| Bacteriology | Announcement

Draft genome sequence of the fungal biocontrol agent, *Bacillus velezensis* Kos

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ABSTRACT   Here, we report the draft genome sequence of *Bacillus velezensis* strain Kos, isolated from casing soil used during *Agaricus bisporus* cultivation in Dublin, Ireland. *B. velezensis* Kos exhibits a suppressive ability toward *Cladobotryum mycophilum*, *Trichoderma aggressivum*, and *Lecanicillium fungicola*, which are common threats to *A. bisporus* production, cultivation, and quality.

S everal fungal pathogens pose a significant threat to the commercially important white mushroom, *Agaricus bisporus* (1). Historically, chemical fungicides have been used to prevent yield reductions and disease outbreaks. Due to environmental/health considerations, there is now pressure to reduce fungicide use (2). The future of mushroom disease treatment will depend upon integrated pest management, including the use of biological control agents (BCAs) (3). Here we report the draft genome sequence of the novel strain *Bacillus velezensis* Kos, which has been shown to have potential as a BCA for mushroom disease (4–6).

The Kos strain was originally isolated during *A. bisporus* cultivation in Dublin, Ireland (global positioning system coordinates 53.38 N 6.33 W"), and taxonomical identification showed that Kos is *Bacillus velezensis* (6), which we further confirmed using the average nucleotide identity (ANI) (see below). *B. velezensis* Kos plate cultures were grown on nutrient agar (Thermo Scientific Oxoid) at 30°C for 24 h. A loopful of culture from plate cultures was added to 50-mL nutrient broth (Thermo Scientific Oxoid) and grown for 24 h at 30°C, 120 rpm. Genomic DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research). The genome was sequenced by Novogene Co. Ltd., with the DNA library prepared using the Novogene NGS DNA library prep set in which the DNA was randomly sheared, end repaired, A-tailed, and then ligated with Illumina adaptors. These sequences were amplified using PCR, and DNA of 350 bp was selected, purified, and sequenced using 150-bp Illumina paired-end sequencing on the Illumina NovaSeq platform. Reads with adapters and low quality were trimmed using Skewer (v.0.2.2) (7).

In total 12,619,076 high-quality paired-end reads were obtained and initially assembled and then annotated using National Center for Biotechnology Information's Read Assembly and Annotation Pipeline Tool with default settings (rapt-45639894). RAPT utilizes the SKESA (v.2.5.1) genome assembler (8), the ANI tool (9) to assign taxonomy and the Prokaryotic Genome Annotation Pipeline (build6771) (10) to functionally annotate the assembly. Genome quality and potential contamination are also assessed using CheckM (v.2015-01-16) (11). In total, the assembly size is 4,194,762 nucleotides in length with a GC content of 45.8%. The N50 and L50 scores are 573,424 and 3, respectively. CheckM showed 98.82% genome completeness and 0% contamination. The longest contig is 1,085,863 nucleotides, and there are 30 contigs in total. The ANI with its

closest strain, *B. velezensis* NRRL B-41580 (12), was 98.264%. A total of 4,248 genes were predicted, including 4,066 protein-coding genes, 99 RNA genes (12 rRNAs, 82 tRNA, and 5 noncoding RNA genes), and 83 pseudogenes. The potential production of secondary metabolites by Kos was analyzed using the antiSMASH tool (v.7.0) with the default setting (13). Genomic clusters with the potential for the biosynthesis of antimicrobial secondary metabolites were predicted. These clusters involve genes encoding surfactin, subtilin, bacillibactin, bacilysin, fengycin, bacillaene, and macrolactin.

The draft genome sequence of *B. velezensis* Kos will help uncover the molecular mechanisms of pathogen suppression and increase its applications in the mushroom industry.

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DATA AVAILABILITY

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession number JBDOQF000000000. The version described in this paper is version JBDOQF010000000. The raw Illumina reads are available at ENA/SRA under accession number SRX24592991.

REFERENCES

1. Fletcher JT, Gaze RH. 2008. Mushroom pest and disease control: a color handbook. Elsevier.
2. Grogan HM. 2008. Challenges facing mushroom disease control in the 21st century, p 120–127.GAMU GmbH, Institut für Pilzforschung, Krefeld
3. Barzman M, Bàrberi P, Birch ANE, Boonekamp P, Dachbrodt-Saaydeh S, Graf B, Hommel B, Jensen JE, Kiss J, Kudsk P, Lamichhane JR, Messéan A, Moonen A-C, Ratnadass A, Ricci P, Sarah J-L, Sattin M. 2015. Eight principles of integrated pest management. Agron Sustain Dev 35:1199–1215. https://doi.org/10.1007/s13593-015-0327-9
4. Clarke J, Grogan H, Fitzpatrick D, Kavanagh K. 2022. Analysis of the effect of *Bacillus velezensis* culture filtrate on the growth and proteome of *Cladobotryum mycophilum*. Fungal Biol 126:11–19. https://doi.org/10.1016/j.funbio.2021.09.003
5. Clarke J, Grogan H, Fitzpatrick D, Kavanagh K. 2022. Characterising the proteomic response of mushroom pathogen *Lecanicillium fungicola* to *Bacillus velezensis* QST 713 and Kos biocontrol agents. Eur J Plant Pathol 163:369–379. https://doi.org/10.1007/s10658-022-02482-1
6. Kosanovic D, Dyas M, Grogan H, Kavanagh K. 2021. Differential proteomic response of *Agaricus bisporus* and *Trichoderma aggressivum* f. *europaeum* to *Bacillus velezensis* supernatant. Eur J Plant Pathol 160:397–409. https://doi.org/10.1007/s10658-021-02252-5
7. Jiang H, Lei R, Ding S-W, Zhu S. 2014. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinformatics 15:182. https://doi.org/10.1186/1471-2105-15-182
8. Souvorov A, Agarwala R, Lipman DJ. 2018. SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol 19:153. https://doi.org/10.1186/s13059-018-1540-z
9. Ciufo S, Kannan S, Sharma S, Badretdin A, Clark K, Turner S, Brover S, Schoch CL, Kimchi A, DiCuccio M. 2018. Using average nucleotide identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. Int J Syst Evol Microbiol 68:2386–2392. https://doi.org/10.1099/ijsem.0.002809
10. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI

prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569

11. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114

12. Huang W, Qu L, Gao P, Du G. 2023. Bioassay and whole-genome analysis of *Bacillus velezensis* FIO1408, a biocontrol agent against pathogenic bacteria in aquaculture. Curr Microbiol 80:354. https://doi.org/10.1007/s00284-023-03423-9

13. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW, Helfrich EJN, van Wezel GP, Medema MH, Weber T. 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Res 51:W46–W50. https://doi.org/10.1093/nar/gkad344