Genome Sequence of the Heteropolysaccharide-Producing Strain *Lactobacillus mucosae* DPC 6426

Paul M. Ryan,[a,b] Caitriona M. Guinane,[a] Lis E. E. London,[c,d] Philip R. Kelleher,[b] Gerald F. Fitzgerald,[b,c] Noel M. Caplice,[c] R. Paul Ross,[d] Catherine Stanton[a,c]

Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland[a]; School of Microbiology, University College Cork, Cork, Ireland[b]; Centre for Research in Vascular Biology, University College Cork, Cork, Ireland[c]; Alimentary Pharmabiotic Centre, Biosciences Institute, University College Cork, Cork, Ireland[d]

Exopolysaccharide-synthesizing *Lactobacillus mucosae* DPC 6426 is a heterofermentative strain, which has demonstrated cholesterol-lowering properties in an animal model of lipid-driven atherosclerosis. The genome revealed a plethora of homologues linked to carbohydrate metabolism and mucin binding.

Address correspondence to Catherine Stanton, catherine.stanton@teagasc.ie.

*L*actobacillus mucosae DPC 6426 is an exopolysaccharide (EPS)-producing strain originally isolated from the bovine gastrointestinal tract (1). *L. mucosae* DPC 6426 exhibits technological and biological robustness compared to a non-EPS-producing *L. mucosae* strain, indicating its versatility as an adjunct culture for functional food applications (1). Additionally, two recent studies have demonstrated the cholesterol-lowering effects of *L. mucosae* DPC 6426 in the atherosclerosis-prone apolipoprotein-E-deficient mouse model (2), as well as its application as an adjunct culture in yogurt for improving technofunctional properties (3).

The draft genome of *L. mucosae* DPC 6426 was sequenced by a combined paired-end 454 pyrosequencing on the FLX sequencer and an Illumina MiSeq approach by Beckman Coulter Genomics, with a final coverage of ~24× being achieved. The reads were assembled using the MIRA software (4) into 72 contigs. The order and orientation of the contigs were determined using the Artemis Comparison Tool (ACT) (5, 6) and progressiveMauve software (7) using the reference genomes of *Lactobacillus fermentum* IFO3956 (GenBank accession no. NC_010610), *Lactobacillus reuteri* JCM1112 (GenBank accession no. NC_010609), and *L. mucosae* LM1 (8). A combination of Glimmer 3.02, Prokaryotic Dynamic Programming Genefinding Algorithm (Prodigal) version 1.20 (http://prodigal.ornl.gov) (9, 10), and Rapid Annotations using Subsystems Technology (RAST) (http://rast.nmpdr.org/) (11) was used to identify and annotate predicted coding sequences (CDSs). The draft genome includes 2,079,103 bp, with an average G+C content of 46.7%. The genome consists of a single circular chromosome and does not appear to harbor plasmids. A total of 2,045 CDSs were predicted, including five rRNA operons, 47 tRNA genes, and a putative complete novel phage of 40.7 kb, Φ6426. A total GC-skew analysis and the open reading frame (ORF) orientation identified the *oriC* proximal to *dnaA* and the *terC* at ~1.06 Mb, and the coding density was predicted to be 85.4%.

The *L. mucosae* species was named as a result of an ability to adhere to intestinal mucosa, due to the apparent ubiquitous expression of mucus binding protein (*mub*) homologues within the species, as well as other adhesin-like proteins (12–14). The *L. mucosae* DPC 6426 genome was found to have predicted CDSs of mucin binding domains with similarities to the *mub* genes in *L. mucosae* and *L. reuteri* strains. Two putative collagen-binding proteins, a fibronectin binding protein (100% coverage and 70% identity to *L. fermentum* IFO3956 adherence protein), and five LPXTG motifs were also identified.

A 24-kbp putative EPS operon was identified, which codes for genes common to EPS-synthesizing lactobacilli, including those involved in regulation, chain length determination, repeating unit biosynthesis, and polymerization. Two additional regions (9.8 kbp and 6 kbp), flanked by putative insertion elements, were also identified as coding for putative glycosyltransferases, dextransucrases, and pectinsucrases.

The availability of the genome sequence of *L. mucosae* DPC 6426 will allow full analysis of the genetics behind its potential health-promoting attributes.

Nucleotide sequence accession numbers. This whole-genome shotgun project of *L. mucosae* DPC 6426 has been deposited at DDBJ/EMBL/GenBank under the accession number JSWI00000000. The version described here is JSWI01000000.

REFERENCES

1. **London LEE, Price NP, Ryan P, Wang L, Auty MA, Fitzgerald GF, Stanton C, Ross RP.** 2014. Characterization of a bovine isolate *Lactobacillus mucosae* DPC 6426 which produces an exopolysaccharide composed predominantly of mannose residues. J Appl Microbiol **117**:509–517. http://dx.doi.org/10.1111/jam.12542.

2. **London LEE, Kumar AH, Wall R, Casey PG, O'Sullivan O, Shanahan F, Hill C, Cotter PD, Fitzgerald GF, Ross RP, Caplice NM, Stanton C.** 2014. Exopolysaccharide-producing probiotic lactobacilli reduce serum cholesterol and modify enteric microbiota in ApoE-deficient mice. J Nutr **144:**1956–1962. http://dx.doi.org/10.3945/jn.114.191627.

3. **London LEE, Chaurin V, Auty MAE, Fenelon MA, Fitzgerald GF, Ross RP, Stanton C.** 2015. Use of *Lactobacillus mucosae* DPC 6426, an exopolysaccharide-producing strain, positively influences the techno-functional properties of yoghurt. Int Dairy J **40:**33–38. http://dx.doi.org/10.1016/j.idairyj.2014.08.011.

4. **Chevreux B.** 2005. MIRA: an automated genome and EST assembler. Ph. D. thesis. The Ruprecht-Karls-University Heidelberg, Heidelberg, Germany.

5. **Carver TJ, Rutherford KM, Berriman M, Rajandream M-A, Barrell BG, Parkhill J.** 2005. ACT: the Artemis Comparison Tool. BioInformatics **21:**3422–3423. http://dx.doi.org/10.1093/bioinformatics/bti553.

6. **Darling AE, Treangen TJ, Messeguer X, Perna NT.** 2007. Analyzing patterns of microbial evolution using the mauve genome alignment system, p 135–152. *In* Bergman N (ed), Comparative genomics, vol 396. Humana Press, New York, NY.

7. **Rissman AJ, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT.** 2009. Reordering contigs of draft genomes using the mauve aligner. BioInformatics **25:**2071–2073. http://dx.doi.org/10.1093/bioinformatics/btp356.

8. **Morita H, Toh H, Fukuda S, Horikawa H, Oshima K, Suzuki T, Murakami M, Hisamatsu S, Kato Y, Takizawa T, Fukuoka H, Yoshimura T, Itoh K, O'Sullivan DJ, McKay LL, Ohno H, Kikuchi J, Masaoka T, Hattori M.** 2008. Comparative genome analysis of *Lactobacillus reuteri* and *Lactobacillus fermentum* reveal a genomic island for reu-

9. **Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics **11:**119. http://dx.doi.org/10.1186/1471-2105-11-119.

10. **Delcher AL, Bratke KA, Powers EC, Salzberg SL.** 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. BioInformatics **23:**673–679. http://dx.doi.org/10.1093/bioinformatics/btm009.

11. **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.

12. **Lee JH, Valeriano VD, Shin Y-R, Chae JP, Kim G-B, Ham J-S, Chun J, Kang D-K.** 2012. Genome sequence of *Lactobacillus mucosae* LM1, isolated from piglet feces. J Bacteriol **194:**4766. http://dx.doi.org/10.1128/JB.01011-12.

13. **Roos S, Karner F, Axelsson L, Jonsson H.** 2000. *Lactobacillus mucosae* sp. nov., a new species with *in vitro* mucus-binding activity isolated from pig intestine. Int J Syst Evol Microbiol **50:**251–258. http://dx.doi.org/10.1099/00207713-50-1-251.

14. **Valeriano VD, Parungao-Balolong MM, Kang DK.** 2014. *In vitro* evaluation of the mucin-adhesion ability and probiotic potential of *Lactobacillus mucosae* LM1. J Appl Microbiol **117:**485–497. http://dx.doi.org/10.1111/jam.12539.