



## Draft Genome Sequence of Bacteriocin-Encoding *Enterococcus faecium* Strain S6, Isolated from Camel Milk

Abrar Akbar, a Rita Rahmeh, Mohamad Kishk, Abdulaziz Alateeqi, Husam Alomirah

<sup>a</sup>Kuwait Institute for Scientific Research, Safat, Kuwait

**ABSTRACT** Enterococcus faecium strain S6 is a newly identified bacteriocin producer isolated from raw camel milk. The draft genome sequence is composed of 2,617,971 bp, with 2,407 coding genes and a G+C content of 37.99%. The genome sequence analysis provided details into the antimicrobial properties of strain S6.

Enterococci are Gram-positive lactic acid bacteria that inhabit the gastrointestinal tracts of different hosts (1). *Enterococcus faecium* strain S6 was isolated from raw camel milk in Kabad, Kuwait (northwest region). The strain exhibited strong antimicrobial activity against three indicator strains, including *Listeria monocytogenes, Salmonella enterica,* and *Escherichia coli* (2). Bacteriocins and bacteriocin-producing bacterial strains can be used in the food industry as food preservatives, and they can substitute conventional antibiotics especially in animal food production (3). This report explains the entry of *E. faecium*, which was isolated from raw camel milk, into the NCBI database genome.

Before isolation, bacteria were grown on MRS medium for 24 h at 37°C under aerobic conditions (4). Genomic DNA was extracted from the cultured bacteria by the GeneElute bacterial genomic DNA kit (Sigma-Aldrich Co. LLC). Barcoded DNA libraries were prepared using the Nextera XT library (Illumina, Inc., San Diego, CA, USA) following the manufacturer's instructions. The 2 imes 250-bp paired-end read sequencing was performed on a HiSeq 2500 instrument (Illumina, Inc.). Read quality was controlled using the FASTQC quality-control tool version 0.11.5. In addition, the sequences were enhanced using the read error correction module BayesHammer in the SPAdes version 3.10 genome assembly tool kit (5), and the high-quality reads were assembled into contigs using SPAdes software. Misassembles and nucleotide disagreement between the Illumina data and the contig sequences were corrected by Pilon (6) version 1.11, which resulted in 37 scaffolds of different sizes (minimum scaffold size, 320 bp; maximum scaffold, 284,269 bp). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation system (PGAP; version 5.2) (7). The genome sequence consists of 2,617,971 bases, with a G+C content of 37.99%. The number of raw reads was 4,437,645, and the assembly  $N_{50}$  score was 191,551 bp with a 408,49 average coverage. A total of 2,453 coding sequences (CDSs) and 55 structural RNAs were predicted. There were 2,407 CDSs with a known function and 129 with a signal peptide. Several CDSs for the production of bacteriocins, namely, enterocin A, enterocin B, two-component enterocin X (X-alfa and X-beta subunits), and lactococcin, were found using BAGEL4 analysis software (8). Virulence factors, such as surface aggregating-protein, gelatinase, and hyaluronidase, were not detected in the sequence using Virulence Finder software (version 2) (9-11). Furthermore, ResFinder software 3.0 (9, 12, 13) was used to identify acquired resistance genes. As shown in Table 1, the resistance genes are tetracycline, macrolide, lincosamide, and streptogramin B. Unless stated otherwise, default parameters were used for all software tools.

This work highlights the potential biotechnological application of this strain for the production of enterocins, which are bacteriocins that can be employed in the food

Editor Catherine Putonti, Loyola University Chicago

**Copyright** © 2022 Akbar et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Abrar Akbar, aajakbar@kisr.edu.kw.

The authors declare no conflict of interest.

Received 13 September 2021 Accepted 23 January 2022 Published 17 February 2022

Gene	Identity (%)	Alignment length	Coverage (×)	Contig	Position in contig	Phenotype
tet(L)	100	1,377/1,377	100	s6_contig_00016	24783854	Tetracycline resistance
tet(M)	96.4583	1,920/1,920	100	s6_contig_00016	40485967	Tetracycline resistance
msr(C)	98.9858	1,479/1,479	100	s6_contig_00007	8026481742	Macrolide resistance
<i>lsa</i> (E)	99.596	1,485/1,485	100	s6_contig_00016	2890730391	Streptogramin B resistance
Inu(B)	99.3781	804/804	100	s6_contig_00016	3044531248	Lincosamide resistance

TABLE 1 Summary of acquired resistance genes using ResFinder software 3.0

industry as a biopreservative against *L. monocytogenes* and as an alternative to classical antibiotics.

**Data availability.** This whole-genome sequencing project has been deposited at DDBJ/ENA/GenBank under the accession JAHCYY000000000.1. The version described in this paper is the first version, JAHCYY010000000. Raw sequencing reads are available in NCBI SRA under accession number SRR17078534.

## ACKNOWLEDGMENTS

This work was funded by Kuwait Foundation for the Advancement of Sciences under project code PR18-12SL-16.

We thank the research core facility unit, Faculty of Medicine, Kuwait University (Jabriya, Kuwait), for their help and support with sequencing.

## REFERENCES

- Franz CMAP, Holzapfel WH, Stiles ME. 1999. Enterococci at the crossroads of food safety? Int J Food Microbiol 47:1–24. https://doi.org/10.1016/S0168 -1605(99)00007-0.
- Rahmeh R, Akbar A, Alonaizi T, Kishk M, Shajan A, Akbar B. 2020. Characterization and application of antimicrobials produced by Enterococcus faecium S6 isolated from raw camel milk. J Dairy Sci 103:11106–11115. https://doi.org/10.3168/jds.2020-18871.
- Ben Lagha A, Haas B, Gottschalk M, Grenier D. 2017. Antimicrobial potential of bacteriocins in poultry and swine production. Vet Res 48:22. https://doi .org/10.1186/s13567-017-0425-6.
- Rahmeh R, Akbar A, Kishk M, Al Onaizi T, Al-Shatti A, Shajan A, Akbar B, Al-Mutairi S, Yateem A. 2018. Characterization of semipurified enterocins produced by Enterococcus faecium strains isolated from raw camel milk. J Dairy Sci 101:4944–4952. https://doi.org/10.3168/jds.2017-13996.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone .0112963.
- 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.

- Van Heel AJ, De Jong A, Song C, Viel JH, Kok J, Kuipers OP. 2018. BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. Nucleic Acids Res 46:W278–W281. https://doi.org/10.1093/nar/gky383.
- Clausen PTLC, Aarestrup FM, Lund O. 2018. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics 19:307. https://doi.org/10.1186/s12859-018-2336-6.
- Tetzschner AMM, Johnson JR, Johnston BD, Lund O, Scheutz F. 2020. In silico genotyping of Escherichia coli isolates for extraintestinal virulence genes by use of whole-genome sequencing data. J Clin Microbiol 58: e01269-20. https://doi.org/10.1128/JCM.01269-20.
- Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol 52:1501–1510. https://doi.org/10.1128/JCM.03617-13.
- Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500. https://doi.org/10 .1093/jac/dkaa345.
- Zankari E, Allesøe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM. 2017. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. J Antimicrob Chemother 72:2764–2768. https://doi.org/10 .1093/jac/dkx217.