

University of Groningen

## Next-Generation Whole-Genome Sequencing of Eight Strains of *Bacillus cereus*, Isolated from Food

Krawczyk, Antonina O; de Jong, Anne; Eijlander, Robyn T; Berendsen, Erwin M; Holsappel, Siger; Wells-Bennik, Marjon H J; Kuipers, Oscar P

*Published in:*  
Genome Announcements

*DOI:*  
[10.1128/genomeA.01480-15](https://doi.org/10.1128/genomeA.01480-15)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Krawczyk, A. O., de Jong, A., Eijlander, R. T., Berendsen, E. M., Holsappel, S., Wells-Bennik, M. H. J., & Kuipers, O. P. (2015). Next-Generation Whole-Genome Sequencing of Eight Strains of *Bacillus cereus*, Isolated from Food. *Genome Announcements*, 3(6), 1-2. [e01480-15].  
<https://doi.org/10.1128/genomeA.01480-15>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Next-Generation Whole-Genome Sequencing of Eight Strains of *Bacillus cereus*, Isolated from Food

Antonina O. Krawczyk,<sup>a,c</sup> Anne de Jong,<sup>a,c</sup> Robyn T. Eijlander,<sup>a,c</sup> Erwin M. Berendsen,<sup>a,b,c</sup> Siger Holsappel,<sup>a</sup> Marjon H. J. Wells-Bennik,<sup>b,c</sup> Oscar P. Kuipers<sup>a,c</sup>

Molecular Genetics, University of Groningen, Groningen, the Netherlands<sup>a</sup>; NIZO food research, Ede, the Netherlands<sup>b</sup>; Top Institute Food and Nutrition (TIFN), Wageningen, the Netherlands<sup>c</sup>

***Bacillus cereus* can contaminate food and cause emetic and diarrheal foodborne illness. Here, we report whole-genome sequences of eight strains of *B. cereus*, isolated from different food sources.**

Received 27 October 2015 Accepted 27 October 2015 Published 17 December 2015

**Citation** Krawczyk AO, de Jong A, Eijlander RT, Berendsen EM, Holsappel S, Wells-Bennik MHJ, Kuipers OP. 2015. Next-generation whole-genome sequencing of eight strains of *Bacillus cereus*, isolated from food. *Genome Announc* 3(6):e01480-15. doi:10.1128/genomeA.01480-15.

**Copyright** © 2015 Krawczyk et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Oscar P. Kuipers, o.p.kuipers@rug.nl.

*Bacillus cereus* is a mesophilic or psychrotrophic, spore-forming bacterium commonly present in soil (1). It occurs in the rhizosphere of plants (2, 3) and as a part of animal intestinal microflora (4). It is opportunistically pathogenic and leads to various infections, including: local infections of wounds, or an eye; bacteremia and septicemia; respiratory infections; central nervous system infections; pericarditis; and endocarditis (5). Due to its presence in soil and production of spores, *B. cereus* often contaminates various food products. Consumption of foods with high levels of *B. cereus* may result in two types of foodborne illness: emetic or diarrheal. The emetic type, characterized by vomiting and nausea, is induced by the cereulide toxin produced by cells growing in food (6–8). The diarrheal illness is caused by enterotoxins, including hemolysin BL, cytotoxin K, and nonhemolytic enterotoxin, which are produced by *B. cereus* cells in the small intestine (6, 7). *B. cereus* is closely related to *Bacillus anthracis*, the causative agent of anthrax, and to the insect pathogen, *Bacillus thuringiensis* (9).

Eight strains of *B. cereus*, isolated from different food sources were sequenced by next-generation whole-genome sequencing. The strains were grown at 30°C with shaking at 220 rpm in heart infusion (BHI) broth (Difco). The overnight cultures were diluted in fresh medium to the optical density at 600 nm (OD<sub>600</sub>) and

harvested by centrifugation at 5,000 relative centrifugal force (RCF). Subsequently, total DNA was isolated by phenol-chloroform extraction as described previously (10). The isolated DNA was sheared to 500-bp fragments in the Covaris (KBioscience) ultrasonication device for preparing the NGS library preps using the paired-end NEB NextGen library preparation kit. The libraries were 101-base paired-end sequenced on an Illumina HiSeq2000. Subsequently, Velvet (11) was used to perform a *de novo* paired-end assembly on each genome resulting in the draft genome sequences. The RAST server (12) and BAGEL3 (13) were used to annotate the genomes and to identify putative bacteriocin gene clusters, respectively.

**Nucleotide sequence accession numbers.** The genome sequence of the eight *Bacillus cereus* strains have been deposited as whole-genome shotgun projects at DDBJ/EMBL/GenBank under the accession numbers listed in Table 1.

## ACKNOWLEDGMENTS

We thank the NGS sequence facility of the University Medical Center of Groningen (UMCG) for performing the sequencing of the strains. We thank Top Institute for Food and Nutrition for contributing to the funding of the project in theme 3: Safety and Preservation.

## 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>.
- Halverson LJ, Clayton MK, Handelsman J. 1993. Population biology of *Bacillus cereus* UW85 in the rhizosphere of field-grown soybeans. *Soil Biol Biochem* 25:485–493. [http://dx.doi.org/10.1016/0038-0717\(93\)90074-L](http://dx.doi.org/10.1016/0038-0717(93)90074-L).
- Zhang S, Liao S, Yu X, Lu H, Xian J, Guo H, Wang A, Xie J. 2015. Microbial diversity of mangrove sediment in Shenzhen Bay and gene cloning, characterization of an isolated phytase-producing strain of SPC09 *B. cereus*. *Appl Microbiol Biotechnol* 99:5339–5350. <http://dx.doi.org/10.1007/s00253-015-6405-8>.
- Margulis L, Jorgensen JZ, Dolan S, Kolchinsky R, Rainey FA, Lo S-C. 1998. The Arthromitus stage of *Bacillus cereus*: intestinal symbionts of animals. *Proc Natl Acad Sci USA* 95:1236–1241. <http://dx.doi.org/10.1073/pnas.95.3.1236>.

TABLE 1 Sequenced strains and their sources<sup>a</sup>

<i>B. cereus</i> strain	Source	Accession no.
B4077	Chilled dessert	LCYI00000000
B4078	Food, undefined	LCYJ00000000
B4080	Dried onion	LCYK00000000
B4086	Boiled rice	LCYL00000000
B4087	Pea soup	LCYM00000000
B4147	Cereals, pasta and pastries	LCYN00000000
B4153	Dairy products	LCYO00000000
B4158	Vegetables	LCYP00000000

<sup>a</sup> B-numbers refer to the strain collection at NIZO food research and the University of Groningen (Molecular Genetics).

5. Drobniński FA. 1993. *Bacillus cereus* and related species. Clin Microbiol Rev 6:324–338.
6. Logan NA. 2012. *Bacillus* and relatives in foodborne illness. J Appl Microbiol 112:417–429. <http://dx.doi.org/10.1111/j.1365-2672.2011.05204.x>.
7. Stenfors Arnesen LP, Fagerlund A, Granum PE. 2008. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 32: 579–606. <http://dx.doi.org/10.1111/j.1574-6976.2008.00112.x>.
8. Granum PE, Lund T. 2006. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Lett 157:223–228. <http://dx.doi.org/10.1111/j.1574-6968.1997.tb12776.x>.
9. Rasko DA, Altherr MR, Han CS, Ravel J. 2005. Genomics of the *Bacillus cereus* group of organisms. FEMS Microbiol Rev 29:303–329. <http://dx.doi.org/10.1016/j.fmrre.2004.12.005>.
10. Krawczyk AO, Berendsen EM, Eijlander RT, de Jong A, Wells-Bennik MHJ, Kuipers OP. 2015. Draft genome sequences of four *Bacillus thermoamylovorans* strains isolated from milk and acacia gum, a food ingredient. Genome Announc 3(2):e00165-15. <http://dx.doi.org/10.1128/genomeA.00165-15>.
11. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
12. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
13. van Heel AJ, de Jong A, Montalbán-López M, Kok J, Kuipers OP. 2013. BAGEL3: automated identification of genes encoding bacteriocins and (non-) bactericidal posttranslationally modified peptides. Nucleic Acids Res 41:W448–W453. <http://dx.doi.org/10.1093/nar/gkt391>.