



Draft Genome Sequence of *Lactobacillus reuteri* 121, a Source of α -Glucan and β -Fructan Exopolysaccharides

Joana Gangoiti, Xiangfeng Meng, Alicia Lammerts van Bueren, Lubbert Dijkhuizen

Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), Groningen, The Netherlands

ABSTRACT The probiotic bacterium *Lactobacillus reuteri* 121 is a well-known producer of diverse homoexopolysaccharides (α -glucans and β -fructans) from sucrose and maltodextrins/starches of interest for food applications. Here, we report the draft genome sequence of this strain, with a focus on carbohydrate-active enzymes.

The exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) are of interest for food applications (1–3). In a previous study, a collection of 182 LAB were screened for the ability to produce EPS in high-sucrose medium (4), resulting in the identification of *Lactobacillus reuteri* 121 as a producer of β -fructans (inulin and levan) and α -glucan (reuteran) EPS. We also have characterized the inulosucrase, levansucrase, and glucansucrase (GS) enzymes converting sucrose into these three different types of EPS (5, 6). *L. reuteri* 121 was also found to encode a GS-like enzyme (designated GtFB) that is inactive on sucrose but displays 4,6- α -glucanotransferase activity (4,6- α -GTase), converting maltodextrins/starch substrates into isomalto-malto polysaccharides (IMMP) (7, 8). Together with this ability to synthesize diverse homo-EPS, *L. reuteri* 121 possesses the generally recognized as safe status, opening great possibilities for its application in the food industry.

Here, we present the draft genome sequence of *L. reuteri* 121, which was obtained from an 8- to 12-kb insert library constructed and sequenced using a PacBio RS II instrument at GATC Biotech AG (Konstanz, Germany). A total of 55,989 reads with a mean size of 5,482 bp were obtained, providing 105,77-fold genome coverage. *De novo* assembly was performed by PacBio SMRT Analysis 2.0 using the HGAP2 protocol (Pacific Biosciences, USA), yielding 14 contigs. The largest contig was 1,570,268 bp long. The genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) (9) and the Rapid Annotations using Subsystems Technology (RAST) server (<http://rast.nmpdr.org/>) (10). The draft genome of *L. reuteri* 121 is 2,302,234 bp in length and has an average G+C content of 39.0%, similar to that of other *L. reuteri* sequenced genomes (11, 12). A total of 2,226 genes (2,027 protein-coding sequences, 105 pseudogenes, and 94 RNA-encoding genes) were annotated using the NCBI annotation pipeline. Because the majority of enzymes involved in EPS synthesis in LAB fall within the carbohydrate active enzyme (CAZy) classification, we analyzed the *L. reuteri* 121 genome by dbCAN (<http://csbl.bmb.uga.edu/dbCAN/>) (13), which resulted in the identification of 26, 25, and 12 putative glycoside hydrolases (GH), glycosyl transferases (GT), and carbohydrate esterases, respectively. Consistent with previous studies, the genes of two GH68 proteins (levansucrase and inulosucrase) and two GH70 proteins (4,6- α -GTase and GS) were identified. However, the *L. reuteri* 121 genome does not appear to encode many (extracellular) enzymes involved in the degradation of β -fructans or α -glucans, and only a single GH31 enzyme was predicted to function as an extracellular α -glucosidase. In contrast, two extracellular β -xylosidases and an extracellular α -N-arabinofuranosidase

Received 12 January 2017 Accepted 12 January 2017 Published 9 March 2017

Citation Gangoiti J, Meng X, Lammerts van Bueren A, Dijkhuizen L. 2017. Draft genome sequence of *Lactobacillus reuteri* 121, a source of α -glucan and β -fructan exopolysaccharides. *Genome Announc* 5:e01691-16. <https://doi.org/10.1128/genomeA.01691-16>.

Copyright © 2017 Gangoiti 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 Lubbert Dijkhuizen, L.Dijkhuizen@rug.nl.

were found belonging to the GH120 and GH43 families, respectively. These enzymes may be involved in the degradation of arabinose- and xylose-containing polysaccharides and/or oligosaccharides, which are recognized as promising prebiotics present in plant cell walls (14–16). Furthermore, genome analysis using antiSMASH 3.0 (17, 18) revealed two heteropolysaccharide biosynthesis gene clusters, both containing several GT enzymes. This finding indicates that *L. reuteri* 121 holds a great potential for the production of both homo- and heteropolysaccharides.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [MKQH00000000](https://doi.org/10.1093/c4fo00529e). The version described in this paper is version MKQH01000000.

ACKNOWLEDGMENTS

J.G., X.M., and L.D. acknowledge funding by the University of Groningen. A.L.V.B. was financially supported by an NWO VENI grant.

REFERENCES

- Ryan PM, Ross RP, Fitzgerald GF, Caplice NM, Stanton C. 2015. Sugar-coated: exopolysaccharide producing lactic acid bacteria for food and human health applications. *Food Funct* 6:679–693. <https://doi.org/10.1039/c4fo00529e>.
- Badel S, Bernardi T, Michaud P. 2011. New perspectives for lactobacilli exopolysaccharides. *Biotechnol Adv* 29:54–66. <https://doi.org/10.1016/j.biotechadv.2010.08.011>.
- Galle S, Arendt EK. 2014. Exopolysaccharides from sourdough lactic acid bacteria. *Crit Rev Food Sci Nutr* 54:891–901. <https://doi.org/10.1080/10408398.2011.617474>.
- van Geel-Schutten GH, Flesch F, ten Brink B, Smith MR, Dijkhuizen L. 1998. Screening and characterization of *Lactobacillus* strains producing large amounts of exopolysaccharides. *Appl Microbiol Biotechnol* 50:697–703. <https://doi.org/10.1007/s002530051353>.
- Ozimek LK, van Hijum SA, van Koningsveld GA, van Der Maarel MJ, van Geel-Schutten GH, Dijkhuizen L. 2004. Site-directed mutagenesis study of the three catalytic residues of the fructosyltransferases of *Lactobacillus reuteri* 121. *FEBS Lett* 560:131–133. [https://doi.org/10.1016/S0014-5793\(04\)00085-7](https://doi.org/10.1016/S0014-5793(04)00085-7).
- Kralj S, van Geel-Schutten GH, van der Maarel MJ, Dijkhuizen L. 2004. Biochemical and molecular characterization of *Lactobacillus reuteri* 121 reuteransucrase. *Microbiology* 150:2099–2112. <https://doi.org/10.1099/mic.0.27105-0>.
- Kralj S, Grijpstra P, van Leeuwen SS, Leemhuis H, Dobruchowska JM, van der Kaaij RM, Malik A, Oetari A, Kamerling JP, Dijkhuizen L. 2011. 4,6-Alpha-glucanotransferase, a novel enzyme that structurally and functionally provides an evolutionary link between glycoside hydrolase enzyme families 13 and 70. *Appl Environ Microbiol* 77:8154–8163. <https://doi.org/10.1128/AEM.05735-11>.
- Leemhuis H, Dobruchowska JM, Ebbelaar M, Faber F, Buwalda PL, van der Maarel MJ, Kamerling JP, Dijkhuizen L. 2014. Isomalto/malto-polysaccharide, a novel soluble dietary fiber made via enzymatic conversion of starch. *J Agric Food Chem* 62:12034–12044. <https://doi.org/10.1021/jf503970a>.
- 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/gkv569>.
- 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. <https://doi.org/10.1186/1471-2164-9-75>.
- Zhang D, Ji H, Liu H, Wang S, Wang J, Wang Y. 2016. Complete genome sequence of probiotic *Lactobacillus reuteri* ZLR003 isolated from healthy weaned pig. *J Biotechnol* 228:69–70. <https://doi.org/10.1016/j.jbiotec.2016.04.044>.
- Leonard MT, Valladares RB, Ardisson A, Gonzalez CF, Lorca GL, Triplett EW. 2014. Complete genome sequences of *Lactobacillus johnsonii* strain N6.2 and *Lactobacillus reuteri* strain TD1. *Genome Announc* 2(3):e00397-14. <https://doi.org/10.1128/genomeA.00397-14>.
- Yin Y, Mao X, Yang J, Chen X, Mao F, Xu Y. 2012. dbCAN: a Web resource for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res* 40:W445–W451. <https://doi.org/10.1093/nar/gks479>.
- Pontonio E, Mahony J, Di Cagno R, O'Connell Motherway M, Lugli GA, O'Callaghan A, De Angelis M, Ventura M, Gobbetti M, van Sinderen D. 2016. Cloning, expression and characterization of a β -D-xylosidase from *Lactobacillus rossiae* DSM 15814^T. *Microb Cell Fact* 15:72. <https://doi.org/10.1186/s12934-016-0473-z>.
- Broekaert WF, Courtin CM, Verbeke K, Van de Wiele T, Verstraete W, Delcour JA. 2011. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Crit Rev Food Sci Nutr* 51:178–194. <https://doi.org/10.1080/10408390903044768>.
- Valls A, Diaz P, Pastor FI, Valenzuela SV. 2016. A newly discovered arabinoxylan-specific arabinofuranohydrolase. Synergistic action with xylanases from different glycosyl hydrolase families. *Appl Microbiol Biotechnol* 100:1743–1751. <https://doi.org/10.1007/s00253-015-7061-8>.
- Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43:W237–W243. <https://doi.org/10.1093/nar/gkv437>.
- Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res* 39:W339–W346. <https://doi.org/10.1093/nar/gkr466>.