





Draft Genome Sequence of Biocontrol Agent Bacillus cereus UW85

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Bacillus cereus UW85 was isolated from a root of a field-grown alfalfa plant from Arlington, WI, and identified for its ability to suppress damping off, a disease caused by *Phytophthora megasperma* f. sp. medicaginis on alfalfa. Here, we report the draft genome sequence of B. cereus UW85, obtained by a combination of Sanger and Illumina sequencing.

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acillus cereus is a Gram-positive, ubiquitous spore-forming bacterium present in the soil, rhizosphere, and guts of several invertebrates (1). B. cereus UW85 was identified from 700 bacterial isolates from the roots of field-grown alfalfa plants. The collection was screened for the suppression of damping off caused by the oomycete Phytophthora megasperma f. sp. medicaginis on alfalfa seedlings (2). Damping off is characterized by browning of stem and root tissues, followed by girdling and seedling death. B. cereus UW85 produces two antibiotics, zwittermicin A (3) and kanosamine (4), which each exhibit broad-spectrum antimicrobial activity that contributes to the suppression of alfalfa seedling damping off, as demonstrated by analysis of mutants deficient in antibiotic production (4, 5). B. cereus UW85 can also protect tobacco seeds from infection by Phytophthora parasitica var. nicotianae (6) and cucumber fruit cotton leak, a disease caused by Phytophthora aphanidermatum (7). In the field, B. cereus UW85 enhances soybean nodulation (8) and can significantly increase the yield of several soybean cultivars (9). In addition, the presence of B. cereus UW85 on soybean seeds shapes the microbial community that develops subsequently in the rhizosphere (10).

The B. cereus UW85 genome was first sequenced using Sanger sequencing of a small-insert library (4 to 5 kb) and a large-insert library (10 to 12 kb), generating a total of ~51,000 reads, which were assembled using the Celera Assembler software (https: //sourceforge.net/projects/wgs-assembler/) (11) into 271 contigs. Contigs were designated as originating from the chromosome or a plasmid with a BLAST comparison to the reference B. cereus isolates ATCC 10987 and ATCC 14579. Chromosomal contigs were ordered by Mauve (12) using the B. cereus ATCC 14579 genome (13) as a reference. A similar approach was used to assemble and order the plasmid contigs using several B. cereus group plasmids as references (14). Contigs were assembled manually by joining neighboring sequences with a linker sequence of unknown nucleotide characters labeled N. Gaps were filled using GapFiller (15) with 9,489,450 paired-end reads of 300 bp from an ~1-kb library generated on an Illumina MiSeq instrument, creating a merged assembly with both Sanger and Illumina data. The resulting assembled chromosome was 5,522,108 bp, consisting of 23 contigs,

with an N_{50} contig size of 240,092 bp. Thirty-one contigs accounting for 881,969 bp, with an N_{50} contig size of 28,451 bp, showed greater similarity with B. cereus plasmid sequences. B. cereus is known to have an extremely varied plasmid profile, with strains carrying mixtures of plasmids ranging in size from 15 to 600 kb (16, 17). The zwittermicin A gene cluster (18, 19) is on one of the larger plasmid contigs, PC_11, which is ~150 kb. A second large plasmid, PC_30, is ~225 kb, has a region of similarity to the Ba-cillus anthracis pXO1 plasmid, and carries an uncharacterized gene cluster with similarity to a cluster encoding biosynthetic pathways for nonribosomal peptide synthesis machinery.

We anticipate that the genome sequence of *B. cereus* UW85, one of the best-characterized biocontrol agents, will facilitate discoveries about its plant growth-promoting activity, disease-suppressive properties, and potential for producing new antibiotics.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. LYVD000000000. The version described in this paper is version LYVD01000000.

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REFERENCES

- Ceuppens S, Boon N, Uyttendaele M. 2013. Diversity of *Bacillus cereus* group strains is reflected in their broad range of pathogenicity and diverse ecological lifestyles. FEMS Microbiol Ecol 84:433–450. http://dx.doi.org/10.1111/1574-6941.12110.
- Handelsman J, Raffel S, Mester EH, Wunderlich L, Grau CR. 1990. Biological control of damping-off of alfalfa seedlings with *Bacillus cereus* UW85. Appl Environ Microbiol 56:713–718.
- 3. He H, Silo-Suh LA, Handelsman J, Clardy J. 1994. Zwittermicin A, an

- antifungal and plant protection agent from *Bacillus cereus*. Tetrahedron Lett 35:2499–2502. http://dx.doi.org/10.1016/S0040-4039(00)77154-1.
- Milner JL, Silo-Suh L, Lee JC, He H, Clardy J, Handelsman J. 1996. Production of kanosamine by *Bacillus cereus* UW85. Appl Environ Microbiol 62:3061–3065.
- Silo-Suh LA, Stabb EV, Raffel SJ, Handelsman J. 1998. Target range of zwittermicin A, an aminopolyol antibiotic from *Bacillus cereus*. Curr Microbiol 37:6–11. http://dx.doi.org/10.1007/s002849900328.
- Handelsman J, Nesmith WC, Raffel SJ. 1991. Microassay for biological and chemical control of infection of tobacco by *Phytophthora parasitica* var. *nicotianae*. Curr Microbiol 22:317–319. http://dx.doi.org/10.1007/ BF02091961.
- Smith KP, Havey MJ, Handelsman J. 1993. Suppression of cottony leak of cucumber with *Bacillus cereus* strain UW85. Plant Dis 77:139–142. http://dx.doi.org/10.1094/PD-77-0139.
- Halverson LJ, Handelsman J. 1991. Enhancement of soybean nodulation by *Bacillus cereus* UW85 in the field and in a growth chamber. Appl Environ Microbiol 57:2767–2770.
- Osburn RM, Milner JL, Oplinger ES, Smith RS. 1995. Effect of *Bacillus cereus* UW85 on the yield of soybean at two field sites in Wisconsin. Plant Dis 79:551–576. http://dx.doi.org/10.1094/PD-79-0551.
- Halverson LJ, Clayton MK, Handelsman J. 1993. Variable stability of antibiotic-resistance markers in *Bacillus cereus* UW85 in the soybean rhizosphere in the field. Mol Ecol 2:65–78. http://dx.doi.org/10.1111/j.1365 -294X.1993.tb00001.x.
- 11. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, et al. 2001. The sequence of the human genome. Science 291:1304–1351. http://dx.doi.org/10.1126/science.1058040.
- 12. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple

- alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. http://dx.doi.org/10.1101/gr.2289704.
- 13. Ivanova N, Sorokin A, Anderson I, Galleron N, Candelon B, Kapatral V, Bhattacharyya A, Reznik G, Mikhailova N, Lapidus A, Chu L, Mazur M, Goltsman E, Larsen N, D'Souza M, Walunas T, Grechkin Y, Pusch G, Haselkorn R, Fonstein M, Ehrlich SD, Overbeek R, Kyrpides N. 2003. Genome sequence of *Bacillus cereus* and comparative analysis with *Bacillus anthracis*. Nature 423:87–91. http://dx.doi.org/10.1038/nature01582.
- 14. Rasko DA, Rosovitz MJ, Økstad OA, Fouts DE, Jiang L, Cer RZ, Kolstø AB, Gill SR, Ravel J. 2007. Complete sequence analysis of novel plasmids from emetic and periodontal *Bacillus cereus* isolates reveals a common evolutionary history among the *B. cereus*-group plasmids, including *Bacillus anthracis* pXO1. J Bacteriol 189:52–64.
- 15. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with Gap-Filler. Genome Biol 13:R56. http://dx.doi.org/10.1186/gb-2012-13-6-r56.
- Helgason E, Caugant DA, Olsen I, Kolstø AB. 2000. Genetic structure of population of *Bacillus cereus* and *B. thuringiensis* isolates associated with periodontitis and other human infections. J Clin Microbiol 38:1615–1622.
- 17. Han CS, Xie G, Challacombe JF, Altherr MR, Bhotika SS, Brown N, Bruce D, Campbell CS, Campbell ML, Chen J, Chertkov O, Cleland C, Dimitrijevic M, Doggett NA, Fawcett JJ, Glavina T, Goodwin LA, Green LD, Hill KK, Hitchcock P, Jackson PJ, Keim P, Kewalramani AR, Longmire J, Lucas S, Malfatti S, McMurry K, Meincke LJ, Misra M, Moseman BL, Mundt M, Munk AC, Okinaka RT, Parson-Quintana B, Reilly LP, Richardson P, Robinson DL, Rubin E, Saunders E, Tapia R, Tesmer JG, Thayer N, Thompson LS, Tice H, Ticknor LO, Wills PL, Brettin TS, Gilna P. 2006. Pathogenomic sequence analysis of Bacillus cereus and Bacillus thuringiensis isolates closely related to Bacillus anthracis. J Bacteriol 188:3382–3390. http://dx.doi.org/10.1128/JB.188.9.3382-3390.2006.
- 18. Emmert EA, Klimowicz AK, Thomas MG, Handelsman J. 2004. Genetics of zwittermicin A production by *Bacillus cereus*. Appl Environ Microbiol 70:104–113. http://dx.doi.org/10.1128/AEM.70.1.104-113.2004.
- Kevany BM, Rasko DA, Thomas MG. 2009. Characterization of the complete zwittermicin A biosynthesis gene cluster from *Bacillus cereus*. Appl Environ Microbiol 75:1144–1155. http://dx.doi.org/10.1128/ AEM.02518-08.