Complete Genome Resource of *Bacillus velezensis* J17-4, an Endophyte Isolated from Stem Tissues of Rice

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Abstract

Dickeya zeae is the causative agent of rice root rot disease and causes severe harvest and economic losses. In this study, the *Bacillus velezensis* strain J17-4 with significant antagonism against *D. zeae* was used to generate DNA for sequencing. After assembly, a high-quality complete genome comprising only a circular chromosome was available. The genome sequence consists of a total of 3,877 prediction coding sequences and nine types of gene clusters involved in secondary metabolite production. This genome data will provide information for understanding the underlying mechanism of strain J17-4 antagonist against *D. zeae* and a new useful source for comparative genomics studies between strains isolated from various habitats.

Genome Announcement

The extensive use of chemical pesticides causes the accumulation of residual chemical compounds in the environment, which further results in environmental pollution and food safety problems. Numerous strains of *Bacillus velezensis* have been reported to suppress the growth of microbial pathogens; therefore, this species is attracting attention as a valuable biocontrol agent to circumvent the current undesirable effects. *B. velezensis* is an aerobic, Gram-positive, plant-associated bacterium which encodes 10% of its genome to the synthesis of antimicrobial molecules (Chen et al. 2007; Chowdhury et al. 2015). These antimicrobial compounds, including surfactin, iturins, bacillomycin-D, fengycins, bacillibactin, bacilysin, macrolactin, bacillaene, and diffidin, synthesized by *B. velezensis* display high antibacterial activity against several pathogenic microbes (Rabbee and Baek 2020). Apart from producing bioactive secondary metabolites against pathogenic microbes, *B. velezensis* was also found to contribute to plant protection by triggering induced systemic resistance in plants (Li et al. 2017), a process that activated the defense-associated genes of jasmonic acid and salicylic acid expression. With the development of sequencing technologies, genome-based approaches have been widely used to obtain the genomic and metabolic characteristics of *Bacillus* spp. (Chun et al. 2019). The first *B. velezensis* genome sequence was performed in 2007 (Chen et al. 2007) and, thus far, more than 150 whole-genome sequences of *B. velezensis* have become available in GenBank. These *B. velezensis* strains were isolated from different geographical locations and diverse environments, most of which were obtained from the plant rhizosphere or soil.

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Keywords

biocontrol agent, cultural and biological practices, endophyte, field crops, genome, prokaryotes

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Table 1. Genomic attributes of endophytic biocontrol *Bacillus velezensis* strains

Features	<i>B. velezensis</i> J17-4	<i>B. velezensis</i> CC09
Isolation source	<i>Oryza sativa</i> stems	<i>Cinnamomum camphora</i> leaves
Genome size (bp)	3,929,768	4,167,153
DNA G+C (%)	46.5	46.1
Total genes	3,877	4,094
Protein encoding genes	3,661	3,928
RNA genes	129	102
Ribosomal RNAs	27	24
Transfer RNAs	86	73
Small RNAs	16	5
Pseudogenes	87	64
Genomic islands	2	–
NCBI accession	CP060420	CP015443

The host range of *Dickeya zeae* is very wide, including monocotyledons and dicotyledons (Hu et al. 2018). As the causative agent of rice root rot disease, *D. zeae* strain EC1 can significantly inhibit the germination of the seeds of many rice varieties with inoculation of only a few bacterial cells (Hussain et al. 2008). Previous studies have shown that some bacteria (i.e., *Pseudomonas fluorescens*, *P. parafulva*, and *B. velezensis*) isolated from soil with antagonistic activities against *D. zeae* have good efficacy in laboratory conditions (Li et al. 2020). Therefore, biocontrol might be a powerful alternative to the use of synthetic chemicals to control rice root rot disease. Recently, we began to screen endophyte bacteria from resistant host plants of *D. zeae* strain EC1 and, providentially, we isolated the J17-4 strain from healthy and sterilized stems of rice variety Guang jinlizhan. This bacterium strain could significantly inhibit the growth of *D. zeae* strain EC1 with the maximum inhibition zones of 21.3 mm via confront culture. Furthermore, the J17-4 strain could significantly reduce the inhibitory effect of *D. zeae* strain EC1 against rice seed germination under laboratory inoculation conditions, and this strain was further identified as *B. velezensis* via taxonomic analysis (unpublished data). Here, we describe the complete genome sequence of this strain.

Genomic DNA of J17-4 was extracted using HiPure Bacterial DNA Kits (Magen, Guangzhou, China) according to the manufacturer's instructions. The whole-genome sequencing was manipulated on single-molecule real-time (SMRT) cells via Pacific Biosciences Sequel (PacBio, Menlo Park, CA, U.S.A.) according to standard protocols. Genome sequencing was also performed on the Illumina Novaseq 6000 sequencer using paired-end technology (PE 150). Raw data attained from SMRT sequencing runs were filtered and, in total, 168,843 subreads with an average read length of 7,041.8 bp were selected for assembly. Then, these subreads were de novo assembled by using the diploid-aware long-read assembler FALCON-Unzip of FALCON (version 0.3.0) (Chin et al. 2016). Raw data from the Illumina platform were filtered using FASTP (version 0.20.0) by removing reads under standard to correct the genome sequences of SMRT sequencing to improve the quality of its assembly and determine the final genome sequences via Pilon (version 1.23) (Chen et al. 2018; Walker et al. 2014). After the complete genome was assembled, BLAST analysis was performed to identify the species that showed similarity to each scaffold. The completeness of strain J17-4 genome assembly was evaluated using the benchmarking universal single-copy orthologs (BUSCO v3.0) with bacilli_odb10 as a reference, based on evolutionarily informed expectations of gene content from near-universal single-copy orthologs (Simão et al. 2015). The analysis showed 299 complete and single-copy BUSCOs (99.01%) and one missing BUSCO (0.33%), indicating that strain J17-4 was a genome with high completeness (99.34% complete).

Protein-coding regions were predicted using Prokka (version 1.11) or NCBI prokaryotic genome annotation pipeline (Seemann 2014; Tatusova et al. 2016). Noncoding RNAs such as ribosomal RNAs (rRNAs) were predicted using rRNAmmer (version 1.2) (Lagesen et al. 2007), while transfer RNAs (tRNA) and small RNAs were identified by tRNAscan (version 1.3.1) and cmScan (version 1.1.2) (Lowe and Chan 2016; Nawrocki and Eddy 2013), respectively. Genomic islands were predicted using IslandViewer (version 4) (Bertelli et al. 2017). Interspersed and tandem repeat elements were identified by RepeatMasker (version 4.0.5) and TRF (Benson 1999; Tarailo-Graovac and Chen 2009), respectively. Secretory protein was predicted using SignalP4.0 (Petersen et al. 2011). Diverse protein databases, including

the NCBI nonredundant protein database, UniProt/Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes, gene ontology, and cluster of orthologous groups of proteins were applied to annotate functional protein categories. The secondary metabolite gene clusters were predicted using antiSMASH (version 4.1.0) (Blin et al. 2017).

The genome of *B. velezensis* strain J17-4 contained only one chromosome with no plasmid. The chromosome of strain J17-4 was 3,929,768 bp in length with 3,877 coding sequences. The additional features of this genome are summered in Table 1. Nine types of gene clusters involved in secondary metabolite production were predicted by genome analysis using antiSMASH. Twelve gene clusters were predicted associated with secondary metabolite production, including one nonribosomal peptide (nrps), two terpene, one T3pks, two transatpks, two transatpks-nrps, one bacteriocin-nrps, one otherks, and one other.

In the present study, we provide the complete genome sequence of *B. velezensis*, a valuable biocontrol agent isolated from healthy and sterilized rice stems. The genome of strain J17-4 represents a new, useful source for future research on comparative genomics studies among stains separated from various environments.

Data Availability

The pure culture of *B. velezensis* J17-4, sequenced in this study, is preserved in the Chinese Culture Collection Center (accession number M2020308), Wuhan, China. The whole genomic data obtained in this study are both available at GenBank under BioProject accession number PRJNA657983 and Bio Sample ID SAMN15854525. The genome accession number described in this article is CP060420.

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