GENOME SEQUENCE OF *BACILLUS VELEZENSIS* W1, A STRAIN WITH STRONG ACARICIDAL ACTIVITY AGAINST TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE)*

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Abstract. *Bacillus velezensis* W1, isolated from two-spotted spider mites that had died naturally, is a patented strain with strong capability to cause mortality of the phytophagous mite *Tetranychus urticae*. The whole genome of W1 was completely sequenced with a combination of an Illumina Miseq platform (400-bp paired-end) with 2 × 250 bases and a Pacific Biosciences (PaBio) RS II Single Molecule Real Time (SMRT) sequencing platform using a 20 kb SMRTbell™ template library. Here, we report the complete genome sequence of *B. velezensis* W1, including one circular chromosome of 4,237,431 bp encoding 4,352 genes with GC content of 45.84%, providing insights into the genomic basis of its acaricidal activity and facilitating its application in red spider mite biocontrol.
Keywords: *Bacillus, two-spotted spider mite, biocontrol, whole genome, Acaricides*

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most polyphagous arthropod herbivores and feeds on more than 1,100 plant species belonging to more than 140 different plant families including species known to produce toxic compounds. It is a major pest in field crops, destroying both annual and perennial crops (Grbic et al., 2011). The use of chemical insecticides and acaricides are currently the primary control method of *T. urticae* (Jafari et al., 2016). The frequent application of these chemicals, paired with several biological aspects of this species, such as its short life cycle, high fecundity and arrhenotokous reproduction, has led to the mites developing resistance to the most pesticide groups (Minazzi et al., 2016). Additionally, chemical pesticides can have unintended consequences, impacting environmental quality, food safety, human health, and biodiversity (Yang et al., 2016).

Recently, biological control agents in the form of parasites, predators and pathogens have gained a lot of attention. Biological control of spider mites using predators or parasites is widely practiced, particularly the use of phytoseiid mites, which are currently sold worldwide (Funayama et al., 2015). Entomopathogenic fungi or entomogenous fungi such as *Hirsutella thompsonii* Fisher (El-Sharabasy, 2015), *Neozygites floridana* Weiser and Muma (Klingen et al., 2008), *Beauveria bassiana*

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(Balsamo) Vuillemin (Ullah and Lim, 2015), *Verticillium lecanii* (Zimm.) (Seiedy, 2015), and *Isaria cateniannulata* (Zhang et al., 2016) are currently the most studied biological control agents of spider mites. Few bacteria, however, have been reported as biological control agents of spider mites owing to the piercing-sucking mouthparts of the mites, which make it difficult for bacteria to infect them. In recent years, researchers have concentrated on intracellular organisms such as *Wolbachia* that may cause distorted sex ratio in the mite offspring, thereby impacting population (Chen et al., 2016), and toxin-producing bacteria such as *Bacillus thuringiensis* that can produce crystal proteins called *δ*-endotoxins that are commonly used as a biological acaricides (Neethu et al., 2016). Additional research has been carried out on potential acaricide producing strains such as *Pseudomonas putida* (Aksoy et al., 2008).

Bacillus velezensis W1 (W1 hereafter), isolated from two-spotted spider mite that had died naturally, is a patented strain (Patent no; ZL201610096541.8) that has a strong capability to cause mortality of the phytophagous mite *T. urticae* (Li et al., 2018). This strain has the potential to be a safe and eco-friendly acaricide. To further explore its biocontrol ability and to reveal its acaricidal activity mechanism, we carried out the complete genome sequencing and analysis of W1.

Materials and methods

Bacterial strain and culture condition

B. velezensis W1 is a Gram-positive rod shaped bacterium averaging 2.5 μm in length and 1 μm in width (*Fig. 1a*). W1 spores are centrally located and average 1.3 μm in length (*Fig. 1b*). The bacteria could grow rapidly in Luria Bertani (LB) liquid medium reaching the stationary phase after 12 h at 35 °C. By comparison, the growth rate in LB solid medium was much slower with the stationary phase attained at 24 h. Optimum growth occur at a temperature 35 °C and pH 8.0 (*Table 1*). The colony morphology of strain W1 grown in solid LB medium is circular convex with undulate beige-opaque margins (*Fig. 1c*).

Phylogenetic analysis

Phylogenetic tree of the *B. velezensis* W1 and other related taxa was constructed with MEGA 7.0 (Kumar et al., 2016) using Neighbor-Joining method (Saitou and Nei, 1987) based on *gyrB* gene, and the distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004), with 1000 replications in the bootstrap test. Bootstrap confidence levels > 30% are indicated at the internodes. The scale bar indicates nucleotide substitutions per nucleotide position.

Genome sequencing information

Genome project history

Strain W1 was selected for sequencing due to its ability to cause mortality of the phytophagous mite, *Tetranychus urticae*. The whole genome was deposited in GenBank under the accession number CP028375. Genome sequencing and assembly was performed at the Wuhan Genoseq Technology Co., Ltd, Wuhan, China. The summary of the project information is shown in *Table 2*.

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Table 1. *Classification and general features of Bacillus velezensis W1 (Field et al., 2008)*

MIGS ID	Property	Term	Evidence code[a]
	Classification	Domain *Bacteria*	TAS (Woese et al., 1990)
		Phylum *Firmicutes*	TAS (Gibbonst and Murray, 1978)
		Class *Baclli*	TAS (Oren and Garrity, 2016)
		Order *Bacillales*	TAS (Skerman et al., 1980)
		Family *Bacillaceae*	TAS (Fischer, 1895)
		Genus *Bacillus*	TAS (Cohn, 1872)
		Species *Bacillus velezensis*	TAS (Dunlap et al., 2016)
		(Type) strain: *W1 (CP028375)*	
	Gram stain	Positive	IDA
	Cell shape	Rod	IDA
	Motility	Motile	IDA
	Sporulation	not reported	
	Temperature range	20-40 °C	IDA
	Optimum temperature	35 °C	IDA
	pH range; Optimum	5–9; 8	IDA
	Carbon source	Heterotrophic	IDA
MIGS-6	Habitat	Plant, spider mite	IDA
MIGS-6.3	Salinity	14% (w/v) NaCl	IDA
MIGS-22	Oxygen requirement	Aerobic	IDA
MIGS-15	Biotic relationship	free-living	IDA
MIGS-14	Pathogenicity	Not reported	
MIGS-4	Geographic location	China	NAS
MIGS-5	Sample collection	2015	NAS
MIGS-4.1	Latitude	25° 01’ N	NAS
MIGS-4.2	Longitude	102° 19’ E	NAS
MIGS-4.4	Altitude	1835	NAS

[a]Evidence codes – MIGS: The minimum information about genome sequence; IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project (Ashburner et al., 2000)

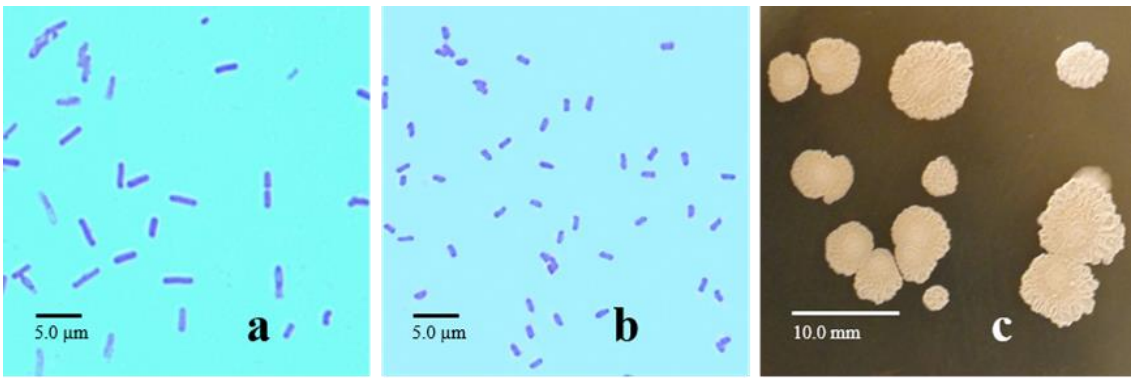

Figure 1. *Cellular and colony morphology of Bacillus velezensis W1.* ***a*** *Vegetative W1 cells grown 72 h, Gram stained, and then at 100x magnification captured with light microscope.* ***b*** *W1 spores at 100x magnification captured light microscope.* ***c*** *Pure culture of W1 grown on nutrient agar plate*

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Table 2. *Genome sequencing project information for Bacillus velezensis W1*

MIGS ID	Property	Term
MIGS 31	Finishing quality	Finished
MIGS 28	Libraries used	a 20 kb SMRTbell™ template library
MIGS 29	Sequencing platforms	Illumina MiSeq + PacBio
MIGS 31.2	Fold coverage	305x
MIGS 30	Assemblers	A5-miseq version 20150522
MIGS 32	Gene calling method	NCBI Prokaryotic Genome Annotation Pipeline
	Locus Tag	/
	Genbank ID	CP028375
	Genbank Date of Release	June 28th, 2018
	GOLD ID	/
	BIOPROJECT	PRJNA445958
MIGS 13	Source Material Identifier	*Bacillus velezensis* W1
	Project relevance	Biocontrol, Agriculture

Growth conditions and genomic DNA preparation

The genomic DNA of W1 was extracted using a QIAamp DNA mini kit (Qiagen, USA), according to the manufacturer's protocols.

Genome sequencing and assembly

The whole genome of W1 was completely sequenced with a combination of an Illumina Miseq platform (400-bp paired-end) with 2 × 250 bases and a Pacific Biosciences (PaBio) RS II Single Molecule Real Time (SMRT) sequencing platform using a 20 kb SMRTbell™ template library. Approximately 1,385.26 Mb with 4,661,344 reads were generated from the Illumina Miseq and PacBio sequencings, respectively. The quality of these reads was assessed by the FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and low quality reads were filtered by Quake (Kelley et al., 2010) and AdapterRemoval (version 2.1.7) (Lindgreen, 2012). The clean reads were *de novo* assembled into a single contig with a genome coverage of 305 folds using A5-miseq version 20150522 (Coil et al., 2015), Canu v1.4 (Koren et al., 2017) and pilon v1.18 (Walker et al., 2014). Glimmer 3.02 (Delcher et al., 1999) was used to predict the open reading frames (ORFs).

Genome annotation

Software packages tRNAscan-SE v. 1.3.1 (Lowe and Eddy, 1997) and RNAmmer v. 1.2 (Lagesen et al., 2007) were used to predict tRNA and rRNA, respectively. The gene function annotations were based on BlastP similarity searches (E-Value < 10^{-6}) against 5 databases: evolutionary genealogy of genes: Non-supervised Orthologous Groups (http://eggnogdb.embl.de/), Kncyclopedia of Genes and Genomes (http://www.genome.jp/kegg/), Non-Redundant GenBank Protein Database databases (www.ncbi.nlm.nih.gov/protein), Swiss-Prot (http://www.uniprot.org/), Gene Ontology Database (http://www.geneontology.org/). Circular genome map was created by cgview (Stothard and Wishart, 2005) with COG function annotation.

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Results and discussion

Sequence analysis using gyrB

The phylogenetic analysis based on *gyrB* gene sequences using MEGA 7.0 [14] showed that *B. velezensis* W1 is evolutionarily positioned between *B. velezensis* and *B. amyloliquefaciens* (*Fig. 2*). In recent studies of genome sequencing and comparative genomics of *B. velezensis* NRRL B-41580, *B. methylotrophicus* KACC 13015 and *B. amyloliquefaciens* subsp. *plantarum* FZB42, it was established that these last two strains are heterotypic synonyms of *B. velezensis* (Dunlap et al., 2016), and based on our results, the classification of strain W1 was confirmed as a member of the species *B. velezensis*. The complete genome sequence of strain *B. velezensis* W1 is deposited at GenBank under accession number CP028375. The strain is available from China General Microbiological Culture Collection Center (CGMCC No. 11949).

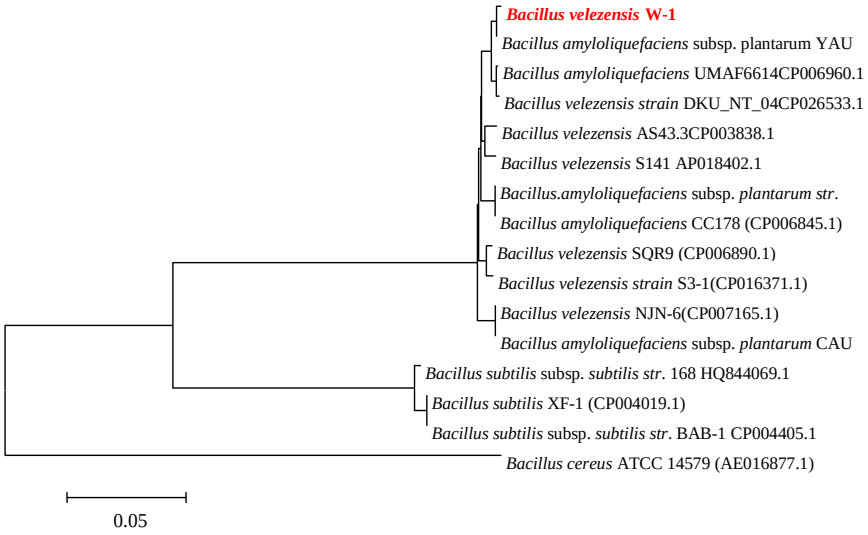

Figure 2. *Phylogenetic trees of the B. velezensis W1 and other related taxa*

Genome properties

The complete genome of W1 consisted of one 4,237,431 bp circular chromosome with an average GC content of 45.84% without a plasmid. Totally, 4,404 protein-coding genes were predicted (*Table 3; Fig. 3*), along with 85 tRNA genes and 27 rRNA genes (*Table 4; Fig. 3*). Among these 4,404 protein-coding genes 4,352 ones (98.82%) were annotated with predicted function (*Table 5*). There were 2879 (65.37%) genes assigned to COG database (*Table 6*).

Table 3. *Genome features of B. velezensis W1*

Features	Value
Genome size (bp)	4,237,431
Average G + C content (%)	45.84
Protein-coding genes	4,404
Longest Protein-coding genes	17,103
Total size of Protein-coding genes	3,761,991
Mean length of Protein-coding genes (bp)	854
Total size of Protein-coding genes % of Genome (%)	88.78

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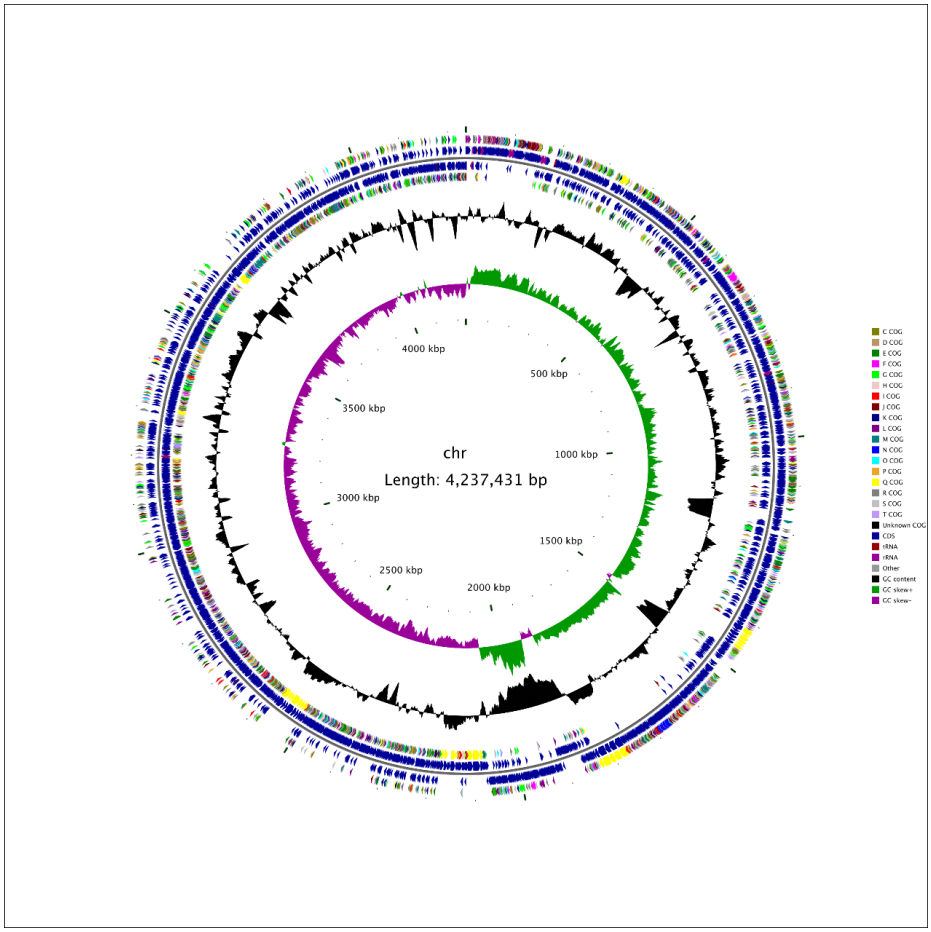

Figure 3. *Circular genome graph of Bacillus velezensis W1. From inside to outside, the first circle represents the scale; the second circle represents the GC Skew; the third circle represents the GC content; the fourth and seventh circles represent each CDS's COG; the fifth and sixth circles represent the position of CDS, tRNA and rRNA on the genome*

Table 4. *Statistics of ncRNA prediction of protein-coding genes of B. velezensis W1*

ncRNA type	Copy	Average length (bp)	Total length (bp)	% of genome
5s rRNA	9	115	1,033	0.024
16s rRNA	9	1,570	14,128	0.333
23s rRNA	9	2,934	26,402	0.623
tRNA	85	77	6,568	0.155
other ncRNA	9	115	1,033	0.024

Table 5. *The statistics of gene function annotation of B. velezensis W1*

Database	Annotated number	% of Genome
NR	4,352	98.82
eggNOG	2,879	65.37
KEGG	2,203	50.02
Swiss-Prot	3,785	85.94
GO	2,485	56.36

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Table 6. *COG categories of B. velezensis W1*

Category	Description	Value	Percent (%)
B	Chromatin structure and dynamics	1	0.02
C	Energy production and conversion	177	4.02
D	Cell cycle control, cell division, chromosome partitioning	33	0.75
E	Amino acid transport and metabolism	340	7.72
F	Nucleotide transport and metabolism	83	1.89
G	Carbohydrate transport and metabolism	250	5.68
H	Coenzyme transport and metabolism	125	2.84
I	Lipid transport and metabolism	119	2.70
J	Translation, ribosomal structure and biogenesis	144	3.27
K	Transcription	279	6.34
L	Replication, recombination and repair	133	3.02
M	Cell wall/membrane/envelope biogenesis	179	4.06
N	Cell motility	57	1.29
O	Posttranslational modification, protein turnover, chaperones	98	2.23
P	Inorganic ion transport and metabolism	205	4.66
Q	Secondary metabolites biosynthesis, transport and catabolism	116	2.63
R	General function prediction only	451	10.24
S	Function unknown	297	6.74
T	Signal transduction mechanisms	159	3.61
U	Intracellular trafficking, secretion, and vesicular transport	49	1.11
V	Defense mechanisms	63	1.43
W	Extracellular structures	3	0.07
	Not in eggNOG	1,525	34.63

Insights from the genome sequence

The virulence factors of pathogenic bacteria (VFDB) of W1 were predicted using BLAST similarity searches (E-Value < 10−6) in Virulence Factors Database (http://www.mgc.ac.cn/VFs/) (Chen et al., 2012). W1 comprises several virulence factors (*Table 7*) which might all contribute to acaricidal activity, such as clpC, clpE, clpP, gale, lplA1, acpXL, bslA/yuaB, cps4I, and ureB.

There is a cluster of three collagen-related structural motif genes found in the genome, i.e., clpC, clpE and clpP. The genes of collagen-like proteins (clPs) have been identified in a broad range of bacteria, including some human pathogens and non-human pathogenic strains such as *B. cereus* and *B. amyloliquefaciens* which are reported to use as biocontrol agents (Zhao et al., 2015). The clPs are important for biofilm formation and bacterial adhesion to host cells and swimming motility (Zhao et al., 2016). In low-GC Gram-positive bacteria, the clpP protease is the main system involved in protein degradation (Vaz Cassenego et al., 2016), and play a role in both virulence and environmental adaptation, and acyldepsipeptides activated by ClpP core were effective in killing persister cells (Springer et al., 2016).

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Biofilms are surface-associated bacterial aggregates, in which bacteria are enveloped by polymeric substances known as the biofilm matrix. Biofilms of biocontrol strains were considered as some significant biocontrol properties, which provide the necessary proximity to the host so that the secretion of secondary metabolites featuring biocontrol activity from biofilm forming cells may act directly on the target (Kröber et al., 2016; Vlamakis et al., 2008). *B. amyloliquefaciens* is a Gram-positive bacterium that forms biofilms, which are created by a heterogeneous population of motile, matrix-producing and sporulating cells. One characteristic feature of biofilms is the extracellular matrix built from EPS combined with macromolecules like proteins and nucleic acids (Kröber et al., 2016). The bslA/yuaB was considered to stand for biofilm surface layer protein and be responsible for the hydrophobic layer on the surface of biofilms (Kobayashi and Iwano, 2012).

The chromosome encodes six chitin deacetylases (chr_orf00199, chr_orf01107, chr_orf03856, chr_orf05798, chr_orf01368, chr_orf02151), which are insect chitin degradation enzymes that catalyze the deacetylation of chitin to form chitosan, and enable the degradation of chitin in the midgut peritrophic membrane of many insects and have been identified as insect virulence factors (Yang et al., 2018; Yu et al., 2016).

Table 7. *Predicted virulence factors of B. velezensis W1*

VFDB ID	Proteins	Annotation	Count
VFG000079 (gi:16802278)	chr_orf00119	clpC	1
VFG000080 (gi:16803037)	chr_orf01991	clpE	1
VFG013286 (gi:16272302)	chr_orf05807	galE	1
VFG002158 (gi:16802971)	chr_orf01464	lplA1	1
VFG011430 (gi:17987758)	chr_orf02383	acpXL	1
VFG045350 (gi:16080160)	chr_orf04548	bslA/yuaB	1
VFG000077 (gi:16804506)	chr_orf05117	clpP	1
VFG001373 (gi:15900286)	chr_orf05282	cps4I	1
VFG000270 (gi:15644702)	chr_orf05452	ureB	1

Conclusions

This report described the complete genome sequence of *B. velezensis* W1. The species has biotechnological potential due to its capability to cause mortality of the phytophagous mite, *Tetranychus urticae*. Its acaricidal activity might be related to function of the virulence factors of pathogenic bacteria, like clpC, clpE, clpP, gale, lplA1, acpXL, bslA/yuaB, cps4I, and ureB, as well as collagen-related structural motif genes clpC, clpE and clpP. Biofilm and chitin deacetylases have been identified as biocontrol properties and insect virulence factors. Moreover, this bacterial strain can be used as microbial acaricides to control more and more acari in the field and greenhouse.

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Conflict of interests. The authors declare that they have no conflict of interests.

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