

# Genome Sequence of *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis* LD61

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***Lactococcus lactis* is widely used in the dairy industry. We report the draft genome sequence of *L. lactis* subsp. *lactis* bv. *diacetylactis* LD61, an industrial and extensively studied strain. In contrast to the closely related and plasmidless strain IL1403, LD61 contains 6 plasmids, and the genome sequence provides additional information related to adaptation to the dairy environment.**

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*Lactococcus lactis* is widely used as a dairy starter in the production of fermented milk products, particularly cheese. *L. lactis* is the model lactic acid bacterium, and the genome of strain IL1403 was the first genome of a lactic acid bacterium (LAB) ever sequenced (1). *L. lactis* subsp. *lactis* IL1403 is a plasmidless dairy-derived strain, and three other sequenced *L. lactis* subsp. *lactis* strains were isolated from nondairy biotopes, i.e., mung bean sprouts for KF147 (2), vaginal flora for CV56 (3), and water of a kitchen sink for IO-1 (4). These three strains contain 1, 5, and 0 plasmids, respectively. These sequences provide interesting genomic data, allowing for the identification of mechanisms of adaptation to peculiar niches, but they do not allow for a fine-tuned analysis of genomic diversity within genetically related clusters. Yet, an integrated approach toward *L. lactis* subsp. *lactis* provided evidence of intrasubspecies genetic diversity (5).

*L. lactis* subsp. *lactis* bv. *diacetylactis* strain LD61 was isolated from a starter culture and has a dairy phenotype. It contains 6 plasmids, the sizes of which were estimated to be 4.5, 6.5, 9, 11, 50, and 55 kb, and which carry important components for sustaining growth in milk (citrate permease operon, lactose operon, and cell wall protease). It has been extensively used in recent works regarding gene expression and the inhibition of *Staphylococcus aureus* virulence expression under various growth conditions (6–8). The whole-genome sequencing of *L. lactis* subsp. *lactis* LD61 reveals genomic diversity of *L. lactis* subsp. *lactis* involved in the different capabilities observed among dairy strains.

The LD61 whole genome was sequenced by using an Illumina HiSeq 2000 (Fasteris, Geneva, Switzerland). Base calling was performed using the HiSeq Control software version 1.4.8. After barcode selection, 9.2 million paired-end reads of 100 bases in length were obtained. The sequence reads were *de novo* assembled using the Edena assembler version 3.131028 (9, 10). Assembly resulted

in 132 contigs (sum, 2.60 Mbp;  $N_{50}$ , 48.2 Kbp; max, 252.7 Kbp; min, 200 bp) with a G+C content of 36.4%. A total of 2,601 coding sequences were detected by using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (11). Over 45% of the genes were assigned to specific subsystem categories by RAST (12). A more detailed analysis of this genome and comparative analyses with other *L. lactis* subsp. *lactis* genomes will provide further insight into the genomic differences and genome evolution within this species.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AXZK000000000](https://www.ncbi.nlm.nih.gov/nuccore/AXZK000000000). The version described in this paper is version AXZK01000000.

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## REFERENCES

1. Bolotin A, Wincker P, Mauger S, Jaillon O, Malarne K, Weissenbach J, Ehrlich SD, Sorokin A. 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res.* 11: 731–753. [http://dx.doi.org/10.1101/gr.GR-1697R](https://doi.org/10.1101/gr.GR-1697R).
2. Siezen RJ, Bayjanov J, Renckens B, Wels M, van Hijum SA, Molenaar D, van Hylckama Vlieg JE. 2010. Complete genome sequence of *Lactococcus lactis* subsp. *lactis* KF147, a plant-associated lactic acid bacterium. *J. Bacteriol.* 192:2649–2650. [http://dx.doi.org/10.1128/JB.00276-10](https://doi.org/10.1128/JB.00276-10).
3. Gao Y, Lu Y, Teng KL, Chen ML, Zheng HJ, Zhu YQ, Zhong J. 2011. Complete genome sequence of *Lactococcus lactis* subsp. *lactis* CV56, a probiotic strain isolated from the vaginas of healthy women. *J. Bacteriol.* 193:2886–2887. [http://dx.doi.org/10.1128/JB.00358-11](https://doi.org/10.1128/JB.00358-11).
4. Kato H, Shiwa Y, Oshima K, Machii M, Arava-Kojima T, Zendo T, Shimizu-Kadota M, Hattori M, Sonomoto K, Yoshikawa H. 2012. Complete genome sequence of *Lactococcus lactis* IO-1, a lactic acid bacte-

- rium that utilizes xylose and produces high levels of L-lactic acid. *J. Bacteriol.* 194:2102–2103. <http://dx.doi.org/10.1128/JB.00074-12>.
5. Tan-a-Ram P, Cardoso T, Daveran-Mingot ML, Kanchanatawee S, Loubière P, Girbal L, Coccain-Bousquet M. 2011. Assessment of the diversity of dairy *Lactococcus lactis* subsp. *lactis* isolates by an integrated approach combining phenotypic, genomic, and transcriptomic analyses. *Appl. Environ. Microbiol.* 77:739–748. <http://dx.doi.org/10.1128/AEM.01657-10>.
  6. Cretenet M, Laroute V, Ulvé V, Jeanson S, Nouaille S, Even S, Piot M, Girbal L, Le Loir Y, Loubière P, Lortal S, Coccain-Bousquet M. 2011. Dynamic analysis of the *Lactococcus lactis* transcriptome in cheeses made from milk concentrated by ultrafiltration reveals multiple strategies of adaptation to stresses. *Appl. Environ. Microbiol.* 77:247–257. <http://dx.doi.org/10.1128/AEM.01174-10>.
  7. Cretenet M, Nouaille S, Thouin J, Rault L, Stenz L, François P, Hennekinne JA, Piot M, Maillard MB, Fauquant J, Loubière P, Le Loir Y, Even S. 2011. *Staphylococcus aureus* virulence and metabolism are dramatically affected by *Lactococcus lactis* in cheese matrix. *Environ. Microbiol. Rep.* 3:340–351. <http://dx.doi.org/10.1111/j.1758-2229.2010.00230.x>.
  8. Even S, Charlier C, Nouaille S, Ben Zakour NL, Cretenet M, Cousin FJ, Gautier M, Coccain-Bousquet M, Loubière P, Le Loir Y. 2009. *Staphylococcus aureus* virulence expression is impaired by *Lactococcus lactis* in mixed cultures. *Appl. Environ. Microbiol.* 75:4459–4472. <http://dx.doi.org/10.1128/AEM.02388-08>.
  9. Hernandez D, François P, Farinelli L, Osterås M, Schrenzel J. 2008. *De novo* bacterial genome sequencing: millions of very short reads assembled on a desktop computer. *Genome Res.* 18:802–809. <http://dx.doi.org/10.101/gr.072033.107>.
  10. Hernandez D, Tewhey R, Veyrieras JB, Farinelli L, Osteras M, Francois P, Schrenzel J. 15 October 2013. *De novo* finished 2.8 Mbp *Staphylococcus aureus* genome assembly from 100 bp short and long range paired-end reads. *Bioinformatics* 30:40–49. <http://dx.doi.org/10.1093/bioinformatics/btt590>.
  11. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. *Omic* 12: 137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
  12. 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>.