






Complete Genome Sequence of *Lactobacillus paracasei* EG9, a Strain Accelerating Free Amino Acid Production during Cheese Ripening

Yui Asahina,^{a,b}  Akino Shiroma,^c  Kazuma Nakano,^{c*} Hinako Tamotsu,^{c*} Noriko Ashimine,^{c*} Misuzu Shinzato,^{c*} Maiko Minami,^{c*} Makiko Shimoji,^c Tetsuhiro Nakanishi,^c Shun Ohki,^c Kuniko Teruya,^c Kazuhito Satou,^{c*} Miho Kobayashi,^b Tatsuro Hagi,^b Naoko Moriya,^b  Chise Suzuki,^b Atsushi Tajima,^a Masaru Nomura,^b Takashi Hirano^{c*}

^aGraduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

^bInstitute of Livestock and Grassland Science, NARO, Tsukuba, Ibaraki, Japan

^cOkinawa Institute of Advanced Sciences, Uruma, Okinawa, Japan

ABSTRACT *Lactobacillus paracasei* EG9 is a strain isolated from well-ripened cheese and accelerates free amino acid production during cheese ripening. Its complete genome sequence was determined using the PacBio RS II platform, revealing a single circular chromosome of 2,927,257 bp, a G+C content of 46.59%, and three plasmids.

Lactobacillus paracasei belongs to the *Lactobacillus casei* group, along with *L. casei* and *L. rhamnosus*, and it inhabits various environments, such as plants, fermented foods, and the intestinal tract of humans and animals (1). *L. paracasei* is widely accepted as one of the dominant species of nonstarter lactic acid bacteria involved in cheese ripening (2, 3). As it influences ripening and flavor development (2–5), attempts have been made to utilize *L. paracasei* as an adjunct starter in cheese ripening (6–8).

L. paracasei EG9 was isolated from well-ripened cheese (9). In a cheese ripening environment at 10°C in the presence of 1.7% NaCl, the growth of EG9 is not suppressed, in contrast to that of the type strain *L. paracasei* subsp. *paracasei* JCM 8130. The addition of EG9 to lactic fermentation starter increased the total free amino acid content of the cheese. Based on these results, EG9 was considered a potential adjunct starter in accelerating cheese ripening. To identify potential genetic determinants that specify the properties of strain EG9, we sequenced the whole genome using single-molecule real-time (SMRT) technology (10). SMRT technology offers advantages, such as long read lengths, high consensus accuracy, and a low degree of bias, and is a powerful tool for sequencing and assembling complete bacterial genomes containing highly repetitive sequences (11, 12).

The genomic DNA was purified from cells in the early log phase using a PowerClean DNA cleanup kit (Mo Bio Laboratories, Carlsbad, CA). This was followed by the construction of a 20-kb library for P6-C4 chemistry with shearing (12). Size selection was not performed. Seven SMRT cells (a 240-min movie per cell) were sequenced using the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA). *De novo* assembly was performed using the Hierarchical Genome Assembly Process version 2 (13). Four circular contigs representing one chromosome (2,927,257 bp; G+C content, 46.59%; coverage, 715.08×) and three plasmids, pEG9A (79,815 bp; G+C content, 43.67%; coverage, 763.18×), pEG9B (55,299 bp; G+C content, 43.10%; coverage, 790.37×), and pEG9C (12,035 bp; G+C content, 40.61%; coverage, 1,408.69×), were obtained.

The numbers of putative coding sequences (CDSs) predicted and annotated by PGAP were 3,025, 102, 72, and 13 for the chromosome, pEG9A, pEG9B, and pEG9C, respectively. Identification using BLAST revealed that the analyzed chromosome was closely related to the chromosome of *L. paracasei* subsp. *paracasei* JCM 8130^T (query

Received 1 June 2018 Accepted 2 June 2018
2018 Published 5 July 2018

Citation Asahina Y, Shiroma A, Nakano K, Tamotsu H, Ashimine N, Shinzato M, Minami M, Shimoji M, Nakanishi T, Ohki S, Teruya K, Satou K, Kobayashi M, Hagi T, Moriya N, Suzuki C, Tajima A, Nomura M, Hirano T. 2018. Complete genome sequence of *Lactobacillus paracasei* EG9, a strain accelerating free amino acid production during cheese ripening. *Genome Announc* 6:e00627-18. <https://doi.org/10.1128/genomeA.00627-18>.

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

Address correspondence to Masaru Nomura, nomura@affrc.go.jp.

* Present address: Kazuma Nakano, Panefri Industrial Co., Ltd., Environmental Science Research Institute, Okinawa, Japan; Hinako Tamotsu, Sentan Pharma, Okinawa, Japan; Noriko Ashimine, Sentan Pharma, Okinawa, Japan; Misuzu Shinzato, Sentan Pharma, Okinawa, Japan; Maiko Minami, Sentan Pharma, Okinawa, Japan; Kazuhito Satou, National Center for Child Health and Development, Tokyo, Japan; Takashi Hirano, Sentan Pharma, Okinawa, Japan.

cover, 89%; identity, 99%; GenBank accession number AP012541) and that pEG9C was closely related to pLBC-2 (query cover, 89%; identity, 100%; GenBank accession number AP012543). pEG9A and pEG9B showed partial homology to the *L. paracasei* N1115 plasmid (query cover, 49%; identity, 96%; GenBank accession number CP007124) and the *L. casei* LC2W plasmid pLC2W (query cover, 22%; identity, 99%; GenBank accession number CP002617), respectively.

The complete genome sequence of *L. paracasei* EG9 will help in elucidating the mechanisms of free amino acid production during cheese ripening and will provide insight into the diversity in *L. paracasei*.

Accession number(s). The complete genome sequence of *L. paracasei* EG9 has been deposited in DDBJ/ENA/GenBank under accession numbers [CP029546](#) (chromosome), [CP029547](#) (pEG9A), [CP029548](#) (pEG9B), and [CP029549](#) (pEG9C).

ACKNOWLEDGMENTS

This work was supported by the Okinawa Prefectural Government.

We declare no conflicts of interest.

We thank Enago (www.enago.jp) for the English language review.

REFERENCES

1. Stefanovic E, McAuliffe O. 2018. Comparative genomic and metabolic analysis of three *Lactobacillus paracasei* cheese isolates reveals considerable genomic differences in strains from the same niche. *BMC Genomics* 19:205. <https://doi.org/10.1186/s12864-018-4586-0>.
2. Beresford TP, Fitzsimons NA, Brennan NL, Cogan TM. 2001. Recent advances in cheese microbiology. *Int Dairy J* 11:259–274. [https://doi.org/10.1016/S0958-6946\(01\)00056-5](https://doi.org/10.1016/S0958-6946(01)00056-5).
3. Gobetti M, De Angelis M, Di Cagno R, Mancini L, Fox PF. 2015. Pros and cons for using non-starter lactic acid bacteria (NSLAB) as secondary/adjunct starters for cheese ripening. *Trends Food Sci Technol* 45: 167–178. <https://doi.org/10.1016/j.tifs.2015.07.016>.
4. Lynch CM, Muir DD, Banks JM, McSweeney PLH, Fox PF. 1999. Influence of adjunct cultures of *Lactobacillus paracasei* ssp. *paracasei* or *Lactobacillus plantarum* on cheddar cheese ripening. *J Dairy Sci* 82:1618–1628. [https://doi.org/10.3168/jds.S0022-0302\(99\)75390-7](https://doi.org/10.3168/jds.S0022-0302(99)75390-7).
5. Crow V, Curry B, Hayes M. 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand cheddar. *Int Dairy J* 11:275–283. [https://doi.org/10.1016/S0958-6946\(01\)00057-7](https://doi.org/10.1016/S0958-6946(01)00057-7).
6. Poveda JM, Chicón R, Cabezas L. 2015. Biogenic amine content and proteolysis in Manchego cheese manufactured with *Lactobacillus paracasei* subsp. *paracasei* as adjunct and other autochthonous strains as starters. *Int Dairy J* 47:94–101. <https://doi.org/10.1016/j.idairyj.2015.03.004>.
7. Van Hoorde K, Van Leuven I, Dirinck P, Heyndrickx M, Coudijzer K, Vandamme P, Huys G. 2010. Selection, application and monitoring of *Lactobacillus paracasei* strains as adjunct cultures in the production of Gouda-type cheeses. *Int J Food Microbiol* 144:226–235. <https://doi.org/10.1016/j.ijfoodmicro.2010.05.007>.
8. Bintsis T, Robinson RK. 2004. A study of the effects of adjunct cultures on the aroma compounds of feta-type cheese. *Food Chem* 88:435–441. <https://doi.org/10.1016/j.foodchem.2004.01.057>.
9. Saiki R, Hagi T, Narita T, Kobayashi M, Sasaki K, Asahina Y, Tajima A, Nomura M. 2018. Effects of the addition of non-starter lactic acid bacteria on free amino acid production during cheese ripening. *Food Sci Technol Res* 24:299–309. <https://doi.org/10.3136/fstr.24.299>.
10. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, et al. 2009. Real-time DNA sequencing from single polymerase molecules. *Science* 323:133–138. <https://doi.org/10.1126/science.1162986>.
11. Nakano K, Shiroma A, Shimoji M, Tamotsu H, Ashimine N, Ohki S, Shinzato M, Minami M, Nakanishi T, Teruya K, Satou K, Hirano T. 2017. Advantages of genome sequencing by long-read sequencer using SMRT technology in medical area. *Hum Cell* 30:149–161. <https://doi.org/10.1007/s13577-017-0168-8>.
12. Nakano K, Shiroma A, Tamotsu H, Ohki S, Shimoji M, Ashimine N, Shinzato M, Minami M, Nakanishi T, Teruya K, Satou K, Suzuki C, Kimoto-Nira H, Kobayashi M, Mizumachi K, Aoki R, Miyata S, Yamamoto K, Ohtake Y, Eguchi-Ogawa T, Moriya N, Hagi T, Nomura M, Hirano T. 2016. First complete genome sequence of the skin-improving *Lactobacillus curvatus* strain FBA2, isolated from fermented vegetables, determined by PacBio single-molecule real-time technology. *Genome Announc* 4:e00884-16. <https://doi.org/10.1128/genomeA.00884-16>.
13. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.