



Genome Sequences of *Bacillus sporothermodurans* Strains Isolated from Ultra-High-Temperature Milk

Rodney Owusu-Darko,^a Mushal Allam,^b  Silvia D. de Oliveira,^c Carlos A. S. Ferreira,^c Sunita Grover,^d Senzo Mtshali,^b Arshad Ismail,^b Rashmi H. Mallappa,^d Frederick Tabit,^e  Elna M. Buys^a

^aDepartment of Consumer and Food Sciences, University of Pretoria, Pretoria, South Africa

^bNational Institute for Communicable Diseases, Sandringham, South Africa

^cSchool of Sciences, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil

^dDairy Microbiology Division, Molecular Biology Unit, National Dairy Research Institute, Karnal, India

^eDepartment of Life and Consumer Sciences, University of South Africa, Pretoria, South Africa

ABSTRACT Here, we report the draft genome sequences of 3 *Bacillus sporothermodurans* strains isolated from ultra-high-temperature milk products in South Africa and Brazil and the type strain MB 581 (DSM 10599). The genomes will provide valuable information on the molecular dynamics of heat resistance in *B. sporothermodurans*.

Bacillus sporothermodurans is a thermoresistant Gram-positive bacterium that can produce highly heat-resistant endospores (HRS) capable of surviving ultra-high-temperature (UHT) heat treatments (1, 2). First detected in UHT milk (3), it has subsequently been isolated from other dairy products, including UHT cream, chocolate milk, evaporated milk, and reconstituted milk (4). Furthermore, *B. sporothermodurans* has been isolated from non-dairy-based foods, including Indian curry (5), as well as from marine sources (6). After heat processing, the surviving spores may germinate and grow in the stored milk (1). The spores of *B. sporothermodurans* grow at low levels ($\approx 10^5$ CFU/ml) and do not affect the pH of the milk (2); as a result, its presence may go unnoticed. However, there are reports of *B. sporothermodurans* strains isolated in Brazil causing significant proteolytic activity leading to UHT milk spoilage (7). If spoilage does occur, though, there may be slight changes in color, off flavors, and the destabilization of casein micelles (2). Consequently, the main concerns to the dairy industry are milk quality, nonsterility of milk products, and biofilm formation in milk processing equipment.

B. sporothermodurans strains SAD and SA01 were isolated from UHT milk produced in South Africa, and *B. sporothermodurans* strain BR12 was isolated from UHT milk from Brazil. The type strain *B. sporothermodurans* DSM 10599 was obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). The enumeration and isolation of strains SAD and SA01 were undertaken as previously described (8), with the substitution of plate count agar for brain heart infusion (BHI) agar (Oxoid, UK). Single colonies of overnight fresh cultures of all four strains of *B. sporothermodurans* were inoculated into BHI broth (Oxoid) and incubated at 37°C for 72 hours. Genomic DNA was extracted using the ZR bacterial DNA miniprep kit (Zymo Research, USA) and quantified using the Qubit instrument and double-stranded DNA (dsDNA) broad-range (BR) assay kit (Life Technologies, USA). Multiplexed paired-end libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina, USA). Genome sequencing was carried out on an Illumina MiSeq system. The paired-end reads (2×300 bp) were checked for quality and trimmed and *de novo* assembled using the CLC genomics workbench version 9

Citation Owusu-Darko R, Allam M, de Oliveira SD, Ferreira CAS, Grover S, Mtshali S, Ismail A, Mallappa RH, Tabit F, Buys EM. 2019. Genome sequences of *Bacillus sporothermodurans* strains isolated from ultra-high-temperature milk. *Microbiol Resour Announc* 8:e00145-19. <https://doi.org/10.1128/MRA.00145-19>.

Editor David A. Baltrus, University of Arizona

Copyright © 2019 Owusu-Darko et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Elna M. Buys, elna.buys@up.ac.za.

Received 13 February 2019

Accepted 6 May 2019

Published 30 May 2019

TABLE 1 Summary report of the *de novo* assembly of four *B. sporothermodurans* strains

Organism	GenBank accession no.	SRA accession no.	Total no. of reads	Genome size (bp)	No. of coding sequences	Coverage (×)	No. of contigs	N_{50} (kpb)
<i>B. sporothermodurans</i> DSM 10599	NAZD000000000	SRR8741694	3,570,064	3,783,858	4,257	226	527	15,402
<i>B. sporothermodurans</i> SAD	NAZB000000000	SRR8732968	2,523,238	3,857,089	4,111	175	110	114,649
<i>B. sporothermodurans</i> SA01	NAZA000000000	SRR8732969	1,858,252	3,414,010	3,768	146	290	22,386
<i>B. sporothermodurans</i> BR12	NAZC000000000	SRR8741693	3,421,418	3,974,872	4,558	193	805	9,377

(Qiagen, Netherlands), with all low-quality ($Q, <20$) data filtered out. The resultant contigs were submitted to GenBank, where gene annotation was implemented using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (9). The annotation was further uploaded to Rapid Annotation using Subsystem Technology (RAST) for subsystems-based annotation (10–12).

The four assembled genomes had an average G+C content of 38.6%. Overall, genomes of *B. sporothermodurans* contain heat shock and hyperosmotic proteins (including DnaJ, GrpE, and GroEL) that will have an influence on the heat resistance and, consequently, the processing dynamics of food products. Additionally, all four strains sequenced contain the biofilm matrix protein component TasA and its homologs, which have been shown to be the major biofilm matrix component (13), especially in *Bacillus subtilis*. Ultimately, the whole-genome sequence of *B. sporothermodurans* will help improve our understanding of the heat resistance of this bacterium with the view of improving milk quality.

Data availability. The genome sequences of all four strains of *B. sporothermodurans* are publicly available at NCBI GenBank under the BioProject accession number [PRJNA379529](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA379529). Raw and trimmed sequencing reads have been deposited in the NCBI SRA under the study accession number [SRP188520](https://www.ncbi.nlm.nih.gov/sra/SRP188520). The GenBank and SRA accession numbers are listed in Table 1. This announcement represents the first version of all four genomes.

ACKNOWLEDGMENTS

R.O.-D. acknowledges support from the Department of Science and Technology (DST), National Research Foundation (NRF), South Africa, in a form of scholarship disbursed through the Institute for Food, Nutrition and Well-being, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa. We also acknowledge the HESA/IBSA Research Cooperation Program.

We have no potential conflicts of interest to disclose.

REFERENCES

- Huemer IA, Klijn N, Vogelsang HWJ, Langeveld L. 1998. Thermal death kinetics of spores of *Bacillus sporothermodurans* isolated from UHT milk. *Int Dairy J* 8:851–855. [https://doi.org/10.1016/S0958-6946\(98\)00129-0](https://doi.org/10.1016/S0958-6946(98)00129-0).
- Klijn N, Herman L, Langeveld L, Vaerewijck M, Wagendorp AA, Huemer I, Weerkamp AH. 1997. Genotypical and phenotypical characterization of *Bacillus sporothermodurans* strains, surviving UHT sterilisation. *Int Dairy J* 7:421–428. [https://doi.org/10.1016/S0958-6946\(97\)00029-0](https://doi.org/10.1016/S0958-6946(97)00029-0).
- Pettersson B, Lembke F, Hammer P, Stackebrandt E, Priest FG. 1996. *Bacillus sporothermodurans*, a new species producing highly heat-resistant endospores. *Int J Syst Bacteriol* 46:759–764. <https://doi.org/10.1099/00207713-46-3-759>.
- Herman L, Heyndrickx M. 2000. The presence of intragenically located REP-like elements in *Bacillus sporothermodurans* is sufficient for REP-PCR typing. *Res Microbiol* 151:255–261. [https://doi.org/10.1016/S0923-2508\(00\)00146-7](https://doi.org/10.1016/S0923-2508(00)00146-7).
- Krawczyk AO, de Jong A, Holsappel S, Eijlander RT, van Heel A, Berendsen EM, Wells-Bennik MHJ, Kuipers OP. 2016. Genome sequences of 12 spore-forming bacillus species, comprising *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus sporothermodurans*, and *Bacillus vallismortis*, isolated from foods. *Genome Announc* 4:e00103-16. <https://doi.org/10.1128/genomeA.00103-16>.
- Ki J-S, Zhang W, Qian P-Y. 2009. Discovery of marine *Bacillus* species by 16S rRNA and rpoB comparisons and their usefulness for species identification. *J Microbiol Methods* 77:48–57. <https://doi.org/10.1016/j.mimet.2009.01.003>.
- Pinto CLO, Souza LV, Meloni VAS, Batista CS, Silva R, Martins EMF, Cruz AG, Martins ML. 2018. Microbiological quality of Brazilian UHT milk: identification and spoilage potential of spore-forming bacteria. *Int J Dairy Technol* 71:20–26. <https://doi.org/10.1111/1471-0307.12339>.
- Scheldeman P, Pil A, Herman L, De Vos P, Heyndrickx M. 2005. Incidence and diversity of potentially highly heat-resistant spores isolated at dairy farms. *Appl Environ Microbiol* 71:1480–1494. <https://doi.org/10.1128/AEM.71.3.1480-1494.2005>.
- 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>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- 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. <https://doi.org/10.1186/1471-2164-9-75>.
12. Disz T, Akhter S, Cuevas D, Olson R, Overbeek R, Vonstein V, Stevens R, Edwards R. a. 2010. Accessing the SEED genome databases via Web services API: tools for programmers. *BMC Bioinformatics* 11:319. <https://doi.org/10.1186/1471-2105-11-319>.
13. Branda SS, Chu F, Kearns DB, Losick R, Kolter R. 2006. A major protein component of the *Bacillus subtilis* biofilm matrix. *Mol Microbiol* 59: 1229–1238. <https://doi.org/10.1111/j.1365-2958.2005.05020.x>.