Complete Genome Sequence of Carotenoid-Producing Enterococcus gilvus CR1, Isolated from Raw Cow’s Milk

Shun Ohki,* Tatsuro Hagi,b Kazuma Nakano,a Akino Shiroma,a Hinako Tamotsu,a Makiko Shimoji,a Misuzu Shinzato,a Noriko Ashimine,a Maiko Minami,a Tetsuhiro Nakamichi,a Kuniko Teruya,a Kazuho Satou,a Naoko Moriya,a Miho Kobayashi,b Masaru Nomura,b Chise Suzuki,b Takashi Hiranoa

*Okinawa Institute of Advanced Sciences, Uruma, Okinawa, Japan
bInstitute of Livestock and Grassland Science, NARO, Tsukuba, Ibaraki, Japan

ABSTRACT  Enterococcus gilvus CR1, isolated from raw cow’s milk, can produce carotenoids. The complete genome sequence of this strain was determined using the PacBio RS II platform. The assembly was found to contain a circular chromosome, including carotenoid biosynthesis genes, and comprises 2,863,043 bp, with a G+C content of 41.86% and three plasmids.

The yellow-pigmented Enterococcus gilvus was first isolated from clinical specimens from humans in 2002 (1). In addition, E. gilvus strains were isolated from foods such as cheese and fermented sausages (2, 3). Therefore, these strains isolated from cheese (or milk) may aid cheese ripening by functioning as nonstarter lactic acid bacteria.

The yellow pigment produced by E. gilvus has been identified as diaponeurosperone, which is related to tolerance of hydrogen peroxide, low pH, bile acids, and lysozyme (4, 5). A study using E. gilvus CR1, isolated from raw cow’s milk, showed that diaponeurosperone synthesis could be strongly induced under aerobic conditions, along with the upregulation of the gene expression level in the isoprenoid biosynthesis pathway and pyruvate dehydrogenase complex (6, 7).

The genome sequence is useful in clarifying the properties associated with fermentation and carotenoid biosynthesis regulation. To identify properties of CR1, the complete genome sequence was determined using single-molecule real-time (SMRT) technology (8). SMRT technology is a powerful tool for sequencing complete bacterial genomes with a highly repetitive sequence (9, 10).

CR1 was grown to early log phase in M17 medium (Difco Laboratories, Detroit, MI) supplemented with 0.5% glucose at 30°C under static condition. The genomic DNA was extracted as previously reported (11) and purified using a PowerClean DNA cleanup kit (Mo Bio Laboratories, Carlsbad, CA), which was followed by a 20-kb library construction for P6-C4 chemistry with shearing. Eight SMRT cells (240-min movie each) were used for sequencing on the RS II platform (Pacific Biosciences, Menlo Park, CA). De novo assembly was constructed using the hierarchical genome assembly process (HGAP) workflow (12) implemented in the SMRT analysis software v2.3.0 patch 5 (Pacific Biosciences) as an RS_HGAP_Assembly.2 protocol. In the protocol, we changed the following parameters from their defaults: compute minimum seed read length, un-checked; minimum seed read length, 10,000 bp; genome size, 4,000,000 bp; and target coverage, 15×. Resulting contigs were circularized using the Minimus2 pipeline from the AMOS v3.1.0 package (13) with its default parameters.

The genome sequence of CR1 contains one chromosome (2,863,043 bp, G+C content of 41.86%, and 1078× coverage) and three plasmids with sizes of 919,333 bp (G+C content of 42.94% and 935× coverage), 80,244 bp (G+C content of 35.03% and 1,434× coverage), and 82,704 bp (G+C content of 36.85% and 1,377× coverage). The PacBio RS II platform produced 1,665,885 reads with a mean length of 2,876 bp. The chromo-
some contains the isoprenoid biosynthesis pathway. The spx genes encoding transcriptional regulators involved in carotenoid biosynthesis (14) were found to be located on both the chromosome and the plasmids. Concerning fermentation properties, genes encoding peptidases and sugar metabolism, such as the phosophoenolpyruvate (PEP)-dependent phosphotransferase system, were also located on both the chromosome and the plasmids.

Further investigation into the CR1 genome will provide more insight into the regulation of carotenoid biosynthesis and milk fermentation.

Data availability. The complete genome sequence of Enterococcus gilvus CR1 has been deposited at DDBJ/ENA/GenBank under accession numbers CP030932 (chromosome), CP030933 (pCR1A), CP030934 (pCR1B), and CP030935 (pCR1C).

ACKNOWLEDGMENTS

This work was supported by the Okinawa Prefectural Government (to all authors) and JSPS KAKENHI grant number 16K08012 (to T. Hagi).

We have no conflicts of interest.

REFERENCES

1. Tyrrell GJ, Turnbull L, Teixeira LM, Lefebvre J, Carvalho MG, Facklam RR, Lowgren M. 2002. Enterococcus gilvus sp. nov. and Enterococcus pallens sp. nov. isolated from human clinical specimens. J Clin Microbiol 40: 1140–1145. https://doi.org/10.1128/JCM.40.4.1140-1145.2002.

2. Martin B, Corominas L, Garriga M, Aymerich T. 2009. Identification and tracing of Enterococcus spp. by RAPD-PCR in traditional fermented sausages and meat environment. J Appl Microbiol 106:66–77. https://doi.org/10.1111/j.1365-2672.2008.03976.x.

3. Ago M, Bonvini B, Carminati D, Giraffa G. 2009. Detection and quantification of Enterococcus gilvus in cheese by real-time PCR. Syst Appl Microbiol 32:514–521. https://doi.org/10.1016/j.syapm.2009.07.001.

4. Taylor RF, Davies BH. 1974. Triterpenoid carotenoids and related lipids. The triterpenoid carotenes of Strepctococcus faecium UNH 564P. Biochem J 139:751–760. https://doi.org/10.1042/bj1390751.

5. Hagi T, Kobayashi M, Kawamoto S, Shima J, Nomura M. 2013. Expression of novel carotenoid biosynthesis genes from Enterococcus gilvus improves the multistress tolerance of Lactococcus lactis. J Appl Microbiol 114:1763–1771. https://doi.org/10.1111/jam.12182.

6. Hagi T, Kobayashi M, Nomura M. 2015. Aerobic conditions increase isoprenoid biosynthesis pathway gene expression levels for carotenoid production in Enterococcus gilvus. FEMS Microbiol Lett 362:fnv075. https://doi.org/10.1093/femsle/fnv075.

7. Hagi T, Kobayashi M, Nomura M. 2018. Whole-transcriptome analysis of oxidative stress response genes in carotenoid-producing Enterococcus gilvus. Biosci Biotechnol Biochem I 82:1053–1057. https://doi.org/10.1080/09168451.2017.1399790.

8. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibiloo 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, Kearnns G, Kong X, Kuske R, Lacroix Y, Liu S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Sheng G, Sorenson J, Tomaney A, Travers K, Trulson M, Viecelli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Koriach J, Turner S. 2009. Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986.

9. Nakano K, Shiroma A, Shimoji M, Tamotsu H, Ashime 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.

10. Nakano K, Shiroma A, Shiroma M, Tamotsu H, Ohki S, Shimoji M, Ashime 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.

11. Saito H, Miura K-I. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim Biophys Acta 72:619–629. https://doi.org/10.1016/0006-8993(63)90386-4.

12. Chin C-S, Alexander DH, 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/nmeth.2474.

13. Sommer DD, Delcher AL, Salzberg SL, Pop M. 2007. Minimus: a fast, lightweight genome assembler. BMC Bioinformatics 8:64. https://doi.org/10.1186/1471-2105-8-64.

14. Engman J, Rognstam A, Frees D, Ingmar H, von Wachenfeldt C. 2012. The Yjhm adaptor protein enhances proteolysis of the transcriptional regulator Spx in Staphylococcus aureus. J Bacteriol 194:1186–1194. https://doi.org/10.1128/JB.06414-11.