





Draft Genome Sequence of Ligilactobacillus salivarius FFIG58, Isolated from the Intestinal Tract of Wakame-Fed Pig

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ABSTRACT Ligilactobacillus salivarius FFIG58 was isolated from the intestine of a wakame-fed pig and sequenced with an Illumina HiSeg system. FFIG58 genome sequencing revealed a genome size of 1,984,180 bp, with 1,994 protein-coding genes and a GC content of 32.9%. This draft genome sequence will contribute to a better understanding of the porcine gut microbiome.

igilactobacillus salivarius (basonym: Lactobacillus salivarius) (1) is considered an important member of the swine intestinal microbiota (2, 3) because of its capacity to protect against intestinal infections through inhibition of the growth of pathogens, competition for mucosal binding sites, or modulation of the immune system (4-7). Our previous work demonstrated that feeding pigs wakame (Undaria pinnatifida), economically important edible algae in Asian countries (8), was able to modify the gastrointestinal microbiota, inducing a significant increase in the abundance of lactobacilli (9). A library of L. salivarius strains isolated from the intestine of pigs fed wakame was constructed (9), and we investigated the capacities of those strains to modulate the innate antiviral immune response in porcine intestinal epithelial (PIE) cells. Among the strains evaluated, L. salivarius FFIG58 had a remarkable capacity to improve interferon beta levels in PIE cells after Toll-like receptor 3 activation and to protect against rotavirus infection (9).

A single colony of FFIG58 cultured on MRS agar (Oxoid, Cambridge, UK) plates was inoculated into MRS broth and incubated at 37°C for 12 h. Genomic DNA isolation was performed by using a lysozyme lysis buffer (75 mM NaCl, 20 mM EDTA, 20 mM Tris-HCl [pH 7.5], 10 mg/ml lysozyme), chloroform-isoamyl alcohol separation, and isopropanol precipitation method, as described previously (10). The genomic DNA of L. salivarius FFIG58 was sequenced with an Illumina HiSeq platform using the 2×300 -bp paired-end read sequencing protocol. The paired-end sequencing library was prepared using the TruSeq DNA HT sample preparation kit (Illumina) according to the manufacturer's protocol. The paired-end reads were filtered with prinseq-lite v.0.20.4 (min_qual_mean, 20; min_len, 75) (11). After filtering, the reads were assembled using A5-miseq v.201690825 (12) with default parameters. The sequencing protocol generated 59.7imes mean coverage of the genome. The FFIG58 draft genome sequence contains 222 contigs, with an average GC content of 32.9% and a total estimated size of 1,984,180 bp.

Sequencing results were analyzed using various software programs with default

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settings, unless otherwise specified. Gene prediction and annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.4.12 (13) and the Rapid Annotations using Subsystems Technology (RAST) server (14). This genome contained 2,041 open reading frames, with 1,994 DNA coding sequences, 36 tRNA coding sequences, and 11 rRNA coding sequences.

RAST system category distribution showed 223 subsystems. The FFIG58 genome contains enzymes for carbohydrate metabolism that are not found in the genomes of *L. salivarius* strains of porcine origin, such as strains JCM1046 (15), CP400 (16), and ZLS006 (17). Among those, the p-galactarate, p-glucarate, and p-glycerate transport and catabolism enzymes could be involved in the ability of FFIG58 to grow efficiently in wakame-based media (9). A gene for a choloylglycine hydrolase was also detected, which could be involved in the ability of *L. salivarius* to survive in the gastrointestinal tract (2). In addition, genes of the SecA2-SecY2 system, which have been proposed as important factors for adaptation to the intestinal tract in *L. salivarius* strains isolated from pigs (2), were found in the genome of FFIG58.

The draft genome sequence of *L. salivarius* FFIG58 will be useful for further studies of specific genetic features and could contribute to a better understanding of the intestinal porcine microbiome.

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number JACBJR000000000. The version described in this paper is version JACBJR010000000. The SRA/DRA/ERA accession number is SRR12128965. The BioProject and BioSample numbers are PRJNA643532 and SAMN15418202, respectively.

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REFERENCES

- Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. 2020. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leu-conostocaceae*. Int J Syst Evol Microbiol 70:2782–2858. https://doi.org/ 10.1099/ijsem.0.004107.
- Lee J-Y, Han GG, Kim EB, Choi Y-J. 2017. Comparative genomics of Lactobacillus salivarius strains focusing on their host adaptation. Microbiol Res 205:48–58. https://doi.org/10.1016/j.micres.2017.08.008.
- Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Møller K. 2002. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl Environ Microbiol 68:673–690. https://doi.org/10.1128/AEM.68.2.673-690.2002.
- Lo Verso L, Lessard M, Talbot G, Fernandez B, Fliss I. 2018. Isolation and selection of potential probiotic bacteria from the pig gastrointestinal tract. Probiotics Antimicrob Proteins 10:299–312. https://doi.org/10 .1007/s12602-017-9309-3.
- Yeo S, Lee S, Park H, Shin H, Holzapfel W, Huh CS. 2016. Development of putative probiotics as feed additives: validation in a porcinespecific gastrointestinal tract model. Appl Microbiol Biotechnol 100: 10043–10054. https://doi.org/10.1007/s00253-016-7812-1.
- Walsh MC, Gardiner GE, Hart OM, Lawlor PG, Daly M, Lynch B, Richert BT, Radcliffe S, Giblin L, Hill C, Fitzgerald GF, Stanton C, Ross P. 2008. Predominance of a bacteriocin-producing *Lactobacillus salivarius* component of a five-strain probiotic in the porcine ileum and effects on host immune phenotype. FEMS Microbiol Ecol 64:317–327. https://doi.org/10 .1111/j.1574-6941.2008.00454.x.

- Sayan H, Assavacheep P, Angkanaporn K, Assavacheep A. 2018. Effect of Lactobacillus salivarius on growth performance, diarrhea incidence, fecal bacterial population and intestinal morphology of suckling pigs chal- lenged with F4+ enterotoxigenic Escherichia coli. Asian Australas J Anim Sci 31:1308–1314. https://doi.org/10.5713/ajas.17.0746.
- Yoshinaga T, Nishiduka H, Nanba N. 2014. Genotype analysis of commercial products of the soft seaweed *Undaria pinnatifida* (Laminariales, Alariaceae). Coast Mar Sci 37:9–15.
- Masumizu Y, Zhou B, Kober AKMH, Islam MA, Iida H, Ikeda-Ohtsubo W, Suda Y, Albarracin L, Nochi T, Aso H, Suzuki K, Villena J, Kitazawa H. 2019. Isolation and immunocharacterization of *Lactobacillus salivarius* from the intestine of wakame-fed pigs to develop novel "immunosynbiotics." Microorganisms 7:167. https://doi.org/10.3390/microorganisms7060167.
- Azcárate-Peril MA, Raya RR. 2001. Methods for plasmid and genomic DNA isolation from lactobacilli, p 135–139. In Spencer JFT, Ragout de Spencer AL (ed), Food microbiology protocols. Humana Press, Totowa, NJ.
- Schmieder R, Edwards R, Bateman A. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10.1093/bioinformatics/btr026.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
- 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.
- 14. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K,

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- 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.
- Raftis EJ, Forde BM, Claesson MJ, O'Toole PW. 2014. Unusual genome complexity in *Lactobacillus salivarius* JCM1046. BMC Genomics 15:771. https://doi.org/10.1186/1471-2164-15-771.
- MacKenzie DA, McLay K, Roos S, Walter J, Swarbreck D, Drou N, Crossman LC, Juge N. 2014. Draft genome sequence of a novel *Lactobacillus salivarius* strain isolated from piglet. Genome Announc 2:e01231-13. https://doi.org/10.1128/genomeA.01231-13.
- Harris HMB, Bourin MJB, Claesson MJ, O'Toole PW. 2017. Phylogenomics and comparative genomics of *Lactobacillus salivarius*, a mammalian gut commensal. Microb Genom 3:e000115. https://doi.org/10.1099/mgen.0.000115.

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