

## **Complete Genome Sequence of Lactobacillus plantarum Strain LQ80, Selected for Preparation of Fermented Liquid Feed for Pigs**

genomeA<sub>nnouncements™</sub>

**Naoko Moriya,a [Kazuma Nakano,](https://orcid.org/0000-0001-9908-6526)b\* [Akino Shiroma,](https://orcid.org/0000-0003-2109-239X)b Misuzu Shinzato,b Noriko Ashimine,b Maiko Minami,b Hinako Tamotsu,b Makiko Shimoji,b Tetsuhiro Nakanishi,b Shun Ohki,b Kuniko Teruya,b Kazuhito Satou,b\* Takashi Hirano,b Tatsuro Hagi,a Miho Kobayashi,a Masaru Nomura,a Hiromi Kimoto-Nira,a Kiyoshi Tajima,a Yimin Cai,c [Chise Suzukia](https://orcid.org/0000-0001-7148-8101)**

aInstitute of Livestock and Grassland Science, NARO, Tsukuba, Ibaraki, Japan <sup>b</sup>Okinawa Institute of Advanced Sciences, Uruma, Okinawa, Japan c Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan

**AMERICAN SOCIETY FOR MICROBIOLOGY** 

**ABSTRACT** Lactobacillus plantarum LQ80 is a strain isolated from liquid feed for pigs. We determined the complete genome sequence of this strain using the PacBio RS II platform. LQ80 contained a single circular chromosome of 3,230,192 bp, with 44.66% G-C content and seven plasmids.

*L*actobacillus plantarum is found in various environmental niches, e.g., vegetable pickles [\(1](#page-1-0)[–](#page-1-1)[3\)](#page-1-2), fermented milk products [\(4\)](#page-1-3), and human gastrointestinal tracts [\(5\)](#page-1-4). Strains of L. plantarum are considered to be useful starters not only in the food industry, but also for the preparation of silage [\(6\)](#page-1-5) and fermented liquid feed [\(7](#page-1-6)[–](#page-1-7)[9\)](#page-1-8) because of their great acidification ability via the production of lactic acid. Furthermore, some strains of L. plantarum are tolerant to gastric juice and bile acid [\(10\)](#page-1-9) and are generally considered probiotics [\(11,](#page-1-10) [12\)](#page-1-11).

L. plantarum LQ80, isolated from liquid feed for pigs, enhances the immune response [\(13\)](#page-1-12) and the growth of intestinal villi [\(14\)](#page-1-13) in weaning piglets. Fermented liquid feed prepared with LQ80 enhances bacterial diversity in the digestive tract of piglets [\(15\)](#page-1-14). Genomic information for this strain would help clarify these potential functions as probiotic lactic acid bacteria. Therefore, we report the genome sequence of strain LQ80.

Strain LQ80 was cultured with de Man-Rogosa-Sharpe medium at 37°C. For the genome sequencing of LQ80, DNA was isolated at the early log phase. DNA was purified using a PowerClean DNA cleanup kit (Mo Bio Laboratories, Carlsbad, CA), followed by 20-kb library construction for P6-C4 chemistry with shearing. Five singlemolecule real-time (SMRT) cells were used for sequencing on the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA) with a 180-min movie time. We obtained 320,152 reads with a mean length of 5,268 bp. De novo assembly was performed using the Hierarchical Genome Assembly Process version 3 (HGAP3) [\(16\)](#page-1-15). Four circular contigs were obtained, representing one chromosome (3.2 Mb) and three plasmids (92, 58, and 30 kb). For sequencing plasmids smaller than 30 kb, plasmid DNA was extracted at the stationary phase per the Qiagen user-developed protocol (see [https://www.qiagen](https://www.qiagen.com/us/resources/resourcedetail?id=8b1b2b4f-3dca-4447-8d21-f6fd64c3a729) .com/us/resources/resourcedetail?id=[8b1b2b4f-3dca-4447-8d21-f6fd64c3a729\)](https://www.qiagen.com/us/resources/resourcedetail?id=8b1b2b4f-3dca-4447-8d21-f6fd64c3a729), and a 5-kb library was constructed with shearing. One SMRT cell was used for sequencing on the PacBio RS II platform with a 360-min movie time. The result of 362,945 reads with a mean length of 1,519 bp was assembled with HGAP3, and five circular contigs were obtained, representing five plasmids (30, 16, 10, 6, and 3.5 kb). From this analysis, another four smaller plasmids were constructed.

The LQ80 chromosome was 3,230,192 bp in length, with a G+C content of 44.66%. In addition, LQ80 had seven plasmids: pLQ801 (92,191 bp), pLQ802 (58,027 bp), pLQ803 **Received** 15 May 2018 **Accepted** 15 May 2018 **Published** 21 June 2018

**Citation** Moriya N, Nakano K, Shiroma A, Shinzato M, Ashimine N, Minami M, Tamotsu H, Shimoji M, Nakanishi T, Ohki S, Teruya K, Satou K, Hirano T, Hagi T, Kobayashi M, Nomura M, Kimoto-Nira H, Tajima K, Cai Y, Suzuki C. 2018. Complete genome sequence of Lactobacillus plantarum strain LQ80, selected for preparation of fermented liquid feed for pigs. Genome Announc 6:e00530-18. [https://doi.org/10.1128/](https://doi.org/10.1128/genomeA.00530-18) [genomeA.00530-18.](https://doi.org/10.1128/genomeA.00530-18)

**Copyright** © 2018 Moriya et al. This is an openaccess article distributed under the terms of the [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) [International license.](https://creativecommons.org/licenses/by/4.0/)

Address correspondence to Naoko Moriya, [hinona@affrc.go.jp.](mailto:hinona@affrc.go.jp)

\* Present address: Kazuma Nakano, Panefri Industrial Co., Ltd., Environmental Science Research Institute, Shuri, Okinawa, Japan; Kazuhito Satou, National Center for Child Health and Development, Setagaya, Tokyo, Japan.

genameAnnouncements<sup>TM</sup>

(30,644 bp), pLQ804 (16,405 bp), pLQ805 (10,218 bp), pLQ806 (6,355 bp), and pLQ807 (3,592 bp). The complete genome was annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [\(17\)](#page-1-16) and Microbial Genome Annotation Pipeline (MiGAP) [\(18\)](#page-1-17). PGAP identified 3,371 genes, including 3,186 coding sequences, 88 RNAs, and 97 pseudogenes. MiGAP identified 3,260 coding sequences and 88 RNAs. Plasmids pLQ801, pLQ802, pLQ803, pLQ804, pLQ805, and pLQ807 contained candidate genes for the plasmid replication initiation proteins.

**Accession number(s).** The complete genome sequences of the chromosome and seven plasmids of L. plantarum LQ80 have been deposited in GenBank under accession numbers [CP028977](https://www.ncbi.nlm.nih.gov/nuccore/CP028977) (chromosome), [CP028978](https://www.ncbi.nlm.nih.gov/nuccore/CP028978) (pLQ801), [CP028979](https://www.ncbi.nlm.nih.gov/nuccore/CP028979) (pLQ802), [CP028980](https://www.ncbi.nlm.nih.gov/nuccore/CP028980) (pLQ803), [CP028981](https://www.ncbi.nlm.nih.gov/nuccore/CP028981) (pLQ804), [CP028982](https://www.ncbi.nlm.nih.gov/nuccore/CP028982) (pLQ805), [CP028983](https://www.ncbi.nlm.nih.gov/nuccore/CP028983) (pLQ806), and [CP028984](https://www.ncbi.nlm.nih.gov/nuccore/CP028984) (pLQ807).

## **ACKNOWLEDGMENT**

This work was supported by the Okinawa Prefectural Government.

## <span id="page-1-0"></span>**REFERENCES**

- 1. Blana VA, Grounta A, Tassou CC, Nychas G-JE, Panagou EZ. 2014. Inoculated fermentation of green olives with potential probiotic Lactobacillus pentosus and Lactobacillus plantarum starter cultures isolated from industrially fermented olives. Food Microbiol 38:208 –218. [https://doi](https://doi.org/10.1016/j.fm.2013.09.007) [.org/10.1016/j.fm.2013.09.007.](https://doi.org/10.1016/j.fm.2013.09.007)
- <span id="page-1-1"></span>2. Plengvidhya V, Breidt F, Jr, Lu Z, Fleming HP. 2007. DNA fingerprinting of lactic acid bacteria in sauerkraut fermentations. Appl Environ Microbiol 73:7697–7702. [https://doi.org/10.1128/AEM.01342-07.](https://doi.org/10.1128/AEM.01342-07)
- <span id="page-1-2"></span>3. Yu J, Gao W, Qing M, Sun Z, Wang W, Liu W, Pan L, Sun T, Wang H, Bai N, Zhang H. 2012. Identification and characterization of lactic acid bacteria isolated from traditional pickles in Sichuan, China. J Gen Appl Microbiol 58:163–172. [https://doi.org/10.2323/jgam.58.163.](https://doi.org/10.2323/jgam.58.163)
- <span id="page-1-3"></span>4. Nacef M, Chevalier M, Chollet S, Drider D, Flahaut C. 2017. MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: the Maroilles. Int J Food Microbiol 247:2-8. [https://doi.org/10.1016/j.ijfoodmicro.2016.07.005.](https://doi.org/10.1016/j.ijfoodmicro.2016.07.005)
- <span id="page-1-4"></span>5. Ahrné S, Nobaek S, Jeppsson B, Adlerberth I, Wold AE, Molin G. 1998. The normal Lactobacillus flora of healthy human rectal and oral mucosa. J Appl Microbiol 85:88 –94. [https://doi.org/10.1046/j.1365-2672.1998](https://doi.org/10.1046/j.1365-2672.1998.00480.x) [.00480.x.](https://doi.org/10.1046/j.1365-2672.1998.00480.x)
- <span id="page-1-5"></span>6. Cai Y, Benno Y, Ogawa M, Kumai S. 1999. Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. J Dairy Sci 82:520 –526. [https://doi.org/](https://doi.org/10.3168/jds.S0022-0302(99)75263-X) [10.3168/jds.S0022-0302\(99\)75263-X.](https://doi.org/10.3168/jds.S0022-0302(99)75263-X)
- <span id="page-1-6"></span>7. Plumed-Ferrer C, Kivelä I, Hyvönen P, von Wright A. 2005. Survival, growth and persistence under farm conditions of a Lactobacillus plantarum strain inoculated into liquid pig feed. J Appl Microbiol 99: 851– 858. [https://doi.org/10.1111/j.1365-2672.2005.02666.x.](https://doi.org/10.1111/j.1365-2672.2005.02666.x)
- <span id="page-1-7"></span>8. Demecková V, Kelly D, Coutts AGP, Brooks PH, Campbell A. 2002. The effect of fermented liquid feeding on the faecal microbiology and colostrum quality of farrowing sows. Int J Food Microbiol 79:85–97. [https://doi.org/10.1016/S0168-1605\(02\)00182-4.](https://doi.org/10.1016/S0168-1605(02)00182-4)
- <span id="page-1-8"></span>9. Heres L, Engel B, van Knapen F, de Jong MC, Wagenaar JA, Urlings HA. 2003. Fermented liquid feed reduces susceptibility of broilers for Salmonella Enteritidis. Poult Sci 82:603– 611. [https://doi.org/10.1093/ps/82.4](https://doi.org/10.1093/ps/82.4.603) [.603.](https://doi.org/10.1093/ps/82.4.603)
- <span id="page-1-9"></span>10. Haller D, Colbus H, Gänzle MG, Scherenbacher P, Bode C, Hammes WP.

2001. Metabolic and functional properties of lactic acid bacteria in the gastro-intestinal ecosystem: a comparative in vitro study between bacteria of intestinal and fermented food origin. Syst Appl Microbiol 24: 218 –226. [https://doi.org/10.1078/0723-2020-00023.](https://doi.org/10.1078/0723-2020-00023)

- <span id="page-1-10"></span>11. Kujawa-Szewieczek A, Adamczak M, Kwiecień K, Dudzicz S, Gazda M, Więcek A. 2015. The effect of Lactobacillus plantarum 299v on the incidence of Clostridium difficile infection in high risk patients treated with antibiotics. Nutrients 7:10179 –10188. [https://doi.org/10.3390/nu7125526.](https://doi.org/10.3390/nu7125526)
- <span id="page-1-11"></span>12. Ducrotté P, Sawant P, Jayanthi V. 2012. Clinical trial: Lactobacillus plantarum 299v (DSM 9843) improves symptoms of irritable bowel syndrome. World J Gastroenterol 18:4012– 4018. [https://doi.org/10.3748/](https://doi.org/10.3748/wjg.v18.i30.4012) [wjg.v18.i30.4012.](https://doi.org/10.3748/wjg.v18.i30.4012)
- <span id="page-1-12"></span>13. Mizumachi K, Aoki R, Ohmori H, Saeki M, Kawashima T. 2009. Effect of fermented liquid diet prepared with Lactobacillus plantarum LQ80 on the immune response in weaning pigs. Animal 3:670-676. [https://doi](https://doi.org/10.1017/S1751731109003978) [.org/10.1017/S1751731109003978.](https://doi.org/10.1017/S1751731109003978)
- <span id="page-1-13"></span>14. Yoshida Y, Tsukahara T, Ushida K. 2009. Oral administration of Lactobacillus plantarum Lq80 and Megasphaera elsdenii iNP-001 induces efficient recovery from mucosal atrophy in the small and the large intestines of weaning piglets. Anim Sci J 80:709 –715. [https://doi.org/10.1111/j.1740](https://doi.org/10.1111/j.1740-0929.2009.00692.x) [-0929.2009.00692.x.](https://doi.org/10.1111/j.1740-0929.2009.00692.x)
- <span id="page-1-15"></span><span id="page-1-14"></span>15. Tajima K, Ohmori H, Aminov RI, Kobashi Y, Kawashima T. 2010. Fermented liquid feed enhances bacterial diversity in piglet intestine. Anaerobe 16:6 –11. [https://doi.org/10.1016/j.anaerobe.2009.04.002.](https://doi.org/10.1016/j.anaerobe.2009.04.002)
- 16. Chin C-S, Alexander D, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. [https://doi.org/10.1038/](https://doi.org/10.1038/nmeth.2474) [nmeth.2474.](https://doi.org/10.1038/nmeth.2474)
- <span id="page-1-16"></span>17. 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](https://doi.org/10.1093/nar/gkw569) [.org/10.1093/nar/gkw569.](https://doi.org/10.1093/nar/gkw569)
- <span id="page-1-17"></span>18. Sugawara H, Ohyama A, Mori H, Kurokawa K. 2009. Microbial Genome Annotation Pipeline (MiGAP) for diverse users, abstr S001-1-2. 20th Int Conf Genome Informatics (GIW 2009), 14 to 16 December 2009, Kanagawa, Japan.