



Complete Genome Sequence of *Lactococcus lactis* subsp. *lactis* G121, an Isolate with Allergy-Protective Features Derived from a Farming Environment

Zhongjie Wang,^a Eva-Maria L. Minarsch,^{a*} Susanne Kublik,^a  Holger Heine,^b Michael Schloter,^{a,c}  Baerbel U. Foesel^a

^aResearch Unit for Comparative Microbiome Analysis, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

^bDivision of Innate Immunity, Research Center Borstel, Airway Research Center North, German Center for Lung Research, Borstel, Germany

^cTechnical University of Munich, Central Institute for Food and Health, Freising, Germany

ABSTRACT Early childhood exposure to a farming environment has been found to be protective against asthma and other atopic disorders. Here, we report the complete genome sequence of *Lactococcus lactis* subsp. *lactis* G121, which was isolated from the kitchen of a farm in Bavaria (Germany) and is recognized for its allergy-protective properties. It could be assembled into one circular chromosome, three circular plasmids, and one linear plasmid.

Lactococcus lactis has been described as an abundant member of the cowshed microflora (1) which is considered an important component for the protective effect of farming environments in early childhood, significantly decreasing the risk of developing atopic disorders later in life (2, 3). A subsequent analysis of an isolate derived from a farm, namely, *Lactococcus lactis* subsp. *lactis* G121, revealed a reduction of allergic reactions in sensitized mice, and the strain was able to activate mammalian cells *in vitro* and to induce a TH1-polarizing program in dendritic cells (1).

Here, we present the complete genome of *L. lactis* subsp. *lactis* G121, which was isolated from a farmer's kitchen in Bavaria, Germany (1). Genome sequencing was carried out on the PacBio Sequel platform (Pacific Biosciences, Menlo Park, CA) using v2 chemistry. Library preparation was performed according to the procedure and checklist for preparing multiplexed microbial SMRTbell libraries for the PacBio Sequel system. Briefly, DNA from cells grown overnight in liquid LB at 30°C was extracted using the Genomic-tip 20/G kit (Qiagen, Hilden, Germany). To increase the extraction efficiency, a preincubation of the bacterial culture with ampicillin (0.6 $\mu\text{g}\cdot\text{liter}^{-1}$) for 3 h prior to cell harvesting was included. Genomic DNA was sheared to approximately 10 kb using g-TUBEs (Covaris, Inc., Woburn, MA) and, without additional size selection, further processed according to the protocol. The library including *L. lactis* subsp. *lactis* G121 was loaded onto two single-molecule real-time (SMRT) cells at concentrations of 3 pM and 6 pM, according to the diffusion loading protocol (PacBio). The movie time was 10 h per SMRT cell after immobilization for 2 h and preextension for 2 h. After data demultiplexing, genome assembly for *L. lactis* subsp. *lactis* G121 (using combined data from the two SMRT cells) was performed with the Microbial Assembly pipeline embedded in SMRT Link v8.0.0.80529 (PacBio) (parameters deviating from default settings were as follows: seed coverage, 45; set genome size, 2.7 Mb), utilizing 194,273 realigned subreads, with an average subread length of 3,534 bp. The mean coverage was 253-fold, and the genome contained five scaffolds after initial assembly, including one linear chromosome, three circular plasmids, and one linear plasmid. The linear chromosomal scaffold was circularized successfully using Circlator v1.5.5 with the parameter `--merge_min_length_merge 1000` (4). Coding sequence (CDS), rRNA, and tRNA genes

Citation Wang Z, Minarsch E-ML, Kublik S, Heine H, Schloter M, Foesel BU. 2020. Complete genome sequence of *Lactococcus lactis* subsp. *lactis* G121, an isolate with allergy-protective features derived from a farming environment. *Microbiol Resour Announc* 9:e00777-20. <https://doi.org/10.1128/MRA.00777-20>.

Editor John J. Dennehy, Queens College

Copyright © 2020 Wang 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 Baerbel U. Foesel, baerbel.foesel@helmholtz-muenchen.de.

* Present address: Eva-Maria L. Minarsch, Justus Liebig University Gießen, Gießen, Germany.

Received 15 July 2020

Accepted 21 September 2020

Published 8 October 2020

TABLE 1 Genome features of *Lactococcus lactis* subsp. *lactis* G121

Name	Contig length (bp)	GC content (%)	Topology
Chromosome.000F	2,573,315	35.18	Circular
Plasmid.001F	69,239	31.36	Linear
Plasmid.002F	42,612	31.80	Circular
Plasmid.003F	11,744	36.55	Circular
Plasmid.004F	9,908	33.62	Circular

were scanned and annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (5). The genome was also functionally annotated using the RAST server v2.0 (6).

The overall genome had a size of 2,712,646 bp (GC content, 35%) and contained 2,783 predicted CDSs. As shown in Table 1, the genome consisted of a chromosome of 2,573,315 bp (GC content, 35.18%) and four plasmids. Plasmid.001F, containing 69,239 bp (GC content, 31.36%), was still linear. The other three plasmids, containing 42,612 bp (GC content, 31.8%), 11,744 bp (GC content, 36.54%), and 9,908 bp (GC content, 33.62%), could be circularized.

Nineteen rRNA genes were annotated by PGAP, including six 16S rRNA genes. In addition, 65 tRNAs were detected, but only 1 transfer-messenger RNA gene was found. Based on the RAST annotation, 27% of the CDSs could be assigned to specific subsystems, including the categories carbohydrates (210 CDSs), amino acids and derivatives (188 CDSs), protein metabolism (123 CDSs), and cofactors, vitamins, prosthetic groups, and pigments (120 CDSs). Within the carbohydrate subsystem category, 40 genes were assigned to fermentation, which is closely related to the described phenotypic properties of this species.

Data availability. The complete genome sequence of *Lactococcus lactis* subsp. *lactis* G121 has been deposited in GenBank under accession numbers [CP053671](#) to [CP053674](#) and [CP061027](#). The raw reads have been deposited in the Sequence Read Archive under BioProject accession number [PRJNA633429](#).

ACKNOWLEDGMENTS

We thank Michael Rothballer, Helmholtz Center Munich, German Research Center for Environmental Health (Neuherberg, Germany), for sharing his protocol for ampicillin preincubation of Gram-positive bacteria prior to DNA extraction. Sören Gaterman, Ruhr University Bochum (Bochum, Germany), and Otto Holst, Research Center Borstel, Leibniz Lung Center (Borstel, Germany), are acknowledged for providing us with the *L. lactis* subsp. *lactis* G121 isolate.

This work has partially been funded by the Helmholtz Center Munich allergy program.

REFERENCES

- Debarry J, Garn H, Hanuszkiwicz A, Dickgreber N, Blümer N, von Mutius E, Bufe A, Gatermann S, Renz H, Holst O, Heine H. 2007. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J Allergy Clin Immunol* 119: 1514–1521. <https://doi.org/10.1016/j.jaci.2007.03.023>.
- Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl R, Nowak D, Von Mutius E. 2001. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 358:1129–1133. [https://doi.org/10.1016/S0140-6736\(01\)06252-3](https://doi.org/10.1016/S0140-6736(01)06252-3).
- Daley D. 2014. The evolution of the hygiene hypothesis: the role of early-life exposures to viruses and microbes and their relationship to asthma and allergic diseases. *Curr Opin Allergy Clin Immunol* 14:390–396. <https://doi.org/10.1097/ACI.0000000000000101>.
- Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biol* 16:294. <https://doi.org/10.1186/s13059-015-0849-0>.
- Tatusova T, Dicuccio M, Badretdin A, Chetvernin 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>.
- Aziz RK, Bartels D, Best A, 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. <https://doi.org/10.1186/1471-2164-9-75>.