Genome Sequence Resource of *Bacillus velezensis* EB14, a Native Endophytic Bacterial Strain with Biocontrol Potential Against the Poplar Stem Canker Causative Pathogen, *Sphaerulina musiva*

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Abstract

Bacillus velezensis EB14, isolated from a leaf of *Populus × jackii*, possesses antagonistic activity against *Sphaerulina musiva*, a fungal pathogen of *Populus* sp. that causes leaf spots and stem cankers on poplars, limiting the utility of hybrid poplars as plantation trees. We sequenced the genome of *B. velezensis* EB14 to gain insights into the underlying basis of its antagonistic activity. Here, we report the complete genome sequence of *B. velezensis* EB14, a gram-positive bacterium of the family *Bacillaceae*. Through antiSMASH analysis, we predicted several gene clusters coding for the biosynthesis of antimicrobial compounds and several genes involved in plant bacterial interactions. These findings support the potential of developing *B. velezensis* EB14 as a biocontrol agent against *S. musiva* in poplar plantations. The genome of *B. velezensis* EB14 along with genome sequences of closely related *B. velezensis* species are invaluable for comparative genomic analyses to gain insights into bacterial, fungal, and host plant interactions.

We isolated *Bacillus velezensis* EB14, an endophytic bacterial strain from the leaves of *Populus × jackii*, and found that *B. velezensis* EB14 is antagonistic to *Sphaerulina musiva*, which causes leaf spot and stem canker in hybrid poplars that limit the use of hybrid poplars in forest plantations. The *B. velezensis* EB14 strain inhibits the growth of the fungal pathogen *S. musiva* through production of several iturinic compounds including Iturin A1, Subtulene A, Iturin A2, Iturin A9, and Fengycin (N. Sachin, S. Palys, D. F. Marcos, A. Tsang, P. Périnet, R. Uma Shaanker, and S. Dayanandan, *unpublished data*). Members of *B. velezensis* belong to the “operational group *B. amyloliquefaciens*” which includes two plant-associated species, *B. amyloliquefaciens* and *B. siamensis* (Fan et al. 2017). Members of the *B. velezensis* species have been isolated from diverse niches ranging from the rhizosphere to the phyllosphere. Following the initial isolation of the *B. velezensis* type strains CR-502T and CR-14b from brackish water samples from the river Velez in southern Spain (Ruiz-García et al. 2005), several strains of *B. velezensis* have been reported from a variety of niches and their genomes show a close evolutionary relatedness (Adeniji et al. 2019; Rabbee et al. 2019). *B. velezensis* PEBA20, isolated from *Populus* stem, contains several genes related to plant growth promotion (Kong et al. 2018), and *B. velezensis* NJN6, isolated from *Musa* sp., produces many iturinic compounds (Zhang et al. 2016). *B. velezensis* RC 218, isolated from wheat anthers, possesses antagonistic activity against *Fusarium graminearum*, the causal agent of Fusarium head blight

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Keywords

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Table 1. Genome summary statistics of *Bacillus velezensis* EB14

Genomic features	Results
Size (bp)	4,007,882
GC content (%)	46.5
Number of contigs (with PEGs)	1
Number of subsystems	346
Number of coding sequences	4,567
Number of RNAs	114
Transfer RNAs	87
16S rRNA copy numbers	9
Hypothetical proteins	801
Proteins with functional assignments	3,766
Benchmarking universal single-copy orthologs (BUSCO)	C:89.2% [S:85.2%, D:0.0%], F:9.7%, M:1.1%, n:526

and known to produce a secondary metabolite, lantibiotic ericin, unique to this strain (Palazzini et al. 2016). *B. velezensis* FZB42 is considered the model strain for growth-promoting *Bacilli* (Chen et al. 2007).

We sequenced the *B. velezensis* EB14 genome to determine its relatedness to other *B. velezensis* strains and gain insights into the underlying molecular basis of their antagonistic activity. The resulting genome sequence data are invaluable for genomic analysis in comparison with its closest relatives to discern unique features of this strain and gain insights into the basis of antagonism against the fungal pathogen *S. musiva*. In addition, Pan and Core genome analysis will provide detailed understanding of the molecular basis for the association of *B. velezensis* with diverse niches.

For sequencing the genome, we cultured *B. velezensis* EB14 on Luria-Bertani (LB) agar and a single colony was used for inoculating 3 ml of LB broth and incubated at 30°C in a shaker at 200 rpm. Then, 1 ml of the overnight culture was inoculated into 50 ml of LB broth at 30°C and 200 rpm for 6 h. About 1,800 microliters of the culture was centrifuged twice at 10,000 × *g* for 30 s, and the pellet was used for DNA extraction. DNA was extracted using the MO BIO (MO BIO Laboratories, Inc.) UltraClean Microbial DNA Isolation kit following the manufacturer's protocol (Catalog number 12224-50). The whole genome was sequenced using the Pacific Biosciences (PacBio) RS II platforms at the Genome Quebec Innovation Centre. The PacBio reads were assembled using the RS hierarchical genome assembly process (HGAP) version 3.0 in SMRT Analysis version 2.2.0 (Pacific Biosciences, Menlo Park, CA). The completeness of the genome assembly was assessed in BUSCO (benchmarking universal single-copy orthologs) with the Firmicutes BUSCO lineage (Simão et al. 2015). The genome completeness of the BUSCO notation are C:89.2% [S:85.2%, D:0.0%], F:9.7%, M:1.1%, n:526. An assembled genome was submitted to the comprehensive genome analysis service at PATRIC (Wattam et al. 2017), and the results are summarized in Table 1.

B. velezensis EB14 genome consists of 4,007,882 bp, with an average GC content of 46.5%. Genome annotation was performed using the RAST tool kit (RASTtk) (Brettin et al. 2015). This genome has 4,567 protein-coding sequences, 87 transfer RNA genes, and 27 ribosomal RNA (rRNA) genes. The annotation included nine 16S rRNA genes, 801 hypothetical proteins, and 3,766 proteins with functional assignments.

In silico analysis of the genome via antiSMASH version 5.0.0 tool (Blin et al. 2019) revealed 12 potential gene clusters that encode for the biosynthesis of surfactin, rhizoactin, bacillibactin, fengycin, and bacillaene, and the antibiotics difficidin, macrolactin, and bacilysin. These secondary metabolites may possess antifungal activity against fungal pathogens.

Proteins involved in plant bacterial interactions were retrieved from the Amylowiki database for *B. velezensis* FZB42, the model strain for plant growth-promoting *Bacilli* (Chen et al. 2007) and analyzed in PATRIC protein family sorter (Wattam et al. 2017), which predicted 550 proteins in the *B. velezensis* EB14 strain involved in plant bacterial interactions. Some of these encode for biofilm formation (*yusV*), indole-3-acetic acid synthesis (*yhcX*, *bsdB*, and *ysnE*), modular polyketide synthases (*dfnH*, *dfnA*, *mlnC*, and *mlnF*), and control of plant pathogens (*dfnB*). In summary, the *B. velezensis* EB14 genome, a native endophytic bacterium of *Populus*, contains

multiple gene clusters predicted to be involved in stimulating plant growth and suppress plant pathogens.

Data availability. The complete genome sequence of strain EB14 can be accessed from NCBI Bioproject ID PRJNA681597.

Literature Cited

- Adeniji, A. A., Loots, D. T., and Babalola, O. O. 2019. *Bacillus velezensis*: Phylogeny, useful applications, and avenues for exploitation. Appl. Microbiol. Biotechnol. 103: 3669-3682.
- Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N., Lee, S. Y., Medema, M. H., and Weber, T. 2019. antiSMASH 5.0: Updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res. 47:W81-W87.
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., et al. 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci. Rep. 5:3865.
- Chen, X. H., Koumoutsis, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I., et al. 2007. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nat. Biotechnol. 25: 1007-1014.
- Fan, B., Blom, J., Klenk, H. P., and Borriss, R. 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. Front. Microbiol. 8:1-15.
- Kong, W. J., Yan, Y. C., Li, X. Y., and Liu, Z. Y. 2018. Draft genome sequence of *Bacillus velezensis* PEBA20, a strain with a plant growth promoting effect and biocontrol potential. Genome Announc. 6:e00286-18.
- Palazzini, J. M., Dunlap, C. A., Bowman, M. J., and Chulze, S. N. 2016. *Bacillus velezensis* RC 218 as a biocontrol agent to reduce Fusarium head blight and deoxynivalenol accumulation: Genome sequencing and secondary metabolite cluster profiles. Microbiol. Res. 192:30-36.
- Rabbee, M. F., Sarafat Ali, M., Choi, J., Hwang, B. S., Jeong, S. C., and Baek, K.-H. 2019. *Bacillus velezensis*: A valuable member of bioactive molecules within plant microbiomes. Molecules 24:1046.
- Ruiz-García, C., Béjar, V., Martínez-Checa, F., Llamas, I., and Quesada, E. 2005. *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Vélez in Málaga, southern Spain. Int. J. Syst. Evol. Microbiol. 55:191-195.
- Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. 2015. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210-3212.
- Wattam, A. R., Davis, J. J., Assaf, R., Boisvert, S., Brettin, T., Bun, C., Conrad, N., Dietrich, E. M., Disz, T., and Gabbard, J. L., et al. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res. 45:D535-D542.
- Zhang, N., Yang, D., Kendall, J. R. A., Borriss, R., Druzhinina, I. S., Kubicek, C. P., et al. 2016. Comparative genomic analysis of *Bacillus amyloliquefaciens* and *Bacillus subtilis* reveals evolutionary traits for adaptation to plant-associated habitats. Front. Microbiol. 7:7.