

Draft Genome Sequences of Four Thermophilic Spore Formers Isolated from a Dairy-Processing Environment

Martien P. M. Caspers,^{a,b} Jos Boekhorst,^{a,c} Anne de Jong,^{a,g} Remco Kort,^{a,b,f} Masja Nierop Groot,^{a,e} Tjakko Abbe^{a,d}

Top Institute (TI) Food and Nutrition, Wageningen, The Netherlands^a; TNO, Microbiology and Systems Biology, Zeist, The Netherlands^b; NIZO food research, Ede, The Netherlands^c; Laboratory of Food Microbiology, Wageningen University and Research Centre, Wageningen, The Netherlands^d; Wageningen UR Food & Biobased Research, Wageningen, The Netherlands^e; Molecular Cell Physiology, Vrije Universiteit (VU) Amsterdam, Amsterdam, The Netherlands^f; Molecular Genetics, Rijks Universiteit Groningen (RUG), Groningen, The Netherlands^g

Spores of thermophilic spore-forming bacteria are a common cause of contamination in dairy products. Here, we report draft genome sequences of four thermophilic strains from a milk-processing plant or standard milk, namely, a *Geobacillus thermoglucosidans* isolate (TNO-09.023), *Geobacillus stearothermophilus* TNO-09.027, and two *Anoxybacillus flavithermus* isolates (TNO-09.014 and TNO-09.016).

Received 20 June 2016 Accepted 21 June 2016 Published 11 August 2016

Citation Caspers MPM, Boekhorst J, de Jong A, Kort R, Nierop Groot M, Abbe T. 2016. Draft genome sequences of four thermophilic spore formers isolated from a dairy-processing environment. *Genome Announc* 4(4):e00757-16. doi:10.1128/genomeA.00757-16.

Copyright © 2016 Caspers 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 Tjakko Abbe, tjakko.abbe@wur.nl.

One of the regular problems in the production of dairy concentrates is contamination by heat-resistant spores from thermophilic bacteria of the genera *Anoxybacillus* and *Geobacillus* (1). Genome sequences of two thermophilic strains previously isolated from fouling samples from two dairy-production plants (1) and with relatively strong biofilm-forming capacities, namely, *Geobacillus thermoglucosidans* TNO-09.020 and *Anoxybacillus flavithermus* TNO-09.006, were reported previously (2, 3). Here, we publish the draft genome sequences of an additional set of four dairy thermophiles from a published study (1).

G. thermoglucosidans TNO-09.023, *G. stearothermophilus* TNO-09.027, *A. flavithermus* TNO-09.014, and *A. flavithermus* TNO-09.016 were pregrown on tryptic soy agar (TSA) plates (at 55°C overnight, in plastic bags with wet tissues to prevent evaporation). Genomic DNA was isolated from pelleted cell material of freshly grown flask cultures in tryptic soy broth (TSB) (at 55°C, with optical density at 600 nm [OD₆₀₀] of 0.8 to 1.0), inoculated from a single representative freshly grown colony from a TSA plate. The method involved a mild degradation step of cell walls by lysozyme at 37°C, followed by lysis of cells by the addition of sarcosyl. Proteins were removed by extraction with phenol-chloroform, RNA was degraded using RNase, and the resulting

DNA was precipitated and washed with isopropanol (50% [vol/vol] and 70% (vol/vol) ethanol, respectively (4). DNA was dissolved in water (1 to 9 μg/μl; size on 0.8% agarose gel, ≥20 kb) and used for sequencing.

The isolated DNA was sheared to 250- to 350-bp fragments and paired-end sequenced on an Illumina HiSeq 2000 out-sourced to BaseClear (Leiden, The Netherlands). Ray 2.3.1 (5) was used for assembly. The RAST server (6) was used to annotate the genomes.

Accession number(s). The genome sequences of the four strains have been deposited at DDBJ/EMBL/GenBank under the accession numbers listed in Table 1. The version described in this paper is the first version.

ACKNOWLEDGMENTS

We thank Carlo Brouwer for performing the strain isolation and Trifa Omer for strain culture and DNA isolation.

The project is funded by TI Food and Nutrition, a public-private partnership on precompetitive research in food and nutrition. The public partners are responsible for the study design, data collection and analysis, decision to publish, and preparation of the manuscript. The private partners have contributed to the project through regular discussion.

REFERENCES

- Zhao Y, Caspers MP, Metselaar KI, de Boer P, Roeselers G, Moezelaar R, Nierop Groot M, Montijn RC, Abbe T, Kort R. 2013. Abiotic and micro-biotic factors controlling biofilm formation by thermophilic sporeformers. *Appl Environ Microbiol* 79:5652–5660. <http://dx.doi.org/10.1128/AEM.00949-13>.
- Zhao Y, Caspers MP, Abbe T, Siezen RJ, Kort R. 2012. Complete genome sequence of *Geobacillus thermoglucosidans* TNO-09.020, a thermophilic sporeformer associated with a dairy-processing environment. *J Bacteriol* 194:4118. <http://dx.doi.org/10.1128/JB.00318-12>.
- Caspers MP, Boekhorst J, Abbe T, Siezen RJ, Kort R. 2013. Complete genome sequence of *Anoxybacillus flavithermus* TNO-09.006, a thermophilic sporeformer associated with a dairy-processing environment.

TABLE 1 Sequenced strains and their sources

Species	Strain	Source of isolation	Accession no.
<i>Geobacillus thermoglucosidans</i>	TNO-09.023	Casein pipeline	LUCT00000000
<i>Geobacillus stearothermophilus</i>	TNO-09.027	Casein pipeline	LUCR00000000
<i>Anoxybacillus flavithermus</i>	TNO-09.014	Standard milk	LUFB00000000
<i>Anoxybacillus flavithermus</i>	TNO-09.016	Evaporator	LUCQ00000000

- Genome Announc 1(1):e00010-13. <http://dx.doi.org/10.1128/genomeA.00010-13>.
4. Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
 5. Boisvert S, Laviolette F, Corbeil J. 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. J Comput Biol 17:1519–1533. <http://dx.doi.org/10.1089/cmb.2009.0238>.
 6. 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>.