



Genome Note

Draft genome sequence of broad-spectrum antifungal-producing *Bacillus velezensis* C4341 isolated from a saline-alkali soil sample in China



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ABSTRACT

Objectives: Bacterial micro-organisms from the genus *Bacillus* have a broad spectrum of antimicrobial activity against plant pathogenic bacteria, fungi and viruses. *Bacillus velezensis* has been extensively used as a biocontrol agent in the agricultural field. In the present study, *B. velezensis* strain C4341 was isolated from saline-alkali soil in Xinjiang, China, producing a wide range of antifungal compounds of different structures.

Methods: The whole genome of *B. velezensis* C4341 was sequenced using an Illumina HiSeq™ 2000 sequencing system, was assembled using SOAPdenovo and was optimised with GapCloser.

Results: The genome size of strain C4341 was calculated at 3 963 750 bp with a G+C content of 46.4%. The whole genome was assembled into 4019 open reading frames (ORFs), of which 5.9% and 1.6% were related to antagonistic secondary metabolites and antimicrobial resistance, respectively.

Conclusion: The whole genome sequence of *B. velezensis* C4341 will be beneficial in comparative analysis of the genus *Bacillus* and shows future prospects in antifungal application for sustainable agriculture.

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Emerging plant diseases caused by soil-borne pathogens pose a threat to crop output, resulting in a remarkable reduction in the yield of profit crops and consequent huge economic losses worldwide every year [1]. Chemical antibiotics have been intensively used to suppress plant pathogens in agriculture, but unfortunately their long-term overuse has inevitably led to the emergence of antimicrobial-resistant pathogens. Therefore, use of biocontrol agents, taking the genus *Bacillus* as an example, has been regarded as one of the most promising alternatives because of the ability of these micro-organisms to secrete a range of structurally different secondary metabolites that exhibit antibacterial and/or antifungal activities with reduced pathogen resistance [2].

For several decades, *Bacillus velezensis*, a recently re-classified synonym of *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens*

subsp. *plantarum* and *Bacillus oryzicola*, has been widely applied as an antimicrobial agent in the agricultural sector [3]. Molecular research confirmed that many genetic regions in *B. velezensis* were linked to the synthesis of antagonistic substances [4]. However, details of the genetic functional basis associated with the biocontrol effect are still unclear due to the few reports referring to the complete genomes of *B. velezensis* strains. Recently, we have isolated a new strain from saline-alkali soil samples from Xinjiang Uygur Autonomous Region, China, identified as *B. velezensis* on the basis of 16S DNA sequence analysis, and finally named *B. velezensis* C4341. Antagonism tests on *B. velezensis* C4341 showed strong efficacy against the green mould *Trichoderma aggressivum*, with an inhibition zone of mycelial growth of 4.4 ± 0.7 mm.

Genomic DNA was extracted from *B. velezensis* C4341 cultivated at 37 °C in Luria–Bertani medium using an E.Z.N.A.® Bacterial DNA Kit (Omega Bio-Tek Inc., Norcross, GA), and the whole genome was sequenced using an Illumina HiSeq™ 2000 next-generation sequencing platform (Shanghai Majorbio Pharmaceutical Technology Co., Ltd., Shanghai, China). Using a Paired-End DNA Sample

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Prep Kit (Illumina Inc., Cambridge, UK), a 400-bp library for Illumina paired-end sequencing was constructed according to the manufacturer's recommendations. The reads were de novo assembled with SOAPdenovo v.2.04, obtaining an N_{50} value of 402 239. Open reading frames (ORFs) were identified by Glimmer v.3.02 and were further annotated by comparison with NCBI-NR, COG, GO, KEGG, EggNOG and SwissProt databases. rRNAs and tRNAs were predicted using barrnap v.0.8 and tRNAscan v.2.0, respectively. Antimicrobial resistance genes were analysed using DARD databases, with a 99% threshold for gene identification.

A total of 6 665 440 paired-end reads were produced with $100\times$ coverage. The whole genome size was calculated at 3 963 750 bp, which was assembled into 105 contigs and 89 scaffolds (>500 bp length), with an average G+C content of 46.4% and an N rate of 0.006%. No plasmids were detected. The number of candidate protein-coding sequences was 4019, the length of which accounted for ca. 87.4% of the whole genome. Overall, the genome comprises 29 tRNA genes and only 1 rRNA region, which is a 23S rRNA region. IslandPath-DIOMB

v.0.2 software was used to reveal at least six genomic islands. The genome map was finally displayed using Circos v.0.64 (Fig. 1).

Based on whole-genome analysis, *B. velezensis* C4341 was predicted to encode 13 putative gene clusters responsible for antimicrobial metabolite biosynthesis using antiSMASH v.4.02 and by manual verification, which was equivalent to >5.9% of the whole genome. Of these, eight gene clusters encode non-ribosomal peptide synthetases (NRPS) (one bacillibactin, one iturin, five surfactin and one fengycin), five encode ribosomal peptides (RPS) (one microcins and four lantipeptide), and one related to biosynthesis of terpene, which is similar to *Bacillus* strains reported elsewhere [5]. In addition, the gene clusters have the ability to produce several polyketide synthetases (PKS), such as oleandomycin, bicyclomycin, tetracenomycin, pristnamycin, lincomycin, β -lactamase and tetracycline, potentially suppressing the growth of Gram-positive bacteria. Genes associated with biofilm formation, iron acquisition, colonisation and volatile organic compound synthesis, which play important roles during the

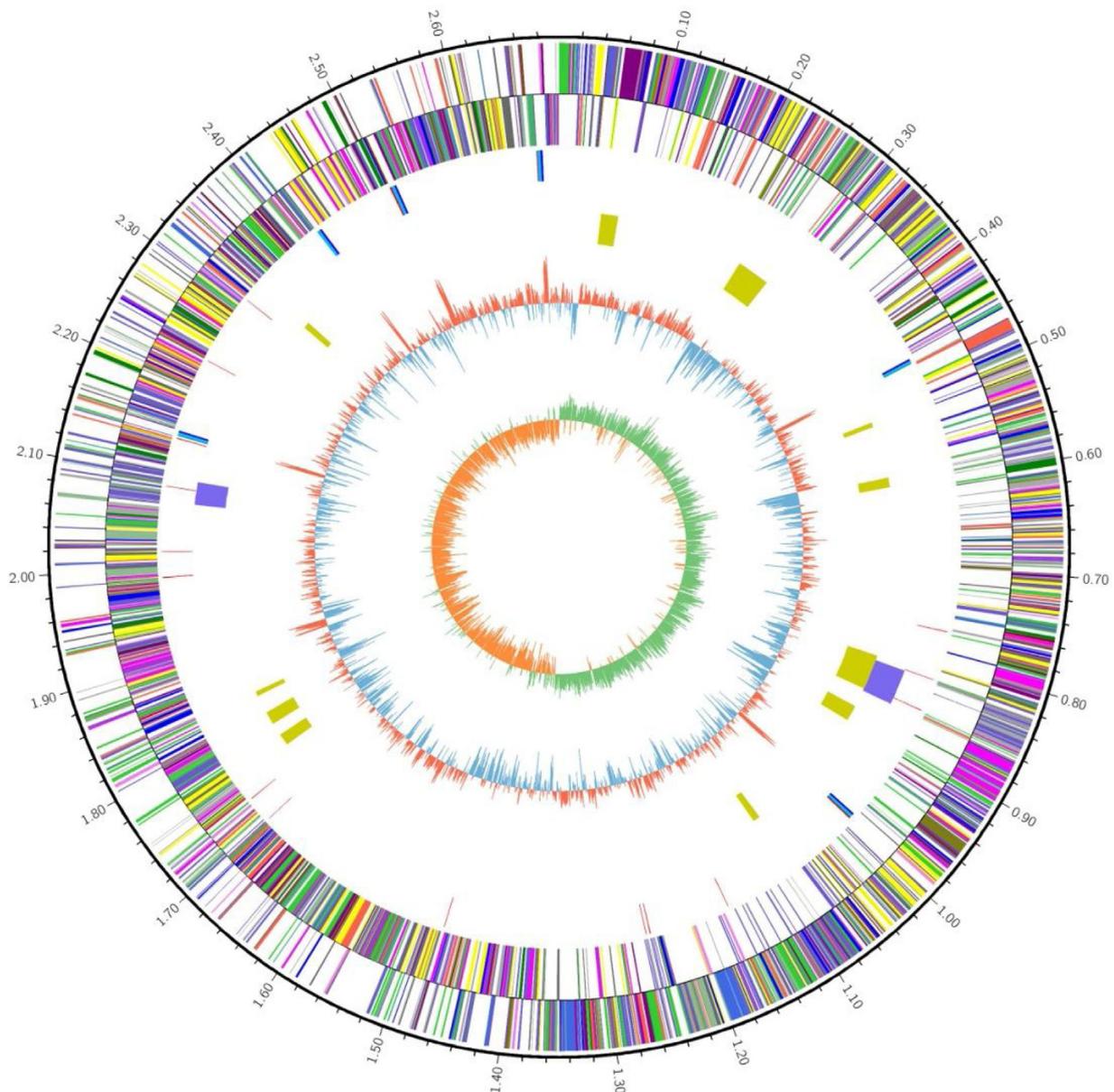


Fig. 1. Circular map of genomic features of the whole genome of *Bacillus velezensis* C4341. The outermost circle is the location of the genome sequence coordinates, and from outside to inside, respectively, coding genes, gene function annotation results, rRNA and tRNA, genomic GC content and genome GC skew value distribution.

biocontrol process, were found in the genome of *B. velezensis* C4341. Moreover, there are a large number of multidrug resistance genes (>1.6% of the whole genome), such as *ykkCD*, *blt*, *norM*, *yoeA*, *ebrAB* and *clbAB*, as well as multidrug transporters, such as *lacEF*, *mgtC* and ABC-type multidrug transport system and a Na⁺-driven multidrug efflux pump.

In brief, this is the first report on the isolation of a new broad-spectrum antifungal-producing strain of the genus *Bacillus* from saline-alkali soil in Xinjiang. Whole-genome sequencing of strain C4341 was aimed at finding correlative proteins affecting antagonistic secondary metabolites and drugs or multidrug resistance. Without a doubt, these genome features make strain C4341 an excellent candidate for biocontrol agents. The above results also provide ample information for deeper research on drug/multidrug resistance mechanisms in *B. velezensis*.

Nucleotide sequence accession no.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession no. **QZCR00000000**. The version described in this paper is version **QZCR01000000**. The BioProject ID in GenBank is **PRJNA491791**.

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Competing interests

None declared.

Ethical approval

Not required.

References

- [1] De Coninck B, Timmermans P, Vos C, Cammue BP, Kazan K. What lies beneath: belowground defense strategies in plants. *Trends Plant Sci* 2015;20:91–101.
- [2] Zhao X, Kuipers OP. Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genomics* 2016;17:882.
- [3] Dunlap CA, Kim SJ, Kwon SW, Rooney AP. *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens*; *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens* subsp. *plantarum* and '*Bacillus oryzicola*' are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics. *Int J Syst Evol Microbiol* 2016;66:1212–7.
- [4] Rückert C, Blom J, Chen X, Reva O, Borriss R. Genome sequence of *B. amyloliquefaciens* type strain DSM7^T reveals differences to plant-associated *B. amyloliquefaciens* FZB42. *J Biotechnol* 2011;155:78–85.
- [5] Kim SY, Lee SY, Weon HY, Sang MK, Song J. Complete genome sequence of *Bacillus velezensis* M75, a biocontrol agent against fungal plant pathogens, isolated from cotton waste. *J Biotechnol* 2017;241:112–5.