

Draft Genome Sequence of Lactobacillus animalis 381-IL-28

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Lactobacillus animalis 381-IL-28 is an integral component of a multistrain commercial culture with food biopreservative and pathogen biocontrol functionality. A draft sequence of the *L. animalis* 381-IL-28 genome is described in this paper.

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Lactobacillus animalis 381-IL-28 is a component of a commercial biocontrol culture. Similar to probiotics (1), biocontrol cultures are living microorganisms that, when applied in adequate amounts, extend the safe storage life of beverages, foods, or feeds without changing their organoleptic properties (2). Some *L. animalis* strains are generally recognized as safe for the biocontrol of *Campylobacter, Escherichia coli* O157:H7, and *Salmonella* organisms in meat and poultry products (3–5) and on fresh-cut spinach (6). The *L. animalis* 381-IL-28 genome was sequenced to determine the genetic basis of its antimicrobial characteristics.

In brief, *L. animalis* 381-IL-28 was cultivated in Menon-Sturino (MS) broth supplemented with 100 mM D-glucose (7), and the genomic DNA was isolated by alkaline lysis (MasterPure Gram-positive DNA purification kit; Epicentre, Madison, WI), ethanol precipitation (8), and solid phase extraction (DNeasy blood purification kit; Qiagen, Valencia, CA). Genomic DNAs were sequenced using two chemistries. A paired-end library was prepared and sequenced using a Genome Analyzer IIx (Illumina, San Diego, CA) at the Genomics and Bioinformatics Center (College Station, TX). Genomic DNA was also primed for shotgun sequencing (Ion Xpress template kit; Ion Torrent, Grand Island, NY), and the library was sequenced by Epoch Life Science, Inc. (Sugar Land, TX) using a Personal Genome Machine (Ion Torrent).

The reads were randomly downsampled using the computational genomics (CG) pipeline (9) to an average 100-fold coverage and assembled *de novo* using Velvet (10) and VelvetOptimiser (11). The assembly was manually validated with AMOS and Hawkeye (12), and read coverage was assessed using SMALT (http://www.sanger.ac.uk/resources/software/smalt/) and SAMtools (13). The final assembly comprised 12 scaffolds (32 contigs) and 68 contigs (1,858,297 nucleotides [nt]). Twenty-seven contigs >10,000 nt covered 97% of the draft genome. Most contigs (93%) were within two standard deviations of the average coverage (110fold), while the minimum coverage was 39-fold.

The size (1.86 Mb) and G+C content (41.1%) of the *L. animalis* 381-IL-28 draft genome were compared to those of other previously sequenced members of the *Lactobacillus salivarius* group, including *L. animalis* KCTC 3501 (1.88 Mb, 41.1% G+C) (16) and *L. salivarius* UCC118 (2.13 Mb, 33% G+C) (17). A functional genome distribution (FGD) analysis (14) was carried out and genome synteny visualized using ACT (15). Gene synteny differed among *L. animalis* 381-IL-28, *L. salivarius* UCC118, and other *L. salivarius* strains. Furthermore, 381-IL-28 harbors 549 strain-specific genes not found in *L. salivarius* UCC118 (e-value cutoff, $1e^{-10}$). In contrast, an FGD comparison between *L. animalis* strains 381-IL-28 and KCTC 3501 showed a high degree of gene synteny within the contigs. An ORFeome comparison highlighted 179 *L. animalis* 381-IL-28-specific genes (e-value cutoff, e^{-100}), including an integrated prophage (open reading frames [ORFs] 822 to 877), transposase elements, and a clustered regularly interspaced short palindromic repeat (CRISPR) system (ORFs 1279 to 1288).

Protein-coding domain sequences were predicted and the draft genome was annotated using GAMOLA version 2 (18). Three L-lactate dehydrogenases (EC 1.1.1.27; ORF_1417, ORF_1456, ORF_1601) and one acetaldehyde dehydrogenase (EC 1.2.1.10; ORF_1385) were among the 1,844 protein-coding genes that were predicted.

Nucleotide sequence accession numbers. This whole-genome sequencing project was deposited at DDBJ/EMBL/GenBank under the accession no. JMHU00000000. The version described in this manuscript is the first version, JMHU01000000.

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REFERENCES

1. Food and Agriculture Organization of the United Nations, World Health Organization. 2001. Health and nutritional properties of powder milk and live lactic acid bacteria: report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. FAO and WHO, Córdoba, Argentina, 1 to 4 October 2001. ftp://ftp.fao.org/es/esn/ food/probio_report_en.pdf.

- Sturino JM, Rajendran M, Altermann E. 2013. Draft genome sequence of the pediocin-encoding biopreservative and biocontrol strain *Pediococcus* acidilactici D3. Genome Announc. 1(3):e00208-13. http://dx.doi.org/ 10.1128/genomeA.00208-13.
- USDA-FSIS. 2012. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Directive 7120:1, Rev. 12. United States Department of Agriculture Food Safety and Inspection Service, Washington, DC. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Rev2.pdf.
- 4. Koo OK, Eggleton M, O'Bryan CA, Crandall PG, Ricke SC. 2012. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes* on frankfurters formulated with and without lactate/diacetate. Meat Sci. 92:533–537. http://dx.doi.org/10.1016/j.meatsci.2012.05.023.
- Dow A, Alvarado C, Brashears M. 2011. Reduction of inoculated Salmonella cocktail in ground turkey and turkey breasts using Lactobacillusbased intervention. Poult. Sci. 90:876–879. http://dx.doi.org/10.3382/ ps.2010-00807.
- Cálix-Lara TF, Rajendran M, Talcott ST, Smith SB, Miller RK, Castillo A, Sturino JM, Taylor TM. 2014. Inhibition of *Escherichia coli* 0157:H7 and *Salmonella enterica* on spinach and identification of antimicrobial substances produced by a commercial lactic acid bacteria food safety intervention. Food Microbiol. 38:192–200. http://dx.doi.org/10.1016/ j.fm.2013.09.006.
- Menon R, Shields M, Duong T, Sturino JM. 2013. Development of a carbohydrate-supplemented semidefined medium for the semiselective cultivation of *Lactobacillus* spp. Lett. Appl. Microbiol. 57:249–257. http:// dx.doi.org/10.1111/lam.12106.
- Sambrook J, Fritsch E, Maniatis T. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Kislyuk AO, Katz LS, Agrawal S, Hagen MS, Conley AB, Jayaraman P, Nelakuditi V, Humphrey JC, Sammons SA, Govil D, Mair RD, Tatti KM, Tondella ML, Harcourt BH, Mayer LW, Jordan IK. 2010. A

computational genomics pipeline for prokaryotic sequencing projects. Bioinformatics 26:1819–1826. http://dx.doi.org/10.1093/bioinformatics/ btq284.

- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res. 18:821–829. http:// dx.doi.org/10.1101/gr.074492.107.
- Zerbino DR. Using the Velvet *de novo* assembler for short-read sequencing technologies. Curr. Protoc. Bioinformatics Chapter 11:Unit 11.5. http://dx.doi.org/10.1002/0471250953.bi1105s31.
- Schatz MC, Phillippy AM, Sommer DD, Delcher AL, Puiu D, Narzisi G, Salzberg SL, Pop M. 2013. Hawkeye and AMOS: visualizing and assessing the quality of genome assemblies. Brief. Bioinform. 14:213–224. http:// dx.doi.org/10.1093/bib/bbr074.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/map format and SAMtools. Bioinformatics 25:2078–2079. http://dx.doi.org/10.1093/bioinformatics/ btp352.
- 14. Altermann E. 2012. Tracing lifestyle adaptation in prokaryotic genomes. Front. Microbiol. 3:48. http://dx.doi.org/10.3389/fmicb.2012.00048.
- Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. 2005. ACT: the Artemis Comparison Tool. Bioinformatics 21:3422–3423. http://dx.doi.org/10.1093/bioinformatics/bti553.
- Nam SH, Choi SH, Kang A, Kim DW, Kim RN, Kim A, Kim DS, Park HS. 2011. Genome sequence of *Lactobacillus animalis* KCTC 3501. J. Bacteriol. 193:1280–1281. http://dx.doi.org/10.1128/JB.01505-10.
- Claesson MJ, Li Y, Leahy S, Canchaya C, van Pijkeren JP, Cerdeño-Tárraga AM, Parkhill J, Flynn S, O'Sullivan GC, Collins JK, Higgins D, Shanahan F, Fitzgerald GF, van Sinderen D, O'Toole PW. 2006. Multireplicon genome architecture of *Lactobacillus salivarius*. Proc. Natl. Acad. Sci. U. S. A. 103:6718–6723. http://dx.doi.org/10.1073/pnas.0511060103.
- Altermann E, Klaenhammer TR. 2003. GAMOLA: a new local solution for sequence annotation and analyzing draft and finished prokaryotic genomes. Omics 7:161–169. http://dx.doi.org/10.1089/153623103322246557.