





Complete Genome Sequence of Lactobacillus plantarum Strain JDARSH, Isolated from Sheep Milk

Abhinandan Patil,^a Anamika Dubey,^c Muneer Ahmad Malla,^d John Disouza,^a Shivaji Pawar,^{a,b} Abdulaziz A. Algarawi,^e

^aCentre for Interdisciplinary Research, D. Y. Patil University, Kolhapur, Maharashtra, India

ABSTRACT Lactobacillus plantarum strain JDARSH, a potential probiotic with a wide range of functions, was isolated from sheep milk. Here, we report the wholegenome sequence of this bacterium. The draft genome yielded a 3.20-Mb genome and 2,980 protein-coding sequences.

actic acid bacteria (LAB) are widely used in various preparations, such as food and food products, and for other raw materials, including vegetables, meat, and plant products. A number of recently conducted studies have led to the conviction that some strains of LAB, in particular, strains from the genus Lactobacillus, may promote health in both humans and animals (1-3); among these, the species Lactobacillus plantarum is the most flexible and versatile. It is a Gram-positive, facultatively anaerobic, rod-shaped, acid-tolerant, and non-spore-forming probiotic bacterium (4-6). The bacterium has wide application in the medical field (7, 8). In this study, we have sequenced the whole genome of an L. plantarum strain isolated from sheep milk.

A total of 180 sheep milk samples (Indian breed) were collected from local places in the Kolhapur, Sangli, and Admapur areas of Maharashtra, India. For bacterial enumeration, milk samples (1 ml) were kept at -80°C in 15% glycerol before use. Isolation and purification were performed on de Man-Rogosa-Sharpe (MRS) broth and agar medium (9). The samples were inoculated on MRS agar medium and incubated for a period of 48 h under microaerophilic conditions. After incubation, the individual colonies formed were transferred into sterile MRS broth medium. The culture is deposited in the public domain as Lactobacillus plantarum strain JDARSH at the National Centre for Microbial Resources (NCMR), Pune, India. Total DNA was extracted using a PureLink genomic DNA extraction kit (Life Technologies), following the manufacturer's instructions. The sequencing libraries were prepared using a Nextera XT library kit, and whole-genome sequencing of L. plantarum strain JRARSH was performed on the Illumina NextSeq 500 sequencing platform (150-bp paired-end reads) with a shotgun strategy (9). A total of 1,203,568 paired-end reads of 150 bp in size were generated. The Illumina-generated sequence reads were quality filtered by FastQC v.0.10.1 (http://www.bioinformatics .babraham.ac.uk/projects/fastqc/), and low-quality reads were removed before assembly. The quality-filtered reads were assembled de novo using SPAdes v.3.9 (10, 11), producing 31 contigs. The gene prediction was performed using the Rapid Annotations using Subsystems Technology (RAST) server v.2.0 (rast.nmpdr.org/), with default parameters (12-14). Predicted genes were annotated using the Prokaryotic Genome

Citation Patil A, Dubey A, Malla MA, Disouza J, Pawar S, Alqarawi AA, Hashem A, Abd_Allah EF, Kumar A. 2020. Complete genome sequence of Lactobacillus plantarum strain JDARSH, isolated from sheep milk. Microbiol Resour Announc 9:e01199-19. https://doi.org/10.1128/MRA

Editor Catherine Putonti, Loyola University

Copyright © 2020 Patil et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Ashwani Kumar, ashwaniiitd@hotmail.com.

Received 24 September 2019 Accepted 4 December 2019 Published 9 January 2020

^bCentre for Innovative and Applied Research, Anekant Education Society, Tuljaram Chaturchand College, Baramati, Maharashtra, India

^cMetagenomics and Secretomics Research Lab, Department of Botany, Dr. Harisingh Gour University (a Central University), Sagar, Madhya Pradesh, India

^dDepartment of Zoology, Dr. Harisingh Gour University (a Central University), Sagar, Madhya Pradesh, India

ePlant Production Department, College of Food and Agriculture Science, King Saud University, Riyadh, Saudi Arabia

^fBotany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

⁹Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt

Patil et al.

Annotation Pipeline (PGAP) v.4.9 (15) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast .cgi). Default parameters were used for all software, unless otherwise specified. The final assembly yielded a 3.20-Mb genome with a mean G+C content of 44.5%, an N_{50} value of 174,093 bp, an L_{50} of 6, and a total of 3,128 genes, of which 2,980 are protein-coding genes, 59 are tRNA genes, 5 are rRNA genes (35S rRNAs, 116S rRNA, and 123S rRNA), and 4 are noncoding RNA (ncRNA) genes. The genome also contained 80 pseudogenes. Within the total of 3,128 genes, biological functions were defined for 2,135 (68.25%). The predicted genes that were identified are associated with cellular metabolism (n =38), protein processing (n = 323), energy (n = 276), stress response defense and virulence (n = 182), DNA processing (n = 181), cellular response (n = 163), RNA processing (91), membrane transport (n = 33), the cellular envelope (n = 28), and regulation and cell signaling (n = 18). Moreover, the genes were each assigned a putative function. Putative functions included cell adhesions, acid tolerance, bile toxicity, molybdenum cofactor biosynthesis, folate and pterine biosynthesis, aromatic compound degradation, exopolysaccharide production, riboflavin and bacteriocin production, thiamine and methionine biosynthesis, phosphate metabolism, sulfur metabolism, dormancy and sporulation, and denitrification. Similar pathways were also found in some other strains of L. plantarum (16–18). This Lactobacillus plantarum bacterial strain also proves to be an attractive candidate for the metabolic engineering of lignocellulosic biomass to biofuels, owing to its characteristic natural abilities to metabolize the hexose sugars and to tolerate high ethanol and acid concentrations (19, 20).

Data availability. The associated BioSample, SRA, and BioProject accession numbers for the sequence reported here are SAMN13106942, SRP226774, and PRJNA579228, respectively.

ACKNOWLEDGMENTS

We extend our sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding to research group number RG-1435–014. A.P. and A.D. acknowledge the Department of Science and Technology (New Delhi, India) for providing financial support under grants IF140s and IF160797, respectively.

REFERENCES

- Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. 2001. Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. Lancet 357:1076–1079. https://doi.org/10.1016/ S0140-6736(00)04259-8.
- de Vries MC, Vaughan EE, Kleerebezem M, de Vos WM. 2006. Lactobacillus plantarum survival, functional and potential probiotic properties in the human intestinal tract. Int Dairy J 16:1018–1028. https://doi.org/10 .1016/j.idairyj.2005.09.003.
- 3. Patil AR, Shinde SS, Kakade PS, D'souza J. 2015. *Lactobacillus* model moiety a new era dosage form as nutraceuticals and therapeutic mediator. *In* Kumar A (ed), Biotechnology and bioforensics: new trends. Springer Singapore, Singapore.
- Teixeira P. 2014. Lactobacillus: Lactobacillus delbrueckii ssp. bulgaricus, p 425–431. In Batt CA (ed), Encyclopedia of food microbiology. Academic Press, Cambridge, MA.
- Douillard FP, de Vos WM. 2014. Functional genomics of lactic acid bacteria: from food to health. Microb Cell Fact 13:S8. https://doi.org/10 .1186/1475-2859-13-S1-S8.
- Ramos CL, Thorsen L, Schwan RF, Jespersen L. 2013. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. Food Microbiol 36:22–29. https://doi.org/10.1016/j.fm.2013.03.010.
- Center MM, Jemal A, Smith RA, Ward E. 2010. Worldwide variations in colorectal cancer. CA Cancer J Clin 59:366–378. https://doi.org/10.3322/ caac.20038
- Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, Sharma S, Khare PK, Khan ML. 2019. Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28:2405. https://doi.org/10.1007/s10531-019-01760-5.

- Minoche AE, Dohm JC, Himmelbauer H. 2011. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and Genome Analyzer systems. Genome Biol 12:R112. https://doi.org/10 .1186/gb-2011-12-11-r112.
- Malla MA, Dubey A, Kumar A, Yadav S, Hashem A, Allah E. 2018. Exploring the human microbiome: rhe potential future role of nextgeneration sequencing in disease diagnosis and treatment. Front Immunol 9:2868. https://doi.org/10.3389/fimmu.2018.02868.
- 11. Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd Allah EF. 2018. Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 9:1132. https://doi.org/10.3389/fmicb.2018.01132.
- 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.
- Kumar A, Vyas P, Malla MA, Dubey A. 2019. Taxonomic and functional annotation of termite degraded *Butea monosperma* (Lam.) Kuntze (flame of the forest). Open Microbiol J 13:154–163. https://doi.org/10 .2174/1874285801913010154.
- Dubey A, Kumar A, Abd_Allah EFA, Hashem A, Khan ML. 2018. Growing more with less: breeding and developing drought resilient soybean to improve food security. Ecol Indic 105:425–437. https://doi.org/10.1016/ j.ecolind.2018.03.003.
- Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic

Volume 9 Issue 2 e01199-19 mra.asm.org **2**



- Genome Annotation Pipeline. Nucleic Acids Res 19:6614–6624. https://doi.org/10.1093/nar/gkw569.
- Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res 45: D535–D542. https://doi.org/10.1093/nar/gkw1017.
- De Vuyst L, Vrancken G, Ravyts F, Rimaux T, Weckx S. 2009. Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. Food Microbiol 26:666–675. https://doi.org/10.1016/j.fm.2009 .07.012.
- Vrancken G, Rimaux T, Wouters D, Leroy F, De Vuyst L. 2009. The arginine deiminase pathway of *Lactobacillus fermentum* IMDO 130101 responds to growth under stress conditions of both temperature and salt. Food Microbiol 26:720–727. https://doi.org/10.1016/j.fm.2009.07.006.
- Flórez AB, Mayo B. 2018. Genome analysis of Lactobacillus plantarum LL441 and genetic characterisation of the locus for the Lantibiotic plantaricin. Front Microbiol 9:1916. https://doi.org/10.3389/fmicb.2018 01916.
- Singh JK, Vyas P, Dubey A, Upadhyaya CP, Kothari R, Tyagi VV, Kumar A.
 Assessment of different pretreatment technologies for efficient bioconversion of lignocellulose to ethanol. Front Biosci (Schol Ed) 10: 350–371. https://doi.org/10.2741/s521.

Volume 9 Issue 2 e01199-19 mra.asm.org **3**