




# Complete Genome Sequence of *Lactococcus lactis* subsp. *lactis* Strain 14B4, Which Inhibits the Growth of *Salmonella enterica* Serotype Poona *In Vitro*

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**ABSTRACT** We present here the complete genome sequence of *Lactococcus lactis* strain 14B4, isolated from almond drupes in northern California. This strain was observed to inhibit the growth of *Salmonella enterica* serotype Poona strain RM3363 *in vitro*.

*Lactococcus lactis* is a Gram-positive nonsporulating aerotolerant anaerobic bacterium. *L. lactis* plays an important role in microbial food safety due to the production of lactic acid and other antimicrobial agents, such as bacteriocins (1). *L. lactis* strain 14B4 was isolated from almond drupes in northern California in April 2014. *L. lactis* strain 14B4 has been screened and characterized *in vitro* for the ability to inhibit the growth of *Salmonella* Poona strain RM3363, an isolate from the 2002 U.S. multistate cantaloupe outbreak (2).

*L. lactis* strain 14B4 was isolated by washing almond drupes in phosphate-buffered saline for 1 h at 25°C with shaking (200 rpm), followed by plating onto de Man, Rogosa, and Sharpe (MRS) agar that was incubated anaerobically for 24 h at 37°C. For DNA extraction, a single colony was transferred to 100 ml of tryptic soy broth (Oxoid, Basingstoke, Hampshire, England) and incubated at 37°C for 24 h. Genomic DNA was extracted using sucrose-Tris with phenol-chloroform cleanup extractions as described previously (3).

Sequencing was carried out using a Pacific Biosciences (PacBio) RS II sequencer using standard protocols as described previously (3). For Illumina sequencing, DNA was sheared at 30 lb/in<sup>2</sup> for 40 s, and 700- to 770-bp fragments were selected and libraries were constructed using standard protocols (3). Sequencing was performed using a 2 × 250-bp MiSeq paired-end reagent kit version 2 on a MiSeq instrument (Illumina, San Diego, CA) following the manufacturer's protocol. The PacBio RS II platform produced 63,099 total reads, 55,427 of which were used for assembly. The Illumina MiSeq platform yielded 849,578 total reads, 829,489 of which were used for assembly. The PacBio reads were assembled using the RS hierarchical genome assembly process (HGAP) version 3.0 in single-molecule real-time (SMRT) analysis version 2.2.0 (Pacific Biosciences, Menlo Park, CA). The PacBio assembly produced a single chromosomal contig and a single plasmid contig. A final base call validation of the PacBio contigs was performed by mapping Illumina MiSeq reads trimmed using a quality score threshold of 30 or higher (Q30) to the PacBio assembly and the reference assembler within Geneious version 11.1.3 (Biomatters, Ltd., Auckland, New Zealand). Single-nucleotide polymorphisms (SNPs) between the PacBio assembly and the MiSeq reads were addressed using the Annotate and Predict/Find SNPs module with a minimum coverage

Received 2 October 2018 Accepted 22 October 2018 Published 15 November 2018

**Citation** Tran TD, Huynh S, Parker CT, Han R, Hnasko R, Gorski L, McGarvey JA. 2018. Complete genome sequence of *Lactococcus lactis* subsp. *lactis* strain 14B4, which inhibits the growth of *Salmonella enterica* serotype Poona *in vitro*. Microbiol Resour Announc 7:e01364-18. <https://doi.org/10.1128/MRA.01364-18>.

**Editor** Catherine Putonti, Loyola University Chicago

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parameter of 50 and a minimum variant frequency parameter of 0.8. PacBio DNA internal control complex P6 was used as an internal sequencing control, and the read quality control was conducted using FastQC (Pacific Biosciences). Protein-, rRNA-, and tRNA-coding genes were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (4) with additional manual annotation based on the genome of *Lactococcus lactis* subsp. *lactis* KF147 (GenBank accession number CP001834).

The whole-genome sequence of *L. lactis* strain 14B4 consists of a single circular chromosome of 2,579,381 bp (95.0× mean coverage combined from PacBio and Illumina platforms; GC content of 35.0%). It is predicted to have 2,486 coding sequences (CDSs), 6 rRNA operons, and 69 tRNAs. The genome contains 1 plasmid, p14B4, which is 59,700 bp long and is predicted to have 63 CDSs. There were 1 intact, 1 questionable, and 3 incomplete prophage regions in the chromosome of *L. lactis* strain 14B4 as indicated by PHAge Search Tool Enhanced Release (PHASTER) (<http://phaster.ca>) (5, 6). No bacteriophages were found in the plasmid p14B4. Determination of insertion sequences (ISs) was performed by ISfinder (<https://www-is.biotoul.fr/>) (7), and 63 ISs were found in the genome of *L. lactis* strain 14B4. A BLASTn search against putative probiotic activity-related genes from *L. lactis* strain RBT018 (8) identified homologous genes encoding a collagen adhesin (GenBank accession number OAZ16633; 98% identical) and a bacteriocin (GenBank accession number OAZ16145; 93% identical).

**Data availability.** The whole-genome sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers CP028160 (chromosome) and CP028161 (p14B4) (BioProject number PRJNA445480; BioSample number SAMN08792430). The raw data are available in SRA under the accession numbers SRR8043672 (Illumina) and SRR8049353 (PacBio).

## ACKNOWLEDGMENT

This work was supported by the United States Department of Agriculture, Agricultural Research Service, National Program 108: Food Safety.

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