genomeA
Journals.ASM.org

Draft Genome Sequence of *Bacillus cytotoxicus* CVUAS 2833, a Very Close Relative to Type Strain NVH 391-98 Isolated from a Different Location

Maria-Elisabeth Böhm, Christopher Huptas, Viktoria Magdalena Krey,* Siegfried Scherer

Lehrstuhl für Mikrobielle Ökologie, Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL), Technische Universität München, Freising, Germany

* Present address: Viktoria Magdalena Krey, BCA-clinic Betriebs GmbH & Co. KG, Augsburg, Germany.

We report the draft genome sequence of *Bacillus cytotoxicus* CVUAS 2833, isolated from potato puree in Germany (2007), which is—despite its clearly different source—very similar to the type strain *B. cytotoxicus* NVH 391-98 isolated in France (average nucleotide identity, 99.5%).

Received 2 July 2015 **Accepted** 10 July 2015 **Published** 20 August 2015

Citation Böhm M-E, Huptas C, Krey VM, Scherer S. 2015. Draft genome sequence of *Bacillus cytotoxicus* CVUAS 2833, a very close relative to type strain NVH 391-98 isolated from a different location. Genome Announc 3(4):e00901-15. doi:10.1128/genomeA.00901-15.

Address correspondence to Siegfried Scherer, siegfried.scherer@wzw.tum.de.

The highly enterotoxic *Bacillus cereus* NVH 391-98 was discovered in vegetable puree, which caused severe food poisoning including fatalities (France, 1998) (1). In this strain, a new cytotoxin CytK-1 was identified. Subsequently, the distantly related homolog CytK-2 was discovered in other strains of the *B. cereus* group which display considerably attenuated toxicity (2). *B. cereus* NVH 391-98 was published in 2013 as the type strain of the new species *Bacillus cytotoxicus* due to the presence of the *cytK-1* gene, its thermotolerance (growth at up to 50°C), a distinctive fatty acid profile, DNA-DNA hybridization, and multilocus sequence typing (MLST) (3). *B. cytotoxicus* CVUAS 2833, which is one of only five known *B. cyototoxicus* strains, was isolated spatially and chronologically separate, but affiliated to *B. cytotoxicus* by 16S rRNA gene sequence and MLST comparison (3, 4). To date (June 2015), only the genome sequence of the type strain has been available.

We sequenced *B. cytotoxicus* CVUAS 2833 on the Illumina MiSeq platform and obtained a library of 3,185,212 reads. About 72.2% of the reads passed quality filtering (length, ≥80%; Phred score, $Q \geq 30$) and were assembled to 36 contigs using ABySS version 1.3.7 with an assembly size of 4,127,075 bp and a G+C content of 35.74%. The assembled contigs were submitted to GenBank and annotated by NCBI's prokaryotic genome annotation pipeline (PGAP). BLAST analysis revealed the presence of the distinct *B. cytotoxicus* variant of the enterotoxin operon *nhe* (5) in strain CVUAS 2833 with only a single nucleotide difference to NVH 391-98 *nhe*. *B. cytotoxicus* CVUAS 2833 does not contain the *ces* cluster encoding the emetic toxin or the *hbl* enterotoxin genes. Whole-genome pairwise average nucleotide identity (ANI) of 99.5% supports the exceptionally high similarity to *B. cytotoxicus* NVH 391-98. Additionally, Gegenees 2.2.1 was used to compare whole-genome similarity in a fragmented alignment (BLAST 2.2.29+) at a fragment size of 200 bp and a comparison step size of 100 bp (6). In contrast to ANI, this approach applies stricter settings (7) and does not exclude dissimilar sequences (8). *B. cytotoxicus* NVH 391-98 and CVUAS 2833 showed 93.5% identity in the fragmented all-all comparison.

These genomic and genetic similarities confirm a clonal character of the *B. cytotoxicus* lineage within *B. cereus sensu lato*. All known *B. cytotoxicus* strains originate from different food sources (1, 9), but are similar on a MLST basis (3). The only well-analyzed clonal phylogenetic cluster in *B. cereus sensu lato* is *Bacillus anthracis*. The *B. anthracis* linage is evolutionarily young and demarcation of *B. anthracis* at a species level is quite debatable (10, 11). In contrast, *B. cytotoxicus* seems to be a much older and clearly discernible branch within *B. cereus sensu lato* (12).

Nucleotide sequence accession numbers. The draft genome sequence of *B. cytotoxicus* CVUAS 2833 has been deposited at DDBJ/EMBL/GenBank under the accession no. JYPG00000000. The version described in this paper is version JYPG01000000.

ACKNOWLEDGMENTS

This research project was supported by the German Ministry of Economics and Energy (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), Project AiF 17506N.

We thank Dr. Jörg Rau (CVUA Stuttgart) for providing the strain *B. cytotoxicus* CVUAS 2833.

REFERENCES

1. **Lund T, De Buyser ML, Granum PE.** 2000. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. Mol Microbiol **38:**254–261. http://dx.doi.org/10.1046/j.1365-2958.2000.02147.x.
2. **Fagerlund A, Ween O, Lund T, Hardy SP, Granum PE.** 2004. Genetic and functional analysis of the cytK family of genes in *Bacillus cereus*. Microbiology **150:**2689–2697. http://dx.doi.org/10.1099/mic.0.26975-0.
3. **Guinebretière MH, Auger S, Galleron N, Contzen M, De Sarrau B, De Buyser ML, Lamberet G, Fagerlund A, Granum PE, Lereclus D, De Vos P, Nguyen-The C, Sorokin A.** 2013. *Bacillus cytotoxicus* sp. nov. is a novel thermotolerant species of the *Bacillus cereus* group occasionally associated with food poisoning. Int J Syst Evol Microbiol **63:**31–40. http://dx.doi.org/10.1099/ijs.0.030627-0.
4. **Rau J, Perz R, Klittich G, Contzen M.** 2009. Cereulide forming presumptive *Bacillus cereus* strains from food—differentiating analyses using cul-

tural methods, LC-MS/MS, PCR, and infrared spectroscopy in consideration of thermotolerant isolates. Berl Munch Tierarztl Wochenschr **122:** 25–36.

5. **Fagerlund A, Brillard J, Fürst R, Guinebretière MH, Granum PE.** 2007. Toxin production in a rare and genetically remote cluster of strains of the *Bacillus cereus* group. BMC Microbiol **7:**43. http://dx.doi.org/10.1186/1471-2180-7-43.
6. **Agren J, Sundström A, Håfström T, Segerman B.** 2012. Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One **7:**e39107. http://dx.doi.org/10.1371/journal.pone.0039107.
7. **Richter M, Rosselló-Móra R.** 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A **106:** 19126–19131. http://dx.doi.org/10.1073/pnas.0906412106.
8. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM.** 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol **57:** 81–91. http://dx.doi.org/10.1099/ijs.0.64483-0.
9. **Guinebretiere MH, Fagerlund A, Granum PE, Nguyen-The C.** 2006. Rapid discrimination of *cytK-1* and *cytK-2* genes in *Bacillus cereus* strains by a novel duplex PCR system. FEMS Microbiol Lett **259:**74–80. http://dx.doi.org/10.1111/j.1574-6968.2006.00247.x.
10. **Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolstø AB.** 2000. *Bacillus anthracis, Bacillus cereus*, and *Bacillus thuringiensis*—one species on the basis of genetic evidence. Appl Environ Microbiol **66:**2627–2630. http://dx.doi.org/10.1128/AEM.66.6.2627-2630.2000.
11. **Zwick ME, Joseph SJ, Didelot X, Chen PE, Bishop-Lilly KA, Stewart AC, Willner K, Nolan N, Lentz S, Thomason MK, Sozhamannan S, Mateczun AJ, Du L, Read TD.** 2012. Genomic characterization of the *Bacillus cereus sensu lato* species: backdrop to the evolution of *Bacillus anthracis*. Genome Res **22:**1512–1524. http://dx.doi.org/10.1101/gr.134437.111.
12. **Guinebretière MH, Thompson FL, Sorokin A, Normand P, Dawyndt P, Ehling-Schulz M, Svensson B, Sanchis V, Nguyen-The C, Heyndrickx M, De Vos P.** 2008. Ecological diversification in the *Bacillus cereus* group. Environ Microbiol **10:**851–865. http://dx.doi.org/10.1111/j.1462-2920.2007.01495.x.