



Complete Genome Sequence of Nisin-Producing *Lactococcus lactis* subsp. *lactis* N8

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ABSTRACT We report here the genome sequence of *Lactococcus lactis* subsp. *lactis* N8, a nisin producer isolated in the 1960s from a dairy product in Finland. The genome consists of a 2.42-Mb chromosome and two plasmids of 80.3 and 71.3 kb.

Lactococcus lactis is an important lactic acid bacterium in the dairy industry (1). Some *L. lactis* strains produce the antimicrobial peptide nisin, which is active against many foodborne pathogens (2, 3). *L. lactis* subsp. *lactis* N8 is a nisin Z producer isolated in the 1960s from a high-quality dairy product by the Finnish dairy company Valio Ltd. (4). We received strain N8 from Valio Ltd. in November 1990, and since then, we have conducted various studies to understand nisin biosynthesis (5–9) and to develop biotechnology tools (10–15).

Genomic DNA (gDNA) was extracted from a 2-ml culture of *L. lactis* N8 grown overnight at 30°C in M17 broth supplemented with 0.5% glucose using a MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, Hilden, Germany) according to the supplier's protocol. A DNA template prep kit, no. 2.0 (Pacific Biosciences, Menlo Park, CA, USA), was used to generate 3- to 10-kb fragments, and DNA/polymerase binding kit P6 (Pacific Biosciences) was used to generate a DNA polymerase/template library complex. The library was sequenced on a PacBio RS II sequencer, generating 103,204 reads (N_{50} , 11,879 bp). Reads were *de novo* assembled using the RS hierarchical genome assembly process (HGAP) version 3.0 implemented in SMRTportal 2.3 (Pacific Biosciences).

Aliquots of gDNA were also used to generate a library with a Nextera XT kit and sequenced on the Illumina MiSeq platform using a 300-bp paired-end strategy with MiSeq reagent kit v3 (Illumina, Inc., San Diego, CA, USA), according to the manufacturer's instruction. The Illumina reads were trimmed with cutadapt (v1.14; minimum length, 50 bp; quality cutoff, 25) (16), resulting in 418,075 R1 plus 418,075 R2 reads for mapping by Burrows-Wheeler Aligner (17) and for polishing the draft assembly by Pilon (18).

Complete genome sequences were circularized using a genome assembly program (Gap4, Staden package) (19), and overlapping ends were removed manually. Default parameters were used for all software unless otherwise noted.

The assembly generated three circular contigs, representing one chromosome and two plasmids, with sizes of 2,421,567 bp (506× PacBio coverage, 85× Illumina coverage), 80,301 bp (pLLN8-1; 178× PacBio coverage, 136× Illumina coverage), and 71,261 bp (pLLN8-2; 178× PacBio coverage, 145× Illumina coverage), respectively. The closed genome was rotated to start at the *dnaA* gene. Like other *L. lactis* subsp. *lactis* strains, strain N8 exhibits a chromosomal G+C content of 35.1%. Its plasmids, pLLN8-1 and pLLN8-2, have G+C contents of 35.1% and 33.7%, respectively.

The genomic sequences were uploaded to NCBI and automatically annotated with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 4.12 (20), which predicted a total of 2,521 gene sequences, including 2,434 coding sequences, 19 rRNAs,

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64 tRNAs, and 4 noncoding RNAs. As expected, the genes *nisZBTCIPRKFE*G, which are needed for nisin biosynthesis, regulation, and immunity, were located in the nisin-sucrose transposon Tn5481 in the chromosome. Interestingly, we also found a chromosomal gene cluster (>40 kb) for a nonribosomal peptide synthetase/polyketide synthase system.

Data availability. The complete genomic sequence of *L. lactis* subsp. *lactis* N8, including its plasmids, pLLN8-1 and pLLN8-2, has been deposited in GenBank and is available under the accession numbers CP059049, CP059050, and CP059051, respectively. Raw reads from PacBio and Illumina sequencing were uploaded to the Sequence Read Archive (SRA) under the accession numbers SRR12756349 and SRR13170739, respectively.

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REFERENCES

- Song AA, In LLA, Lim SHE, Rahim RA. 2017. A review on *Lactococcus lactis*: from food to factory. *Microb Cell Fact* 16:55. <https://doi.org/10.1186/s12934-017-0669-x>.
- Campion A, Casey PG, Field D, Cotter PD, Hill C, Ross RP. 2013. *In vivo* activity of nisin A and nisin V against *Listeria monocytogenes* in mice. *BMC Microbiol* 13:23. <https://doi.org/10.1186/1471-2180-13-23>.
- Stevens KA, Sheldon BW, Klapes NA, Klaenhammer TR. 1991. Nisin treatment for inactivation of *Salmonella* species and other Gram-negative bacteria. *Appl Environ Microbiol* 57:3613–3615. <https://doi.org/10.1128/AEM.57.12.3613-3615.1991>.
- Graeffe T, Rintala H, Paulin L, Saris PEJ. 1991. A natural nisin variant, p 260–268. In Jung G, Sahl H-G (ed), *Nisin and novel lantibiotics*. Excom, Leiden, The Netherlands.
- Immonen T, Saris PEJ. 1998. Characterization of the *nisFEG* operon of the nisin Z producing *Lactococcus lactis* subsp. *lactis* N8 strain. *DNA Seq* 9:263–274. <https://doi.org/10.3109/10425179809008466>.
- Qiao M, Immonen T, Koponen O, Saris PEJ. 1995. The cellular location and effect on nisin immunity of the NisI protein from *Lactococcus lactis* N8 expressed in *Escherichia coli* and *L. lactis*. *FEMS Microbiol Lett* 131:75–80. <https://doi.org/10.1111/j.1574-6968.1995.tb07757.x>.
- Qiao M, Saris PEJ. 1996. Evidence for a role of NisT in transport of the lantibiotic nisin produced by *Lactococcus lactis* N8. *FEMS Microbiol Lett* 144:89–93. <https://doi.org/10.1111/j.1574-6968.1996.tb08513.x>.
- Tolonen M, Saris PEJ, Siika-Aho M. 2004. Production of nisin with continuous adsorption to Amberlite XAD-4 resin using *Lactococcus lactis* N8 and *L. lactis* LAC48. *Appl Microbiol Biotechnol* 63:659–665. <https://doi.org/10.1007/s00253-003-1413-5>.
- Xuanyuan Z, Wu Z, Li R, Jiang D, Su J, Xu H, Bai Y, Zhang X, Saris PE, Qiao M. 2010. Loss of *IrpT* function in *Lactococcus lactis* subsp. *lactis* N8 results in increased nisin resistance. *Curr Microbiol* 61:329–334. <https://doi.org/10.1007/s00284-010-9615-4>.
- Li R, Takala TM, Qiao M, Xu H, Saris PEJ. 2011. Nisin-selectable food-grade secretion vector for *Lactococcus lactis*. *Biotechnol Lett* 33:797–803. <https://doi.org/10.1007/s10529-010-0503-6>.
- Wahlstrom G, Saris PEJ. 1999. A nisin bioassay based on bioluminescence. *Appl Environ Microbiol* 65:3742–3745. <https://doi.org/10.1128/AEM.65.8.3742-3745.1999>.
- Wu Z, Xuanyuan Z, Li R, Jiang D, Li C, Xu H, Bai Y, Zhang X, Turakainen H, Saris PE, Savilahti H, Qiao M. 2009. Mu transposition complex mutagenesis in *Lactococcus lactis*—identification of genes affecting nisin production. *J Appl Microbiol* 106:41–48. <https://doi.org/10.1111/j.1365-2672.2008.03962.x>.
- Zhu D, Liu F, Xu H, Bai Y, Zhang X, Saris PEJ, Qiao M. 2015. Isolation of strong constitutive promoters from *Lactococcus lactis* subsp. *lactis* N8. *FEMS Microbiol Lett* 362:fnv107. <https://doi.org/10.1093/femsle/fnv107>.
- Takala TM, Saris PEJ. 2002. A food-grade cloning vector for lactic acid bacteria based on the nisin immunity gene *nisI*. *Appl Microbiol Biotechnol* 59:467–471. <https://doi.org/10.1007/s00253-002-1034-4>.
- Wan X, Usvalampi AM, Saris PEJ, Takala TM. 2016. A counterselection method for *Lactococcus lactis* genome editing based on class IIa bacteriocin sensitivity. *Appl Microbiol Biotechnol* 100:9661–9669. <https://doi.org/10.1007/s00253-016-7828-6>.
- Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 17:10–12. <https://doi.org/10.14806/ej.17.1.200>.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Staden R, Judge DP, Bonfield JK. 2003. Managing sequencing projects in the GAP4 environment, p 327–344. In Krawetz SA, Womble DD (ed), *Introduction to bioinformatics*. Springer, New York, NY.
- Tatusova T, DiCuccio M, Badretdin A, Chetverin 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>.