



Draft Genome Sequences of *Fructobacillus fructosus* DPC 7238 and *Leuconostoc mesenteroides* DPC 7261, Mannitol-Producing Organisms Isolated from Fructose-Rich Honeybee-Resident Flowers on an Irish Farm

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ABSTRACT Certain bacterial species, including some fructophilic lactic acid bacteria, are known to naturally produce the sugar alcohol mannitol. Here, we announce the draft genome sequences of the mannitol-producing organisms *Fructobacillus fructosus* DPC 7238 and *Leuconostoc mesenteroides* DPC 7261, which were isolated from fructose-rich honeybee-resident flowers found on an Irish farm.

Fructophilic lactic acid bacteria (FLAB), which are present on fructose-rich fruits, flowers, and vegetables, are capable of tolerating high concentrations of fructose (1–3). Certain FLAB produce sugar alcohols, such as mannitol, which has attracted interest as a sugar substitute for diabetics and those with sugar intolerance due to its low calorie content (4). We previously isolated *Fructobacillus fructosus* DPC 7238 and *Leuconostoc mesenteroides* DPC 7261 from fructose-rich honeybee-resident flowers. Both strains display fructose utilization, with high mannitol yield (5). These strains have revealed their potential as application-specific starters in the development of innovative dairy products naturally sweetened with this low-calorie sugar (5).

F. fructosus DPC 7238 and *L. mesenteroides* DPC 7261 were grown overnight at 30°C in MRS broth (Becton, Dickinson and Co., Wokingham, Berkshire, UK) containing 10 g/liter fructose. Genomic DNA was extracted using the UltraClean microbial DNA isolation kit (MO BIO Laboratories, Cambridge, UK) and purified with the Isolate II PCR and gel kit (Bioline, Dublin, Ireland) according to the manufacturers' instructions. Genomic DNA libraries were prepared using a Nextera XT library preparation kit (Illumina, San Diego, CA) and following the manufacturer's protocol, with the following modifications: 2 ng of DNA instead of 1 ng was used as the input, and the PCR elongation time was increased from 30 s to 1 min. Libraries were sequenced on the Illumina HiSeq platform using a 250-bp paired-end read protocol (MicrobesNG, University of Birmingham, Birmingham, UK). Read quality was assessed using FastQC v0.11.7 (6). *De novo* assembly was performed with KmerGenie v1.6982 (7), Velvet v1.2.10 (8), SSPACE v3.0 (9), and GapFiller v1-10 (10). Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.3 (11). The final draft genomes were estimated to be ≥95% complete with ≤3% contamination using CheckM v1.0.12 (12). Default settings were used for all software.

The sequencing data statistics are shown in Table 1. The total numbers of coding genes and protein-coding regions in *F. fructosus* DPC 7238 are 1,444 and 1,368, respectively. Multiple RNAs were identified, i.e., 6 rRNA types, 49 tRNAs, and 3 noncoding RNAs. In the case of *L. mesenteroides* DPC 7261, 3,406 total coding genes and 3,229

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TABLE 1 Genomic features of the FLAB strains used in this study

Organism	Draft genome size (Mb)	No. of contigs	N_{50} (bp)	G+C content (%)	Mean coverage (X)	Total no. of reads	SRA accession no.	GenBank accession no.
<i>F. fructosus</i> DPC 7238	1.34	28	155,038	44.68	30	1,380,363	SRR12976643	JACTNH000000000
<i>L. mesenteroides</i> DPC 7261	3.24	56	157,699	40.34	30	3,343,285	SRR12976642	JACXBX000000000

protein-coding genes were identified, with 120 RNA regions including 13 rRNA types, 5 5S rRNAs, 101 tRNAs, and 6 noncoding RNAs. The numbers of pseudogenes (18 pseudogenes) and non-protein-coding sequences (18 sequences) determined for *F. fructosus* DPC 7238 are comparatively lower than those in *L. mesenteroides* DPC 7261 (57 each).

Detailed analysis of the draft genome sequences of these fructose-tolerating, mannitol-producing organisms will shed further light on their ability to adapt to a fructose-rich environment and to produce mannitol from this substrate. These strains have the potential to be used as starter cultures or adjunct cultures for the manufacture of mannitol-enriched fermented dairy products and beverages.

Data availability. The draft whole-genome shotgun projects were deposited in DDBJ/ENA/GenBank. The SRA and GenBank accession numbers for *F. fructosus* DPC 7238 and *L. mesenteroides* DPC 7261 are listed in Table 1.

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We declare no conflicts of interest for this work.

REFERENCES

- Ruiz Rodriguez LGR, Mohamed F, Bleckwedel J, Medina R, De Vuyst L, Hebert EM, Mozzi F. 2019. Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in northern Argentina. *Front Microbiol* 10:1091. <https://doi.org/10.3389/fmicb.2019.01091>.
- Filannino P, Di Cagno R, Tlais AZA, Cantatore V, Gobbetti M. 2019. Fructose-rich niches traced the evolution of lactic acid bacteria toward fructophilic species. *Crit Rev Microbiol* 45:65–81. <https://doi.org/10.1080/1040841X.2018.1543649>.
- Gustaw K, Michalak M, Polak-Berecka M, Waško A. 2018. Isolation and characterization of a new fructophilic *Lactobacillus plantarum* FPL strain from honeydew. *Ann Microbiol* 68:459–470. <https://doi.org/10.1007/s13213-018-1350-2>.
- Tyler C, Kopit I, Doyle C, Yu A, Hugenholtz J, Marco M. 2016. Polyol production during heterofermentative growth of the plant isolate *Lactobacillus florum* 2F. *J Appl Microbiol* 120:1336–1345. <https://doi.org/10.1111/jam.13108>.
- Behare PV, Mazhar S, Pennone V, McAuliffe O. 2020. Evaluation of lactic acid bacteria strains isolated from fructose-rich environments for their mannitol-production and milk-gelation abilities. *J Dairy Sci* 103:11138–11151. <https://doi.org/10.3168/jds.2020-19120>.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Chikhi R, Medvedev P. 2014. Informed and automated *k*-mer size selection for genome assembly. *Bioinformatics* 30:31–37. <https://doi.org/10.1093/bioinformatics/btt310>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- 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>.
- Boetzer M, Pirovano W. 2012. Toward almost closed genomes with Gap-Filler. *Genome Biol* 13:R56. <https://doi.org/10.1186/gb-2012-13-6-r56>.
- 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>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.