





Draft Genome Sequence of the Immunobiotic Strain *Lactobacillus jensenii* TL2937

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ABSTRACT The genome of the immunomodulatory strain *Lactobacillus jensenii* TL2937 is described here. The draft genome has a total length of 1,678,416 bp, a G+C content of 34.3%, and 1,470 predicted protein-coding sequences. The genome information will be useful for gaining insight into the immunomodulatory properties of the TL2937 strain in the porcine host.

Probiotics able to modulate the immune system (immunobiotics) are of value for reducing intestinal inflammation in pigs (1). *Lactobacillus jensenii* TL2937 inhibits nuclear factor κB and mitogen-activated protein kinase signaling pathways in porcine intestinal epithelial (PIE) cells after the activation of TLR4 through an upregulation of the negative regulators MKP-1, A20, and Bcl-3, conferring protection against inflammatory damage (2). Microarray analysis indicated that *L. jensenii* TL2937 stimulation decreased the expression of cytokines, chemokines, and adhesion molecules in PIE cells (3). *L. jensenii* TL2937 is also able to modulate antigen-presenting cells (APCs) from porcine Peyer's patches (PPs) and blood (4, 5). Stimulation of PPs and blood porcine APCs with the TL2937 strain resulted in a differential cytokine profile in response to TLR4 activation (4). This effect was partially dependent on TLR2 activation and completely dependent on efficient phagocytosis (5). Our *in vivo* experiments in pigs demonstrated that the administration of TL2937 improved immune health, growing performance, and productivity of piglets (6).

The genome of *L. jensenii* TL2937 was sequenced using a whole-genome shotgun strategy on an Illumina MiSeq sequencer. Paired reads with lengths of 300 bp were obtained corresponding to a 1,267-fold coverage. Quality-filtered reads were assembled using Ngen version 12.2.0 software (DNASTAR). This genome was assembled into 69 contigs (mean coverage of 1,267.0×). The functional annotation of predicted genes in the *L. jensenii* TL2937 genome was achieved using the RAST server and the NCBI's Prokaryotic Genome Annotation Pipeline (7). tRNAs and rRNAs were identified by tRNAscan-SE and RNAmmer, respectively (8, 9).

The draft genome of *L. jensenii* TL2937 consists of 1,678,416 bp with a mean G+C content of 34.3%. A total of 1,470 coding sequences (CDSs), 53 structural tRNAs, and nine rRNAs were predicted. Among all CDSs, 1,157 (70%) were assigned to known protein functions, while the remaining 313 (30%) were identified as hypothetical proteins. Additionally, there are 251 RAST subsystems represented in the genome, which represent only 47% of the assigned sequences. Interestingly, *in silico* genomic studies revealed an open reading frame encoding a putative fibronectin-binding

Received 3 January 2017 **Accepted** 5 January 2017 **Published** 2 March 2017

Citation Villena J, Masumizu Y, Iida H, Ikeda-Ohtsubo W, Albarracin L, Makino S, Ohkawara S, Kimura K, Saavedra L, Hebert EM, Kitazawa H. 2017. Draft genome sequence of the immunobiotic strain *Lactobacillus jensenii* TL2937. Genome Announc 5:e00005-17. https://doi.org/10.1128/genomeA.00005-17.

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protein (BFX48_RS04115) consisting of 563 amino acids that appears to be highly conserved among *Lactobacillus* species (10). Fibronectin-binding proteins are extensively described in bacterial pathogens; however, it was postulated that in probiotic strains these adhesion molecules are essential for attachment to their ecological niches (10). Buck et al. (11) reported that a *L. acidophilus* mutant, with inactivated fbpA, exhibited a significant decrease in adhesion to epithelial cells *in vitro* (11). Based on these data, ongoing research is focused on the functional characterization of this putative adhesive molecule.

The draft genome sequence of *L. jensenii* TL2937 will be useful for further studies of specific genetic features of this strain, for understanding the mechanisms of its immunobiotic properties in the porcine host, and for its biotechnological application in the development of novel functional feeds.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number MDTN01000000. The version described in this paper is the first version, MDTN01000000.1.

ACKNOWLEDGMENTS

This work was supported by CERELOMICS (PICT 2011-0175, BID), a Grant-in-Aid for Scientific Research (B)(2) (24380146, 16H05019), Challenging Exploratory Research (23658216, 26660216, 16K15028), and Open Partnership Joint Projects of JSPS Bilateral Joint Research Projects from the Japan Society for the Promotion of Science (JSPS) to H. Kitazawa.

REFERENCES

- Villena J, Kitazawa H. 2014. Modulation of intestinal TLR4-inflammatory signaling pathways by probiotic microorganisms: lessons learned from Lactobacillus jensenii TL2937. Front Immunol 4:1–12. https://doi.org/ 10.3389/fimmu.2013.00512.
- Shimazu T, Villena J, Tohno M, Fujie H, Hosoya S, Shimosato T, Aso H, Suda Y, Kawai Y, Saito T, Makino S, Ikegami S, Itoh H, Kitazawa H. 2012. Immunobiotic *Lactobacillus jensenii* elicits anti-inflammatory activity in porcine intestinal epithelial cells by modulating negative regulators of the Toll-like receptor signaling pathway. Infect Immun 80:276–288. https://doi.org/10.1128/IAI.05729-11.
- Kobayashi H, Albarracin L, Sato N, Kanmani P, Kober AK, Ikeda-Ohtsubo W, Suda Y, Nochi T, Aso H, Makino S, Kano H, Ohkawara S, Saito T, Villena J, Kitazawa H. 2016. Modulation of porcine intestinal epitheliocytes immunetranscriptome response by *Lactobacillus jensenii* TL2937. Benef Microbes 7:769–782. https://doi.org/10.3920/BM2016.0095.
- Villena J, Suzuki R, Fujie H, Chiba E, Takahashi T, Tomosada Y, Shimazu T, Aso H, Ohwada S, Suda Y, Ikegami S, Itoh H, Alvarez S, Saito T, Kitazawa H. 2012. Immunobiotic *Lactobacillus jensenii* modulates the Toll-like receptor 4-induced inflammatory response via negative regulation in porcine antigen-presenting cells. Clin Vaccine Immunol 19:1038–1053. https://doi.org/10.1128/CVI.00199-12.
- Tsukida K, Takahashi T, Iida H, Kanmani P, Suda Y, Nochi T, Ohwada S, Aso H, Ohkawara S, Makino S, Kano H, Saito T, Villena J, Kitazawa H. 2016. Immunoregulatory effects triggered by immunobiotic *Lactobacillus jensenii* TL2937 strain involve efficient phagocytosis in porcine antigen

- presenting cells. BMC Immunol 17:21. https://doi.org/10.1186/s12865-016-0160-1.
- Suda Y, Villena J, Takahashi Y, Hosoya S, Tomosada Y, Tsukida K, Shimazu T, Aso H, Tohno M, Ishida M, Makino S, Ikegami S, Kitazawa H. 2014. Immunobiotic *Lactobacillus jensenii* as immune-health promoting factor to improve growth performance and productivity in post-weaning pigs. BMC Immunol 15:24. https://doi.org/10.1186/1471-2172-15-24.
- 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.
- Lowe TM, Eddy SR. 1997. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964. https://doi.org/10.1093/nar/25.5.0955.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. https://doi.org/10.1093/nar/gkm160.
- Hymes JP, Klaenhammer TR. 2016. Stuck in the middle: fibronectinbinding proteins in Gram-positive bacteria. Front Microbiol 7:1504. https://doi.org/10.3389/fmicb.2016.01504.
- Buck BL, Altermann E, Svingerud T, Klaenhammer TR. 2005. Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. Appl Environ Microbiol 71:8344–8351. https://doi.org/10.1128/AEM .71.12.8344-8351.2005.

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