PROKARYOTES



Whole-Genome Sequence of *Bacillus cereus* AR156, a Potential Biocontrol Agent with High Soilborne Disease Biocontrol Efficacy and Plant Growth Promotion

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ABSTRACT *Bacillus cereus* AR156 was originally isolated from the forest soil of Zhenjiang, a city in China. To shed new light on the molecular mechanisms underlying the biological control of soilborne pathogens, the whole genome of this strain was sequenced. Here, we report the draft genome sequence of this strain, consisting of a single circularized contig measuring 5.66 Mb, with an average GC content of 35.5% and 5,367 open reading frames.

acillus cereus AR156 is a plant growth-promoting rhizobacterium, originally isolated m D from a garden tree soil rhizosphere in Nanjing, China, that could protect tomato plants against bacterial wilt caused by Ralstonia solanacearum and the root knot nematode Meloidogyne incognita. We previously found that B. cereus AR156 can induce systemic resistance to Pseudomonas syringae pv. tomato DC3000 by simultaneously activating the SA and JA/ET signaling pathways via an NPR1-dependent mechanism (1-3). The biocontrol mechanisms of B. cereus AR156 are currently unclear. To better understand the biocontrol and plant growth-promoting mechanisms of B. cereus AR156, the whole genome of the strain was determined by 454 sequencing. Total DNA was prepared as described in the GS FLX Titanium general library preparation kit (454 sequencing) and then sequenced according to the GS FLX Titanium sequencing method manual. The 239,826 generated reads were assembled into contigs using the GS de novo assembler. The genome coverage, which was based on the sequenced DNA, was found to be approximately 15.3-fold. Primer walking and PCR-based techniques were applied to close the remaining gaps. All of the manual editing steps were performed using the Consed software package (4, 5). Prediction of the operons in the B. cereus AR156 chromosomes was performed with an algorithm that combines intergenic distance and phylogenetic information (6), and tRNAs and transfer-messenger RNAs (tmRNAs) were predicted using the ARAGORN program (7); rRNA was obtained by using RNAmmer version 1.2 (8).

The 5.66-Mb *B. cereus* AR156 genome was composed of four replicons, a circular chromosome that encodes 5,367 open reading frames (ORFs) and three plasmids, pAR460, pAR41, and pAR10. Among the 5,367 ORFs, 3,333 clusters of orthologous groups were identified. Riboswitches play a very important role in a number of cellular processes, such as regulation of gene expression, tRNA processing, and protein secretion (9). The riboswitches in the *B. cereus* AR156 genome sequence were predicted with a method using pHMM. By applying default parameters, 36 riboswitches were identified

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in the *B. cereus* AR156 genome (eight binding *S*-adenosylmethionine, six specific for thiamine pyrophosphate, eight for purines, eight for PreQ, two for flavin mononucleotide, two for lysine, and one each for Glms and Cob). *B. cereus* AR156 displays a robust swarming phenotype. In *B. cereus* AR156, there are four genes whose products are weak and similar to those of *swr* in *B. subtilis*. It has been reported that the hook-associated flagellar proteins HAP1 and HAP2 are affected differently by exudates secreted by plant roots (10). There are four hook-associated flagellar proteins identified in *B. cereus* AR156 chromosome, its pAR460 and pAR41 plasmids also contain peptide antibiotics; pAR460 encodes four other bacteriocins. The pAR460 plasmid also encodes one camelysin and one microbial collagenase. Several genes similar to linear gramicidin synthetase subunits and linear gramicidin dehydrogenase LgrE were found in pAR460. Linear gramicidin is a pentadecapeptide antibiotic that forms a membrane channel (11, 12). The other plasmid, pAR41, also encodes four lantibiotic acids as modification enzymes and six bacteriocins.

Accession number(s). The complete genome sequence for *B. cereus* AR156 reported here has been deposited in GenBank under the accession numbers CP015589 (chromosome), CP015590 (plasmid pAR10), CP015591 (plasmid pAR41), and CP015592 (plasmid pAR460).

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REFERENCES

- Niu DD, Liu HX, Jiang CH, Wang YP, Wang QY, Jin HL, Guo JH. 2011. The plant growth-promoting rhizobacterium *B. cereus* AR156 induces systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signalling pathways. Mol Plant Microbe Interact 24:533–542. https://doi.org/10.1094/MPMI-09-10 -0213.
- Niu DD, Wang CJ, Guo YH, Jiang CH, Zhang WZ, Wang YP, Guo JH. 2012. The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces resistance in tomato with induction and priming of defence response. Biocontrol Sci Technol 22:991–1004. https://doi.org/10.1080/ 09583157.2012.706595.
- Jiang CH, Huang ZY, Xie P, Gu C, Li K, Wang DC, Yu YY, Fan ZH, Wang CJ, Wang YP, Guo YH, Guo JH. 2016. Transcription factors WRKY70 and WRKY11 served as regulators in rhizobacterium *Bacillus Cereus* AR156induced systemic resistance to *Pseudomonas syringae* pv. tomato DC3000 in arabidopsis. J Exp Bot 67:157–174. https://doi.org/10.1093/ jxb/erv445.
- Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. Genome Res 8:195–202. https://doi.org/10.1101/gr.8.3 .195.
- Gordon D. 2003. Viewing and editing assembled sequences using Consed. Curr Protoc Bioinform Chapter 11:Unit 11.12. https://doi.org/10 .1002/0471250953.bi1102s02.
- Bergman NH, Passalacqua KD, Hanna PC, Qin ZS. 2007. Operon prediction for sequenced bacterial genomes without experimental informa-

tion. Appl Environ Microbiol 73:846-854. https://doi.org/10.1128/AEM .01686-06.

- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. https://doi.org/10.1093/nar/gkh152.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. https://doi.org/10.1093/nar/ gkm160.
- 9. Storz G. 2002. An expanding universe of noncoding RNAs. Science 296:1260–1263. https://doi.org/10.1126/science.1072249.
- Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I, Morgenstern B, Voss B, Hess WR, Reva O, Junge H, Voigt B, Jungblut PR, Vater J, Süssmuth R, Liesegang H, Strittmatter A, Gottschalk G, Borriss R. 2007. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nat Biotechnol 25:1007–1014. https://doi.org/10.1038/nbt1325.
- Kessler N, Schuhmann H, Morneweg S, Linne U, Marahiel MA. 2004. The linear pentadecapeptide gramicidin is assembled by four multimodularnon ribosomal peptide synthetases that comprise 16 modules with 56 catalytic domains. J Biol Chem 279:7413–7419. https://doi.org/10.1074/ jbc.M309658200.
- Kubota K. 1987. Membranous phosphoglyceride-linked biosynthesis of pentadeca peptide, linear gramicidin, by *Bacillus brevis*, ATCC 8185. Biochem Biophys Res Commun 144:203–209. https://doi.org/10.1016/ S0006-291X(87)80496-5.