



# Draft Genome Sequences of *Lactococcus lactis* Strains MS22314, MS22333, MS22336, and MS22337, Isolated from Fermented Camel Milk in Ethiopia

Esben Bragason,<sup>a</sup> Christina Aaby Svendsen,<sup>a</sup> Mitiku Eshetu Guya,<sup>b</sup> Tesfemariam Berhe,<sup>b</sup>  Egon Bech Hansen<sup>a</sup>

<sup>a</sup>National Food Institute, Technical University of Denmark, Lyngby, Denmark

<sup>b</sup>Haramaya University, School of Animal and Range Sciences, Dire Dawa, Ethiopia

**ABSTRACT** The genome sequences of four *Lactococcus lactis* strains isolated from fermented camel milk were sequenced using paired-end Illumina MiSeq reads. The genome size of each strain was about 2.6 Mb, and three of the strains were annotated with *tet(S)* coding for tetracycline resistance.

*Lactococcus lactis* is a well-known acidifying Gram-positive bacterium, approved with qualified presumption of safety (QPS) status by the European Food Safety Authority and used in starter cultures to make dairy products (1).

Here, we report the draft genome sequences of *L. lactis* strains MS22314, MS22333, MS22336, and MS22337. All strains were isolated from camel milk in Ethiopia. The new strains demonstrate superior fermentation qualities in camel milk of exponential cell growth, acidification, and decrease in redox potential, comparable to what other strains have shown in bovine milk (2). Starter cultures used for bovine-based products have shown poor fermentation results in camel milk (3, 4).

Camel milk samples ( $n = 29$ ) were collected from several farms in the Babile area of Ethiopia and incubated at 30°C or 42°C for 48 h to stimulate fermentation. Samples with a pH of  $< 5$  after 48 h were plated and restreaked 5 times onto De Man, Rogosa, and Sharpe (MRS) agar, M17 agar containing 0.5% lactose, or Prussian blue agar, all containing 20  $\mu\text{g ml}^{-1}$  natamycin for fungal inhibition (5, 6).

Single colonies from 114 isolates on one of the agar plates were chosen for 16S rRNA gene sequencing as described by Fugl et al. (2).

Based on phenotypic characterization (2), single colonies from each of four isolates, MS22314, MS22333, MS22336, and MS22337, were clean streaked at 30°C for whole-genome sequencing onto M17-lac agar plates. DNA was extracted following the manufacturer's protocol (NORGEN milk bacterial DNA isolation kit 21550).

DNA concentrations were measured on the Qubit fluorometer using the double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Invitrogen). Libraries for paired-end sequencing were constructed using the Nextera XT kit (Illumina, CA, USA) guide 15031942v01. The pooled Nextera XT libraries were loaded onto an Illumina MiSeq reagent cartridge using the MiSeq reagent kit v3 and 500 cycles with a standard flow cell. Both sequencing and assembly were done using default settings unless otherwise indicated. Sequencing was carried out using an Illumina MiSeq benchtop sequencer with an average read length of 210 bp, which yielded 1,868,468 to 2,187,598 reads. The coverages ranged between 130.2 $\times$  and 173.8 $\times$ .

The raw Illumina reads were filtered and trimmed using Assembler v1.0 (<https://cge.cbs.dtu.dk/services/Assembler/>) (7). The trimmed reads were assembled using Velvet v1.1.04 (8) with the standard quality control parameters included in the software. The genome statistics are reported in Table 1.

The contigs were annotated using the NCBI Prokaryotic Genome Annotation

**Citation** Bragason E, Svendsen CA, Guya ME, Berhe T, Hansen EB. 2020. Draft genome sequences of *Lactococcus lactis* strains MS22314, MS22333, MS22336, and MS22337, isolated from fermented camel milk in Ethiopia. *Microbiol Resour Announc* 9:e00862-20. <https://doi.org/10.1128/MRA.00862-20>.

**Editor** J. Cameron Thrash, University of Southern California

**Copyright** © 2020 Bragason 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 Egon Bech Hansen, [egbh@food.dtu.dk](mailto:egbh@food.dtu.dk).

**Received** 24 July 2020

**Accepted** 21 October 2020

**Published** 19 November 2020

**TABLE 1** Characteristics and accession numbers of the *L. lactis* strains isolated from spontaneous fermented camel milk from Ethiopia

| Species and strain       | Genome size (bp) | GC content (%) | No. of contigs | No. of CDS <sup>a</sup> | N <sub>50</sub> (bp) | No. of reads | Coverage (×) | GenBank accession no. | SRA accession no. |
|--------------------------|------------------|----------------|----------------|-------------------------|----------------------|--------------|--------------|-----------------------|-------------------|
| <i>L. lactis</i> MS22314 | 2,694,284        | 35.0           | 236            | 2,684                   | 66,984               | 1,940,308    | 153.9        | WWDH000000000         | SRR11713472       |
| <i>L. lactis</i> MS22333 | 2,689,322        | 35.1           | 340            | 2,669                   | 34,236               | 2,187,598    | 173.8        | WWDI000000000         | SRR11713471       |
| <i>L. lactis</i> MS22336 | 2,692,760        | 35.0           | 273            | 2,690                   | 60,629               | 1,667,736    | 130.2        | WWDJ000000000         | SRR11713470       |
| <i>L. lactis</i> MS22337 | 2,659,725        | 35.1           | 233            | 2,674                   | 76,645               | 1,868,468    | 147.6        | WWDK000000000         | SRR11713469       |

<sup>a</sup>CDS, coding DNA sequences.

Pipeline (PGAP) v4.11. A Swiss-Prot (9) entry (accession number [Q48712](#)) was found to have 99.8% identity to a gene coding for tetracycline resistance *tet(S)* in MS22314, MS22336, and MS22337, which should be considered when developing starter cultures for camel dairy applications.

The draft genome sequences of *L. lactis* strains MS22314, MS22333, MS22336, and MS22337 are valuable for future manufacturing of effective and safe starter cultures specific to the camel dairy industry.

**Data availability.** The genome sequences of MS22314, MS22333, MS22336, and MS22337 have been deposited in DDBJ/ENA/GenBank under the BioSample numbers [SAMN13701540](#), [SAMN13701541](#), [SAMN13701542](#), and [SAMN13701543](#). The raw read data have been uploaded to the NCBI Sequence Read Archive (10) and can be found at GenBank under the accession numbers listed in Table 1, together with the Illumina paired-end contigs.

## ACKNOWLEDGMENTS

We acknowledge financial support from the Danish Development Fund, Danida, through grant DFC 12-017DTU, and from Innovation Fund Denmark through grant 7045-00021.

We thank Bodil Madsen for expert technical assistance.

## REFERENCES

1. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). 2011. Scientific opinion on the safety and efficacy of *Lactococcus lactis* (NCIMB 30117) as a silage additive for all animal species. *EFSA J* 9:2448. <https://doi.org/10.2903/j.efsa.2011.2448>.
2. Fugl A, Berhe T, Kiran A, Hussain S, Laursen MF, Bahl MI, Hailu Y, Sørensen KI, Guya ME, Ipsen R, Hansen EB. 2017. Characterisation of lactic acid bacteria in spontaneously fermented camel milk and selection of strains for fermentation of camel milk. *Int Dairy J* 73:19–24. <https://doi.org/10.1016/j.idairyj.2017.04.007>.
3. Berhe T, Seifu E, Ipsen R, Kurtu MY, Hansen EB. 2017. Processing challenges and opportunities of camel dairy products. *Int J Food Sci* 2017:9061757. <https://doi.org/10.1155/2017/9061757>.
4. Berhe T, Ipsen R, Seifu E, Kurtu MY, Eshetu M, Hansen EB. 2018. Comparison of the acidification activities of commercial starter cultures in camel and bovine milk. *LWT* 89:123. <https://doi.org/10.1016/j.lwt.2017.10.041>.
5. Pedersen JC. 1992. Natamycin as a fungicide in agar media. *Appl Environ Microbiol* 58:1064–1066. <https://doi.org/10.1128/AEM.58.3.1064-1066.1992>.
6. Saito M, Seki M, Iida K-I, Nakayama H, Yoshida S-I. 2007. A novel agar medium to detect hydrogen peroxide-producing bacteria based on the Prussian blue-forming reaction. *Microbiol Immunol* 51:889–892. <https://doi.org/10.1111/j.1348-0421.2007.tb03971.x>.
7. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 50:1355–1361. <https://doi.org/10.1128/JCM.06094-11>.
8. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
9. Boeckmann B, Bairoch A, Apweiler R, Blatter M-C, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M. 2003. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. *Nucleic Acids Res* 31:365–370. <https://doi.org/10.1093/nar/gkg095>.
10. Leinonen R, Sugawara H, Shumway M, International Nucleotide Sequence Database Collaboration. 2011. The Sequence Read Archive. *Nucleic Acids Res* 39:D19–D21. <https://doi.org/10.1093/nar/gkq1019>.