after performing ANI analysis. *B. amyloliquefaciens* strains were then excluded, since the ANIb for the pairwise comparison of these genomes against each of the five genomes of *B. velezensis* strain was less than 98.00%. Analysis of mobile genetic elements in genomes *B. velezensis* KS04AU, JS25R, FZB42, ONU-553, and SRCM102752 shows the number of IS-elements—47, 39, 53, 47, and 60, respectively (Table S2). Among these genomes, IS-elements were more present in SRCM102752. The comparative analysis revealed that all genomes shared 22 IS elements (Figure 7). Two IS elements (ISMetp1 and ISIlo12) are only found in KS04AU; ten IS elements (ISIse1, ISChh1, ISDpr8, ISOba2, ISAeme4, ISCysp21, ISGob7, IS231W, IS231V, IS231K) in SRCM102752; eight (ISBth19, ISPa72, ISDph1, ISShes11, ISSso4, ISM1, IS1221I, IS1221G) in FZB42; five (ISAur1, ISCosp2, ISMlo5, ISCth11, ISNg1) in JS25R; and ISSpo1 IS-element in ONU-553. Strains SRCM102752 and FZB42 shared thirteen (ISBce5, ISBce7, ISBth4, IS231Y, ISBce8, MICBce5, MICBce6, MICBth1, ISBce2, MICBce2, IS231D, ISBce4, ISOih1) IS-Elements; KS04AU and ONU-553 shared five (ISDpr6, ISDpr5, ISFnu8, ISAba5, IS1182) IS-Elements; ONU-553 and FZB42 shared one (ISRba1) IS-Elements; four IS-elements (ISPlu5, ISSsu4, ISAau4, ISRru1) are shared between ONU-553 and JS25R.

The full spectrum of the pan-genome (based on Roary analysis) contained 4771 protein-coding genes. Among these, 3334 genes are present in all seven strains (core genes), and the remaining 1437 genes belong to shell genes. Cloud and soft-core genes were not found. Analysis of orthologous gene clusters using OrthoVenn2 revealed that a core of 3371 orthologous genes is shared among seven genomes (Figure 8 and Table 10). In addition, 158, 33, 82, 137, 126, and 100 singleton gene clusters are present in KS04AU, ONU-553, FZB42, JS25R, SRCM102752, IT-45, and IT-45 (Table 10). No unique orthologue gene cluster was found in seven genomes, whereas the genomes of strains KS04AU, JS25R, FZB42, ONU-553, and SRCM102752 contained 26, 10, 7, 3, and 10 unique genes, respectively (Figure 8A). The strain KS04AU shared 2 homologous gene clusters with SRCM102752, 60 with ONU-553, 30 with JS25R, and 27 homologous gene clusters with IT-45 (Figure 8B).

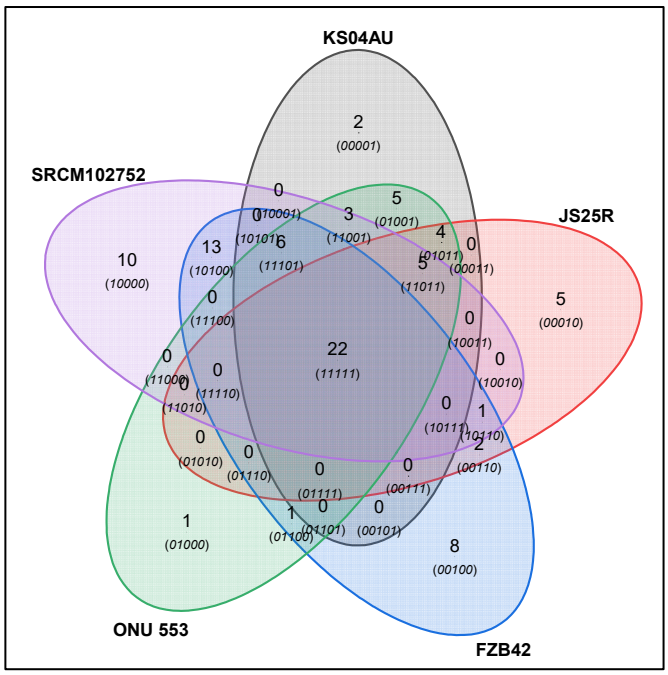

Figure 7. Comparative analysis of mobile genetic elements (IS-Elements) of genome *B. velezensis* strains KS04AU, JS25R, FZB42, ONU-553, and SRCM102752. Data in italics represent the group number with IS-elements shared between genomes (Table S3).

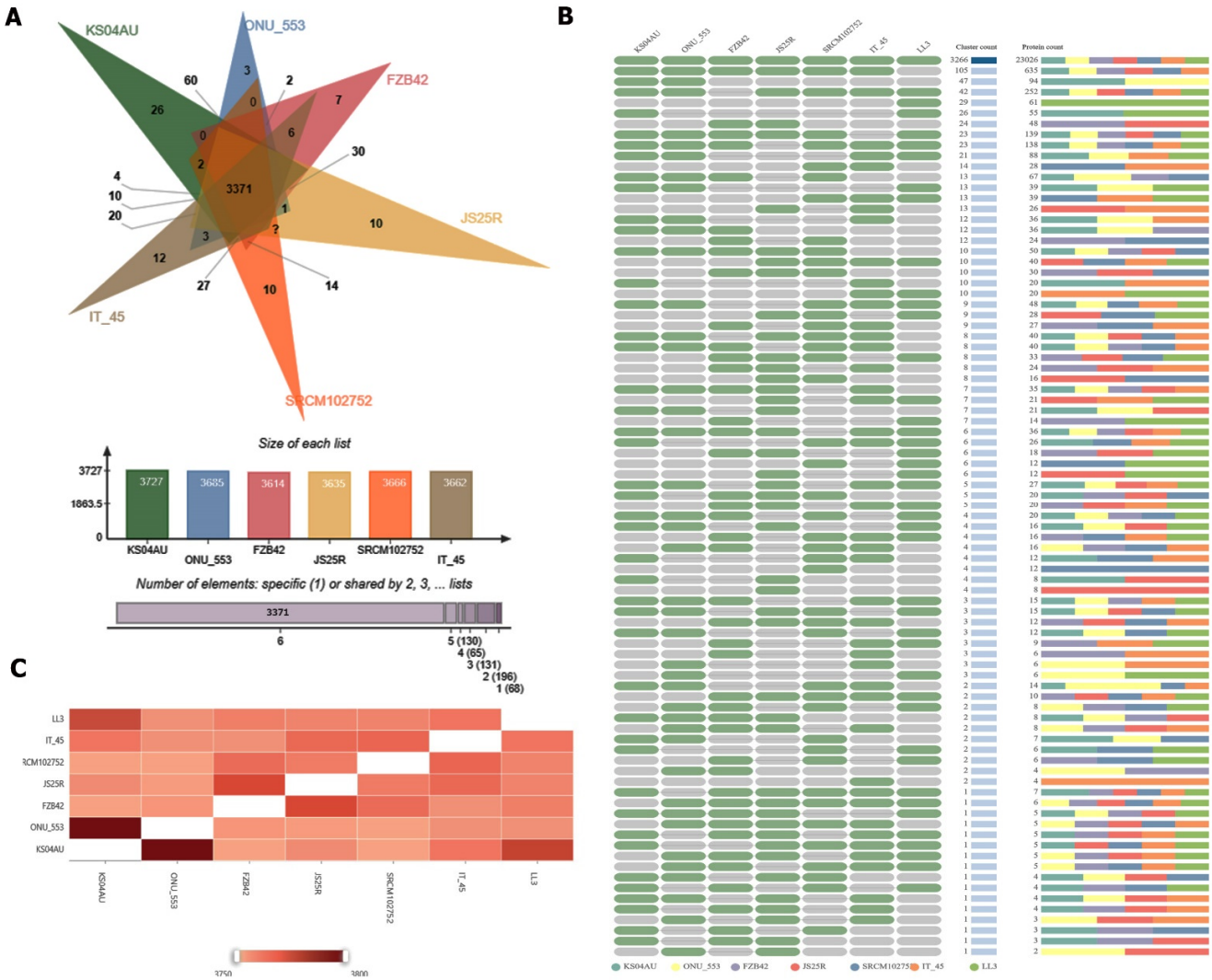

Figure 8. Genomic analysis using Orthovenn2. Venn diagram (**A**) displays the distribution of shared orthologous clusters among *B. velezensis* and *amyloliquefaciens* strains. (**B**) The occurrence table shows the occurrence pattern of shared orthologous groups among selected strains. (**C**) Pairwise heatmap of overlapping cluster numbers of compared genomes. Each cell shows the overlap cluster between species. The overlapping cluster numbers refer to the number of gene clusters shared among *B. velezensis* and *amyloliquefaciens* strains.

Table 10. Comparative analysis of gene clusters using OrthoVenn2.

Species	Gene Clusters	Singletons
KS04AU	3727	158
ONU-553	3685	33
FZB42	3614	82
JS25R	3635	137
SRCM102752	3666	126
IT-45	3662	100

3.2. Antagonistic and Hydrolytic Activities

The antagonistic activity of *B. velezensis* KS04AU against phytopathogenic fungi are presented in Figure 9. The results after incubation showed inhibition effects of KS04AU on the growth of all phytopathogenic fungi tested in this study, since the zones of inhibition were observed against *A. alternata*, *F. graminarium*, *F. oxysporum*, and *P. syringae* (Figure 9),

compared with the positive and negative control strains, *B. mojavensis* PS17 and *P. putida* PCL1760. The ability of *B. velezensis* KS04AU to produce hydrolytic enzymes is represented in Figure 9b. The results obtained show the lipase, chitinase, protease, cellulase, amylase, and phytase activity of KS04AU (Figure 10).

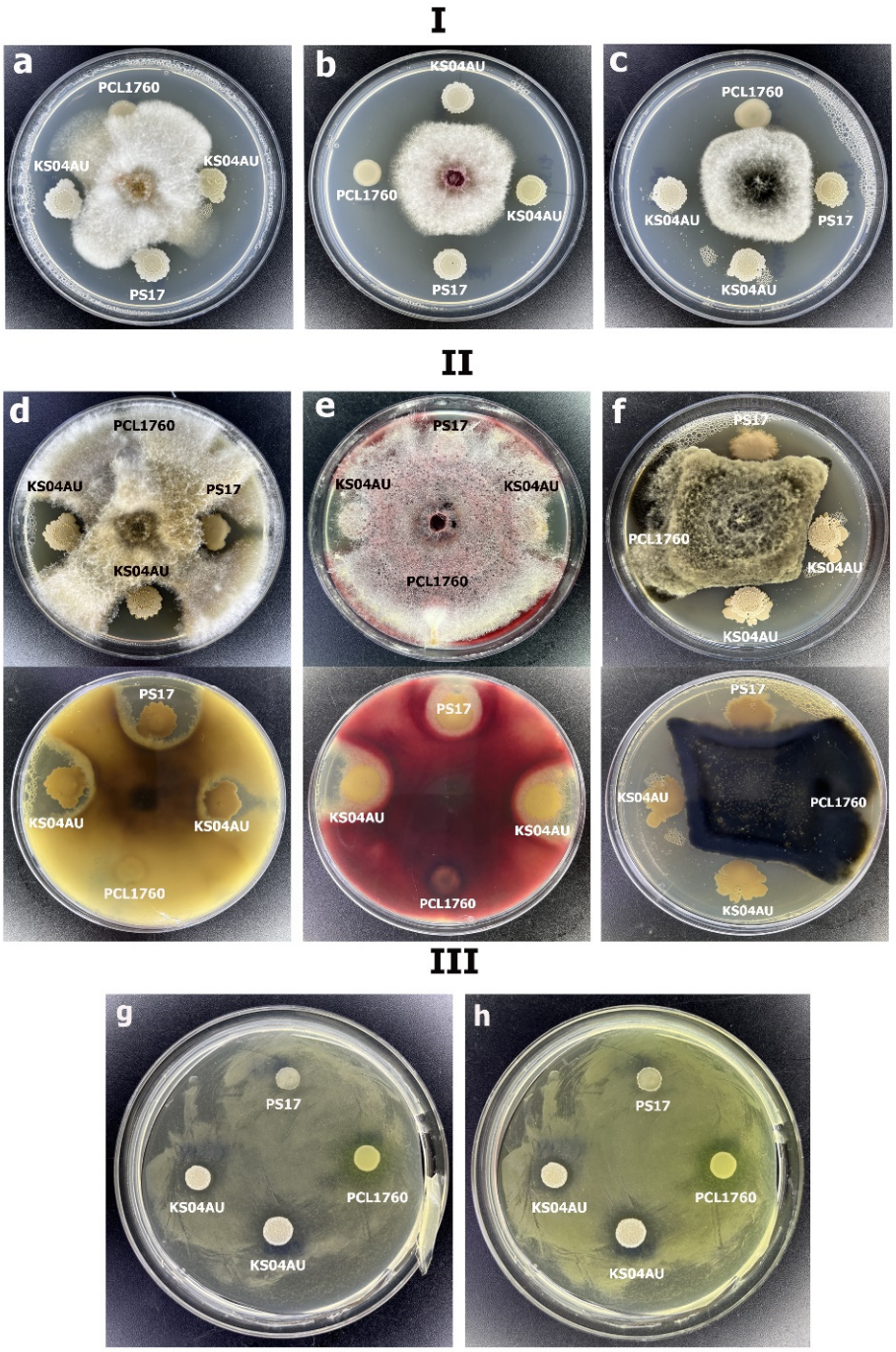

Figure 9. The antagonistic activity of *B. velezensis* KS04AU against phytopathogenic fungi *F. oxysporium* (**a**,**d**), *F. graminarium* (**b**,**e**), *A. alternata* (**c**,**f**) after 5 days (**I**) and 14 days (**II**) of incubation. The antagonistic activity of *B. velezensis* KS04AU against phytopathogenic bacteria *P. syringae* (**III**) after 1 day (**g**) and 2 days (**h**) of incubation.

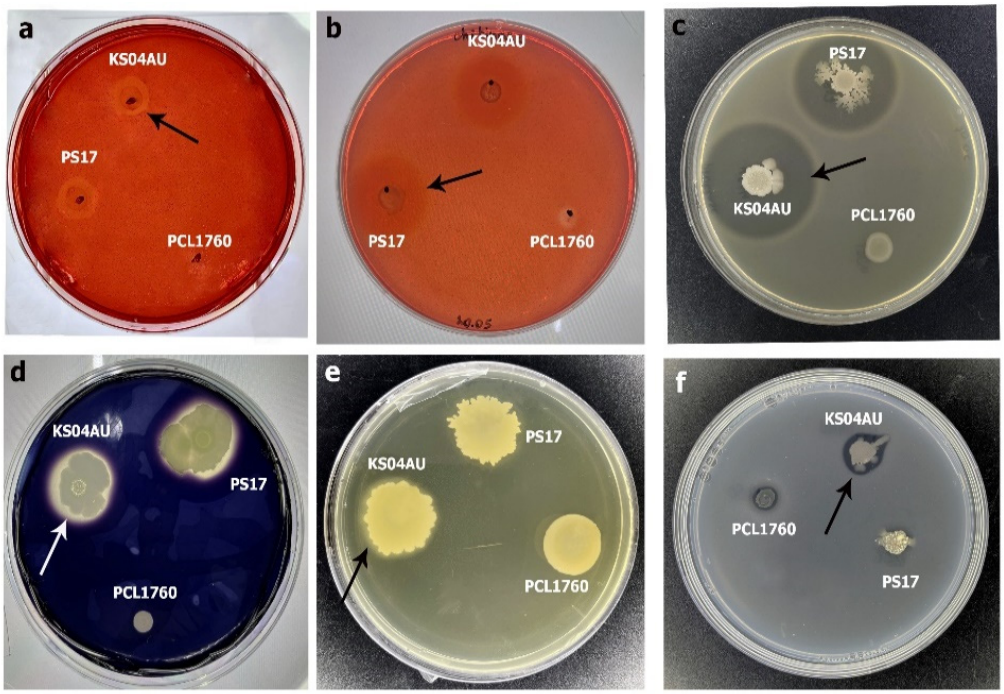

Figure 10. Hydrolytic enzymatic activity of *B. velezensis* KS04AU with arrows indicating enzymatic zone of activity. Plates showing cellulase (**a**), chitinase (**b**), protease (**c**), amylase (**d**), lipase (**e**), and phytase (**f**).

3.3. Biocontrol Ability of B. velezensis KS04AU to Suppress Tomato Foot and Root Rot

The ability of *B. velezensis* KS04AU to inhibit the growth of root disease cause by *Forl* is shown in Figure 11. After 21 days of incubation, the disease index in the group of plants treated with *B. velezensis* KS04AU was statistically lower compared with the control: *B. velezensis* KS04AU (disease index of 0.61 ± 0.12188) in comparison with control without treatment (disease index of 1.13 ± 0.0839) and *P. putida* PCL1760 with (disease index of 0.50 ± 0.13541), respectively. More importantly, compared to the well-known biocontrol *P. putida* PCL1760, no statistical difference ($p < 0.05$) was observed in terms of its biocontrol ability in tomato plants against *Forl* ZUM2407.

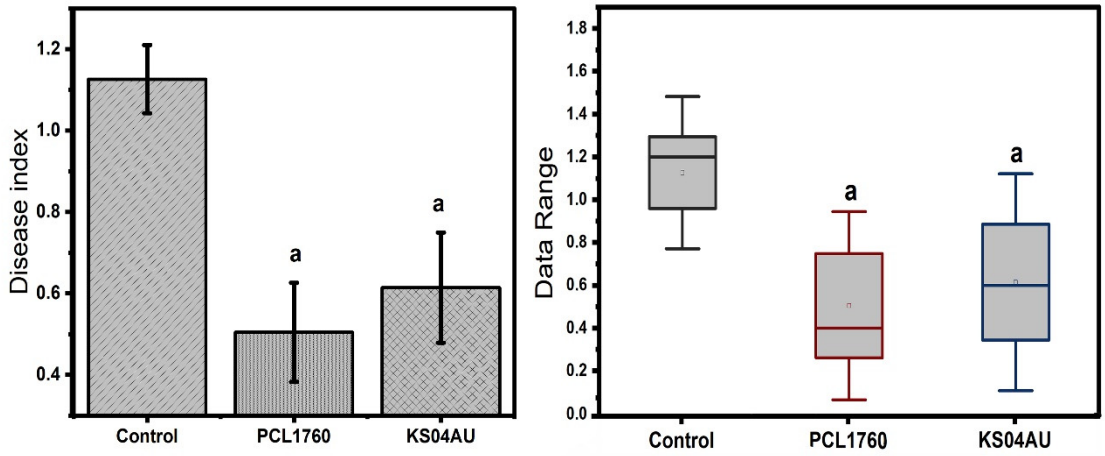

Figure 11. Biocontrol ability of *B. velezensis* KS04AU in suppression of tomato root rot disease diseases caused by *Forl* ZUM2407. Different letters above the bars indicate a statistically significant difference between groups at $p < 0.05$.

4. Discussion

Bacillus velezensis strains received much attention in the past two decades for their enzymatic properties and their wide spectrum range of antagonistic activity against phytopathogens, including *F. oxysporum*, *F. graminearum*, *Botrytis cinerea*, *A. alternata*, *Fulvia fulva*, and *Ustilaginoidea virens* [35,36]. Since microbial strain activities are normally regulated by the substances produced by other microbes in their community (biotic) and the environmental conditions (abiotic), there is a constant mutation in their genomes. These mutations include deletions, insertions, and translocations by mobile elements contributing to the unique features of a strain [37]. Although the phylogenetic tree based on the 16S rRNA genes showed that KS04AU is 98.90% identical to *B. subtilis*, a similar report showed that *B. methylotrophicus* KACC 13105 is closely related to *B. subtilis* [38].

A work published by Borris et al. [39] explains that neither the minimal description of new taxa based on phenotypic characteristics, nor the 16S rRNA nucleotide sequence is enough for strain discrimination of close-related bacteria. The authors used phylogenetic analysis of gyrase subunit A (*gyrA*) and histidine kinase (*cheA*) as a complementary approach to discriminate close-related *Bacillus* strains. Taking this into account, the best method for the discrimination of close-related *Bacillus* species by full genome analysis was adopted in this work. Full genome analysis of ANI genes did prove that the identity of our strain is closely related to *B. velezensis* ONU-553, but the genome comparison based on functional group of genes performing a particular biological function showed differences in potassium metabolism, phages, prophages, transposable elements, plasmids, and protein metabolism (Table S1). Likewise, PHASTER analysis showed KS04AU to have a high number of identified phages (four) followed by species, but *B. amyloliqueficiens* LL3, and ONU-553 but with two identical phages, showed the possibility of higher immunity of our strain to such phages in comparison with strains of the same species.

Recently, the rate of duplication of cells was calculated with the number of phages constituent in a bacterium [40]. The authors confirmed that fast-growing bacteria contain more prophages in their genome, which might be one characteristic of KS04AU. Out of the four phage regions found in the KS04AU genome, the two intact phages were identical to the thermophyllic bacteriophage *Aeribacillus* bacteriophage AP45 [41], first isolated from the Kamchatka region, Russia, and *Paenibacillus* larvae bacteriophage Tripp from North Carolina, USA [42]. The two phage regions contain no phage lytic enzyme (lysin) gene to lyse the host strain; we, therefore, suggest KS04AU to be immune to these phages, making it a stable strain in relation to subsequent infections. The key adaptive resistant mechanism of bacteria and archea as a form of systematic immune manipulations depends on clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (*Cas*) genes when the phage absorption or superinfection exclusion (Sie) system is inevitable [43,44]. CRISPR–Cas component system of KS04AU was identical in all parameters, with the exception of *B. velezensis* JS25R and *B. amyloliquefaciens* IT-45. The strain KS04AU can comparably be considered as a robust strain in relation to JS25R and IT-45, since the main element responsible for immunity is as targets against phages is the spacer genes.

Secondary metabolites that are responsible for antagonism against phytopathogens (fungal, bacterial, and viral), induction of ISR, and iron-chelating (siderophore) genes are primarily associated with *Bacillus* of this related strain [45–47]. Compared with its closely related strains, the lantibiotic mersacidin gene cluster is only present in the KS04AU strain. The unique property of this secondary metabolite is its antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria [48]. Although mersacidin was reported not to be synthesized in FZB42, a fragment of the gene was found in its genome [49], but in our case the antiSMASH program showed no identity of the gene cluster. The expression of mersacidin in FZB42 was achieved only after the transfer of the biosynthetic part to its gene cluster [50]. In KS04AU, the similarity percentage is 100%, showing the presence of a full gene cluster of this secondary metabolite in this strain.

Most transpositions of IS elements are thought to induce mutations which are capable of altering the fitness of the cell host [51,52], which make them the main factor involved in

the evolution and diversification of the bacterial genome. In this study, the comparative analysis revealed 47 IS-Elements in KS04AU compared with its closest-related strain, ONU-553, which has 53.

The ability for *B. velezensis* to protect plants as an inoculant in the soil depends on its viability in relation to other antibiotic-producing microbes that can inhibit its growth by the different classes of antibiotics they produce. For this purpose, the presence of an antimicrobial resistant gene family was analyzed, and the results confirmed the resistance of KS04AU to different drug classes of antibiotics targeting the alteration gene family Cfr 23S ribosomal RNA methyltransferase (clbA) and the major facilitator superfamily (MFS) antibiotic efflux pump *tet* (45). These genes were reported for other strains of *B. velezensis* showing a high resistance to most antibiotics and used as a biocontrol agent against *Erwinia amylovora* [53].

Finally, the phenotypic parameters to attest to the biocontrol and the plant growth-promoting ability of *B. velezensis* KS04AU can be seen by its antagonistic activity against the selected phytopathogens (*Forl* ZUM2407, *F. graminearum*, *A. alternata*, *P. syringae*) and the solubilization of phosphate. Although *P. putida* PCL1760 was used as a negative control in our antagonistic and enzymatic activity experiments, it was able to inhibit the bacterial pathogen *P. syringae* in comparison with *B. mojavensis* PS17 (positive control). Ye et al. [54] reported on the *P. putida* strain W15Oct28 that was able to inhibit the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and the plant pathogen *P. syringae*, stating this characteristic as unusual. There is no report on *P. putida* PCL1760 producing active metabolic compounds able to inhibit the plant pathogen *P. syringae*, but there are several reports of it as a good root-colonizing bacteria able to control tomato foot and root rot [55]. As a biocontrol agent against *Forl* ZUM2407, *B. velezensis* KS04AU was able to control the disease in tomato and did not differ statistically from the positive control PCL1760.

5. Conclusions

To summarize, characterization of Bacillus species by phenotypic analysis and 16S rRNA is inadequate without multi-loci or genomic sequencing analysis. The genomic characteristics of *B. velezensis* KS04AU, in comparison with its strains of the same species and related species, provides an overview of the unique characteristics of the strain. The enzymatic activity of strain KS04AU attests the absence of down-regulation in relation to the genes responsible for the tested exoenzymes and secondary metabolites. The planta experiment of its biocontrol ability also shows that *B. velezensis* KS04AU is a good candidate for biopreparation against plant pathogens. Reportedly, most *B. velezensis* strains have these abilities, and genomic analysis of KS04AU provides comprehensive information on the unique characteristics of this strain.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijpb13030018/s1, Figure S1: Intact Prophage founded in *B. velezensis* KS04AU. PHAGE_*Aeriba*-_AP45_NC_048651 (region 2) and PHAGE_*Paenib_Tripp*_NC_02893 (region 4), Table S1: Comparative analysis of functional subsystem category in genomes of *B. velezensis* KS04AU, SCRM102752, FZB42, ONU553, and *B. amyloliquefaciens* LL3 and IT-45 based on SEED servers, Table S2: Analysis of mobile genetic elements (IS-Elements) of genome *Bacillus velezensis* KS04AU, JS25R, FZB42, ONU-553, and SRCM102752. Table S3: Mobile genetic elements (IS-Elements) shared between genomes used in this study.

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