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PROKARYOTES

Draft Genome Sequence of the Plasmid-Free *Lactococcus lactis* subsp. *lactis* Strain LMG 19460

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ABSTRACT We report here the draft genome sequence of the plasmid-free *Lactococcus lactis* subsp. *lactis* strain LMG 19460. This strain has potential application for a cost-effective production of food-grade plasmid DNA to use in DNA vaccines, produce recombinant proteins, and be used as a mucosal delivery vehicle of therapeutic molecules.

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Lactic acid bacteria (LAB) have a huge relevance in health, food, and industrial applications, being used traditionally as fermentation starters and recently as probiotics (1), mucosal delivery vehicles of therapeutic molecules (2), and recombinant protein producers (1).

Lactococcus lactis is the model LAB, being broadly used in the dairy industry (3). The availability of several molecular biology tools to manipulate this bacterium, together with its food-grade and generally recognized as safe (GRAS) status, raises even more the industrial value of this species (2).

We report here the genome sequence of *L. lactis* subsp. *lactis* LMG 19460, which is a plasmid-free strain, formerly known as *Streptococcus lactis* subsp. *diacetylactis* Bu2-60. This strain is derived from *S. lactis* subsp. *diacetylactis* Bu2 that was exposed to high sublethal temperatures to cure its six plasmids (4–6). The parental strain was isolated from starter cultures of German cheese factories between 1971 and 1979 (7).

Although the LMG 19460 strain lacks the plasmid-coded metabolic functions such as carbohydrate and citrate metabolism, which make it infeasible to be used as a starter culture in dairy fermentation, it has several advantages. This strain is ideal for DNA transfer and gene cloning studies, since issues due to the presence of other plasmids of the same compatibility group are avoided, as well as heterologous DNA degradation problems derived from the presence of two plasmid-encoded restriction-modification systems (8). Studies have been performed using this strain as a recipient for conjugation (4, 9, 10) and for plasmid transfer via transduction (11).

A less explored application of this strain is its potential as a host for the production of food- and pharmaceutical-grade plasmids for use in DNA vaccines (12, 13). Together with being GRAS, the plasmid-free status of this strain allows a cost-effective purification process, due to the absence of copurifying endogenous plasmids and/or pathogenic contaminants (12). Also, the decrease in the metabolic burden could lead to an increase in the plasmid yields and also in the production of pharmaceutical-grade recombinant proteins.

Whole-genome sequencing of *L. lactis* subsp. *lactis* LMG 19460 was performed using the Illumina MiSeq platform (Instituto Gulbenkian de Ciência, Portugal), which yielded a total of 753,312 paired-end reads (totaling ~140× coverage). Paired-end reads were analyzed for Phred quality, trimmed and filtered using Fastq-Mcf version 1.04.676 (14), and assembled using SPAdes version 3.8.0 (15) and HGA version 1.0 (16). Generated contigs were scaffolded using SSPACE version 3.0 (17) followed by automated improvement using iCORN (18). The *L. lactis* subsp. *lactis* LMG 19460 genome was assembled in

41 contigs accounting for 2,260,841 bp and an estimated G+C content of 35.1%. The draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline, which predicted a total of 2,164 protein-coding sequences, 84 pseudogenes, six rRNAs, and 51 tRNAs.

Further experimental and comparative genomic analyses will provide new insights into the use of this strain to produce recombinant proteins and as a delivery vehicle for therapeutic molecules.

Accession number(s). This whole-genome shotgun sequence project has been deposited at DDBJ/ENA/GenBank under the accession number MUBH00000000. The version described in this paper is the first version, MUBH01000000.

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REFERENCES

1. D'Souza R, Pandeya DR, Hong ST. 2012. Review: *Lactococcus lactis*: an efficient gram positive cell factory for the production and secretion of recombinant protein. Biomed Res 23:1–7.
2. Bermúdez-Humarán LG, Kharrat P, Chatel JM, Langella P. 2011. Lactococci and lactobacilli as mucosal delivery vectors for therapeutic proteins and DNA vaccines. Microb Cell Fact 10:S4. https://doi.org/10.1186/1475-2859-10-S1-S4.
3. Pontes DS, de Azevedo MSPD, Chatel JM, Langella P, Azevedo V, Miyoshi A. 2011. *Lactococcus lactis* as a live vector: heterologous protein production and DNA delivery systems. Protein Expr Purif 79:165–175. https://doi.org/10.1016/j.pep.2011.06.005.
4. Neve H, Geis A, Teuber M. 1984. Conjugal transfer and characterization of bacteriocin plasmids in group N (lactic acid) streptococci. J Bacteriol 157:833–838.
5. Pechmann H, Teuber M. 1980. Plasmid pattern of group N (lactic) streptococci. Zbl Bakt Mik Hyg I C 101:133–136.
6. Jahns A, Schäfer A, Geis A, Teuber M. 1991. Identification, cloning and sequencing of the replication region of *Lactococcus lactis* ssp. *lactis* biovar. diacetylactis Bu2 citrate plasmid pSL2. FEMS Microbiol Lett 64:253–258. https://doi.org/10.1111/j.1574-6968.1991.tb04671.x.
7. Loof M, Teuber M. 1986. Heteroduplex analysis of the genomes of *Streptococcus lactis* "*subsp. diacetylactis*" bacteriophages of the P008-type isolated from German cheese factories. Syst Appl Microbiol 8:226–229. https://doi.org/10.1016/S0723-2020(86)80082-0.
8. Vos WM. 1987. Gene cloning and expression in lactic streptococci. FEMS Microbiol Lett 46:281–295. https://doi.org/10.1111/j.1574-6968.1987.tb02466.x.
9. Gevers D, Huys G, Swings J. 2003. In vitro conjugal transfer of tetracycline resistance from *Lactobacillus* isolates to other Gram-positive bacteria. FEMS Microbiol Lett 225:125–130. https://doi.org/10.1016/S0378-1097(03)00505-6.
10. Boguslawska J, Zycka-Krzesinska J, Wilcks A, Bardowski J. 2009. Intra- and interspecies conjugal transfer of Tn*916*-like elements from *Lactococcus lactis* in vitro and in vivo. Appl Environ Microbiol 75:6352–6360. https://doi.org/10.1128/AEM.00470-09.
11. Ammann A, Neve H, Geis A, Heller KJ. 2008. Plasmid transfer via transduction from *Streptococcus thermophilus* to *Lactococcus lactis*. J Bacteriol 190:3083–3087. https://doi.org/10.1128/JB.01448-07.
12. Platteeuw C, van Alen-Boerrigter I, van Schalkwijk S, de Vos WM. 1996. Food-grade cloning and expression system for *Lactococcus lactis*. Appl Environ Microbiol 62:1008–1013.
13. Bins AD, van den Berg JH, Oosterhuis K, Haanen JB. 2013. Recent advances towards the clinical application of DNA vaccines. Neth J Med 71:109–117.
14. Aronesty E. 2013. Comparison of sequencing utility programs. Open Bioinforma J 7:1–8. https://doi.org/10.2174/1875036201307010001.
15. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
16. Al-Okaily AA. 2016. HGA: *de novo* genome assembly method for bacterial genomes using high coverage short sequencing reads. BMC Genomics 17:193. https://doi.org/10.1186/s12864-016-2515-7.
17. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 27:578–579. https://doi.org/10.1093/bioinformatics/btq683.
18. Otto TD, Sanders M, Berriman M, Newbold C. 2010. Iterative correction of reference nucleotides (iCORN) using second generation sequencing technology. Bioinformatics 26:1704–1707. https://doi.org/10.1093/bioinformatics/btq269.