1 Complete genome sequence of *Bacillus velezensis* JT3-1, a microbial germicide isolated from yak
2 feces

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15 **Abstract:**

16 *Bacillus velezensis* JT3-1 is a probiotic strain isolated from feces of the domestic yak (*Bos*
 17 *grunniens*) in the Gansu province of China. It has strong antagonistic activity against *Listeria*
 18 *monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Typhimurium, *Mannheimia*
 19 *haemolytica*, *Staphylococcus hominis*, *Clostridium perfringens*, and *Mycoplasma bovis*. These
 20 properties have made the JT3-1 strain the focus of commercial interest. In this study, we describe
 21 the complete genome sequence of JT3-1, with a genome size of 3,929,799 bp, 3761 encoded genes
 22 and an average GC content of 46.50%. Whole genome sequencing of *Bacillus velezensis* JT3-1
 23 will lay a good foundation for elucidation of the mechanisms of its antimicrobial activity, and for
 24 its future application.

25 **Keywords:** *Bacillus velezensis* JT3-1; Genome; Antimicrobial activity; Secondary metabolite

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Antibiotics utilized in animal husbandry introduce various problems while improving animal productivity (Sharma et al., 2017). In many countries, the current tendency in animal production is towards a reduction or prohibition of the use of antibiotics, with an increase in application of non-antibiotic methods (Lillehoj and Lee, 2012). Of the non-antibiotic methods, probiotics are a safe alternative to antibiotics, and the dietary supplementation of probiotics can improve the productivity and immunological status of livestock (Abd El-Tawab et al., 2016; Khan et al., 2016; Reuter, 2001). Probiotics are dietary supplements that are by definition “live microorganisms, which, upon ingestion in sufficient numbers, exert health benefits” (Schrezenmeir and de Vrese, 2001). In ruminants, probiotics can change the rumen microbial ecosystem, and promote nutrient digestibility and feed efficiency. The most commonly used probiotics are *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and yeast strains. Supplemental *B. subtilis* increases growth performance, disease resistance and immune response in animals (Guo et al., 2016, 2017). Previous reports have demonstrated that *Bacillus velezensis* QST713 (Pandini et al., 2018), *Bacillus velezensis* M75 (Kim et al., 2017), and *Bacillus velezensis* S3-1 (Jin et al., 2017) showed a strong ability to form biofilm and inhibit pathogens.

B. velezensis JT3-1 was isolated from the feces of domestic yak (*Bos grunniens*) in Gansu Province, China. Our study showed that the *B. velezensis* JT3-1 strain had strong antagonistic activity against various intestinal pathogenic flora, and stimulation of animal (such as sheep, goat, mouse, pig and cattle) growth (not published). However, there are no published reports on the novel *B. velezensis* JT3-1 strain and its genome sequence is not found in Genbank. In this study, the genome sequence of the novel strain JT3-1 was determined, the secondary metabolite clusters in the genome of *B. velezensis* JT3-1 were analyzed, and an antimicrobial activity assay was

performed to investigate the abilities of the biocontrol strain.

1. General genomic features of *Bacillus velezensis* JT3-1

Genomic DNA was extracted using Gentra Puregene Yeast/Bact (Qiagen, Germany), and the whole-genome sequencing of *B. velezensis* JT3-1 was performed using a PacBio Sequel sequencing platform at Beijing Genewiz Bioinformatics Technology Co., Ltd. A library with a 10 kb insert size was constructed for the PacBio Sequel platform, and the library was sequenced in a PacBio SMRT (Chin et al., 2013) instrument. Subsequently, the PacBio reads were assembled using SmrtLink, and a total of 199380 reads, in about 550 Mb, were obtained. Finally, we obtained a single circular gapless chromosome. Prodigal software (Hyatt et al., 2010) was utilized to find coding genes in the bacterium. Transfer RNAs (tRNAs) were detected in the genome using the program blast based on Rfam (Eric et al., 2014). The coding genes were annotated with the National Center for Biotechnology Information (NCBI) nr database by Diamond (Buchfink et al., 2015). Afterwards, the functions of genes were annotated using the Gene Ontology database, and the pathways were annotated using the Kyoto Encyclopedia of Genes and Genomes database. The proteins encoded by the genes were phylogenetically classified by the Clusters of Orthologous Groups database. The potential genes involved with the biosynthesis of bacteriocins were identified with antiSMASH4.0 (Blin et al., 2017) (<http://antismash.secondarymetabolites.org/>). The complete genome of *B. velezensis* JT3-1 contains one gapless circular chromosome of 3,929,799 bp, 3761 protein encoding genes, 86 tRNA genes, 90 rRNA genes and 83 other ncRNA; it shows a G + C content of 46.50%, and no plasmids were found (**Table 1**). The circular genome map of the *B. velezensis* JT3-1 complete genome is displayed in **Fig. 1**.

2. Antimicrobial activity of *Bacillus velezensis* JT3-1

The antimicrobial activity of *Bacillus velezensis* JT3-1 was investigated; an agar well diffusion assay was utilized to test the antibacterial properties of the JT3-1 culture supernatant, as previously described (Liu et al., 2017). The Nutrient Agar (NA) medium was utilized to culture the remaining bacteria, and the PPLOB medium was utilized to culture *Mycoplasma bovis*. The diameter of the inhibition zones was calculated in millimeters, and the plus sign shows the range of the inhibition zone. The three-plus sign (+++) means that the diameter of the inhibition zone is more than 15 mm; the two-plus sign (++) means that the diameter of the inhibition zone is between 10 mm and 15 mm; and the one-plus sign (+) means that the diameter of the inhibition zone is less than 10 mm (the diameter of the wells was 5 mm). Each test was repeated three times, and the mean values were adopted. Our study identified that *B. velezensis* JT3-1 showed strong antimicrobial activity against some Gram-negative human or animal pathogens (such as *E. coli* ATCC43888, *E. coli* ATCC25922, *E. coli* LQ1-2, *Yersinia enterocolitis* 23715, *Proteus vulgaris* ATCC29905, *Shigella boydii* ATCC9207, *Salmonella* Typhimurium ATCC13311, and *Mannheimia haemolytica* ATCC29696), several Gram-positive animal and human pathogenic bacteria (such as *Streptococcus pyogenes* ATCC19615, *Listeria monocytogenes* ATCC19115, *Staphylococcus hominis* ZSY2, *Staphylococcus aureus* ATCC6538, and *Clostridium perfringens* MQF5), and *Mycoplasma bovis* CYF strain (**Table 2**). Our previous study identified that the *B. velezensis* JT3-1 strain plays an important role in preventing and curing calf and lamb diarrhea caused by bacteria or by the weaning period of young animals (not published).

3. Secondary metabolite clusters

Through the antiSMASH 4.0 genome analysis tool (Blin et al., 2017), 30 clusters of secondary metabolites were identified in the genome of the JT3-1 strain: two transATPKS-NRPS, two

transATPKS (trans-acyl transferase polyketide synthetase), one lantipeptide, one encoding NRPS (non-ribosomal peptide synthetase), one T3PKS, one bacteriocin-Nrps, two terpenes, one other KS, three Cf_fatty_acid, five Cf_saccharide, and 11 Cf_putative (**Table 3**). Eight clusters were clearly involved in the synthesis of difficidin, surfactin, fengycin, butirosin, macrolactin, bacilysin, bacillaene and bacillibactin, and they were closely related with antimicrobial activities (Chen et al., 2009; Niazi et al., 2014; Ongena et al., 2005; Liu et al., 2017; Kim et al., 2017; Stein, 2005; Pandin et al., 2018). This analysis showed that at least 17% of the JT3-1 genome contributed to the transport and regulation of antibiotic biosynthesis.

4. Genes involved in promoting animal growth

Besides producing many secondary metabolites with antimicrobial activity, several *Bacillus* spp. possess high secretory ability and some strains utilize “cell factories” to produce industrial enzymes (Ruiz-Garcia et al., 2005; Niazi et al., 2014). Several *B. subtilis* strains can colonize the intestinal microvilli of the animal, support nutrition, and trigger a healthy immune system (Guo et al., 2016, 2017; Abd El-Tawab et al., 2016). Several strains of *B. amyloliquefaciens* subsp. *plantarum* can improve plant growth and confer protection by producing antimicrobial materials and phytohormones (Chen et al., 2006; Koumoutsis et al., 2004). *B. velezensis* strains are able to suppress plant or animal pathogens, increase plant or animal growth, and very efficiently colonize rhizosphere soil and the intestinal tract of the host (Ruiz-Garcia et al., 2005; Jin et al., 2017; Chen et al., 2017; Wang et al., 2018; Yi et al., 2018). Our previous study showed that *B. velezensis* JT3-1 can efficiently colonize the intestinal microvilli of an animal, promote growth, and trigger the immune system (not published). The genome of the *B. velezensis* JT3-1 strain contains many genes involved in biofilm formation and animal intestinal colonization, including the *alsD*, *bdhA*,

sacB, *xynA*, *xynD*, 3-hydroxy-2-butanone, 2,3-butanediol, Bacillibactin, indole acetic acid, trehalose, and phytase genes. Several genes of *B. velezensis* JT3-1 were related to the enzymes encoded to use derived substrates; *xynD* and *xynA*, which encode xylanase, are related to the use of hemicelluloses and celluloses presented in the animal intestinal tract. *B. velezensis* JT3-1 also harbors several genes involved in the synthesis of acetoin, 3-hydroxy-2-butanone and 2,3-butanediol (*bdhA* and *alsD*), which are able to improve animal and plant growth efficiency and trigger systemic defenses against pathogenic agents. Bacillibactin, which is a nonribosomal peptide previously discovered in *B. subtilis*, can transfer iron to iron acquisition mechanisms that are essential to animal and plant life (Zhou et al., 2018). Trehalose is a non-reducing disaccharide that plays an important role in protecting cells from stress and maintaining cell structure (Wang et al., 2019). Phytase is secreted by the intestinal bacteria of ruminant animals (such as camels, deer, cattle, goats and sheep), and it can degrade phytate and improve the efficacy of phosphorus metabolism and the performance of animals and plants (Frias et al., 2003). At present, phytase is widely used as an animal feed supplement. Meanwhile, *B. velezensis* JT3-1 also harbors the genes that encode ATP-dependent Clp protease (*clpE*), α -glucosidase (*glyA*), β -glucosidase (*gmuD*), catalase (*katE*), and thiol peroxidase (*tpx*), among others, demonstrating that *B. velezensis* JT3-1 is able to use many organic materials as nutritional supplies to promote its survivability.

Until now, 167 *B. velezensis* strains have been sequenced and released in the NCBI genome database. The availability of the complete genome sequence of *B. velezensis* JT3-1 will not only enrich the genome database but also provide some valuable information regarding the molecular mechanism of antimicrobial action and build the foundation for the utilization of JT3-1 as a biocontrol agent in animal husbandry.

5. Nucleotide sequence accession number

The complete genome sequence of *B. velezensis* JT3-1 was submitted to GeneBank under the accession number CP032506. This strain was deposited at the China General Microbiological Culture Collection Center under the accession number CGMCC No.15545.

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241 **Legends**

242 **Table 1** General genome features of *Bacillus velezensis* JT3-1.

243 **Table 2** Secondary metabolite clusters identified in the genome of *B. velezensis* JT3-1.

244 **Table 3** Antimicrobial activity of the *Bacillus velezensis* JT3-1 strain.

245 ^a NA: Nutrient Agar.

246 ^b PPLOB: PPLOB medium was utilized to culture *Mycoplasma bovis*.

247 **Fig. 1.** Circular genome maps of the *Bacillus velezensis* JT3-1 complete genome. From the
 248 outermost circle to the inner, circle 1, the size of complete genome; circles 2 (red) and 3 (green),
 249 the predicted protein-encoding genes on the + and – strands, respectively; circle 4, rRNA/tRNA;
 250 circle 5, G + C ratio; circle 6, repetitive sequence.

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1 **Table 1** General genome features of *B. velezensis* JT3-1

Feature	Value
Genome size (bp)	3,929,799
GC content [%]	46.50
Predicted genes	3761
Total ncRNA	259
tRNA genes	86
rRNA genes	90
Other ncRNA	83

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Table 2 Antimicrobial activity of the *Bacillus velezensis* JT3-1 culture supernatant.

Pathogens	Broth medium	Antimicrobial activity
Gram-negative bacteria		
<i>E.coli</i> ATCC43888	NA ^a	+++
<i>E.coli</i> ATCC25922	NA	+++
<i>E.coli</i> LQ1-2	NA	+++
<i>Yersinia enterocolitis</i> 23715	NA	++
<i>Proteus vulgaris</i> ATCC29905	NA	+
<i>Shigella boydii</i> ATCC9207	NA	+
<i>Salmonella typhimurium</i> ATCC13311	NA	++
<i>Mannheimia haemolytica</i> ATCC29696	NA	++
Gram-positive bacteria		
<i>Listeria monocytogenes</i> ATCC19115	NA	+++
<i>Staphylococcus aureus</i> ATCC6538	NA	+++
<i>Streptococcus pyogenes</i> ATCC19615	NA	+++
<i>Staphylococcus hominis</i> ZSY2	NA	+++
<i>Clostridium perfringens</i> MQF5	NA	+++
<i>Mycoplasma bovis</i> CYF	PPLOB ^b	+++

^a NA: Nutrient Agar.

^b PPLOB: PPLOB medium was utilized to culture *Mycoplasma bovis*.

Table 3 Secondary metabolite clusters identified in the genome of *B. velezensis* JT3-1

Cluster Number	Sequence size (bp)	Synthetase	Metabolites	MIBiG BGC-ID (Simility)	Bioactivity
1	25038	Cf_fatty_acid	-	-	Not determined
2	29590	Cf_saccharide	-	-	Not determined
3	22551	Cf_putative	-	-	Not determined
4	41244	Otherks	Butirosin	BGC0000693_c1(7%)	Antimicrobial
5	20977	Cf_fatty_acid	-	-	Not determined
6	7135	Cf_putative	-	-	Not determined
7	20740	Terpene	-	-	Not determined
8	24825	Cf_fatty_acid	Citrulline	BGC0000895_c1(18%)	Not determined
9	8911	Cf_putative	-	-	Not determined
10	17703	Cf_putative	Molybdenum	BGC0000916_c1(11%)	Not determined
11	45150	Lantipeptide	-	-	Not determined
12	85905	Transatpks	Macrolactin	BGC0000181_c1(100%)	Antimicrobial
13	102674	Transatpks-Nrps	Bacillaene	BGC0001089_c1(100%)	Antimicrobial
14	137801	Transatpks-Nrps	Fengycin	BGC0001095_c1(100%)	Antimicrobial
15	21883	Terpene	-	-	Not determined
16	4727	Cf_putative	-	-	Not determined
17	41100	T3pks	-	-	Not determined
18	100453	Transatpks	Difficidin	BGC0000176_c1(100%)	Antimicrobial
19	8466	Cf_putative	-	-	Not determined
20	12930	Cf_putative	-	-	Not determined
21	66791	Bacteriocin-Nrps	Bacillibactin	BGC0000309_c1(100%)	Antimicrobial
22	11114	Cf_putative	-	-	Not determined
23	25411	Cf_saccharide	-	-	Not determined
24	54812	Cf_saccharide	Teichuronic_acid	BGC0000868_c1(100%)	Not determined

25	14022	Cf_putative	-	-	Not determined
26	72378	Cf_saccharide	Bacilysin	BGC0001184_c1(100%)	Antimicrobial
27	24874	Cf_saccharide	-	-	Not determined
28	6391	Cf_putative	-	-	Not determined
29	65407	Nrps	Surfactin	BGC0000433_c1(82%)	Antimicrobial
30	25038	Cf_putative	-	-	Not determined