AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

PROKARYOTES

Complete Genome Sequences of *Enterobacter cancerogenus* CR-Eb1 and *Enterococcus* sp. Strain CR-Ec1, Isolated from the Larval Gut of the Greater Wax Moth, *Galleria mellonella*

Joon-hui Chung,[a] Haeyoung Jeong,[b,c] Choong-Min Ryu[b,c]

[a]The Agricultural Genome Center, National Institute of Agricultural Sciences, Rural Development Administration (RDA), Jeonju, Republic of Korea

[b]Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea

[c]Department of Biosystems and Bioengineering Program, Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, Korea University of Science and Technology (UST), Daejeon, Republic of Korea

ABSTRACT *Enterobacter cancerogenus* CR-Eb1 and *Enterococcus* sp. CR-Ec1 were isolated from the larval gut of *Galleria mellonella*, the greater wax moth. Here, we report the completed and annotated genome sequences of insect gut-dwelling bacteria.

The larvae of the greater wax moth (*Galleria mellonella* L.) have been used as a model animal organism for studying the pathogenicity and host-gut microbiome interaction (1, 2). Recent data suggest that the gut bacteria from *G. mellonella* confers wax degradation (3). To investigate the dynamics of gut microbiota and identify potential gut bacteria responsible for wax degradation, we isolated culturable bacteria from the larval gut. Third- to fourth-instar *G. mellonella* larvae were purchased from S-WORM (Cheonan, Republic of Korea). The larvae were fed an artificial diet (600 g rice bran, 600 g wheat bran, 4.5 g yeast extract, 2 g $CaCO_3$, 250 ml glycerol, 600 ml honey, 600 mg vitamin B complex, and 175 ml distilled water) at 37°C (4). The samples from one larval gut were macerated with glass beads and phosphate-buffered saline buffer and streaked onto 10-fold diluted tryptic soybean agar. *Enterobacter cancerogenus* CR-Eb1 and *Enterococcus* sp. CR-Ec1 were isolated and identified from the larval gut samples. *E. cancerogenus* (syn. *E. taylorae*) and *Enterococcus* spp. have been reported to be opportunistic human pathogens that infect the urinary tract and open wounds (5–9).

Genome sequencing was performed on a PacBio RS II platform using P6-C4 chemistry, with one single-molecule real-time (SMRT) cell per sample, at the National Instrumentation Center for Environmental Management, Seoul National University (Seoul, Republic of Korea). Sequencing coverages for CR-Eb1 and CR-Ec1 were 90.7-fold and 222.9-fold, respectively. Genome assemblies obtained with the RS_HGAP_Assembly.2 protocol under SMRT Analysis version 2.3.0 (Pacific Biosciences, Menlo Park, CA, USA), followed by circularization using Circlator (10), were further corrected by running two successive rounds of the RS_Resequencing.1 protocol. The CR-Eb1 genome has a 4,796,512-bp chromosome (55.78% G+C content), while CR-Ec1 has a 3,819,143-bp chromosome (42.4% G+C content) and a 70,706-bp plasmid (36.48% G+C content), where all replicons have circular structure. CR-Eb1 was classified as *E. cancerogenus* on the basis of the average nucleotide identity by orthology (OrthoANI) algorithm (99.88%) (11) and the Genome-to-Genome Distance Calculator (90.80% of DDH estimate; https://ggdc.dsmz.de/distcalc2.php) using the genome sequence of type strain ATCC 33241 (GCA_900185905) as the reference. CR-Ec1, however, could not be

Received 12 January 2018 **Accepted** 16 January 2018 **Published** 15 February 2018

Citation Chung J-H, Jeong H, Ryu C-M. 2018. Complete genome sequences of *Enterobacter cancerogenus* CR-Eb1 and *Enterococcus* sp. strain CR-Ec1, isolated from the larval gut of the greater wax moth, *Galleria mellonella*. Genome Announc 6:e00044-18. https://doi.org/10.1128/genomeA.00044-18.

Address correspondence to Choong-Min Ryu, cmryu@kribb.re.kr.

J.-H.C. and H.J. contributed equally to this work.

assigned species-level taxonomy because the analyzed values were below the cutoff, *Enterococcus casseliflavus* ATCC 49996^T (GCA_000393915) being the closest strain.

Genome sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok) and the Rapid Annotations using Subsystems Technology (RAST) server (12). Island Viewer (13) analysis identified no virulence factors or antimicrobial resistance genes in either genome, and antiSMASH (14) predicted biosynthetic gene clusters for enterobactin (CR-Eb1) and dehydrosqualene (CR-Ec1). In conclusion, the complete genome sequences of these two bacterial isolates will provide insights into the infection and control of microbiota by host and/or dietary factors.

Accession number(s). The complete genome sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers CP025225 (*E. cancerogenus* CR-Eb1) and CP025223 and CP025224 (*Enterococcus* sp. CR-Ec1).

ACKNOWLEDGMENTS

This work was supported by the Agenda Project (PJ012814) of the Rural Development Administration (RDA) and the KRIBB Research Initiative Program of the Ministry of Science and ICT, Republic of Korea.

REFERENCES

1. Ramarao N, Nielsen-Leroux C, Lereclus D. 2012. The insect galleria mellonella as a powerful infection model to investigate bacterial pathogenesis. J Vis Exp 70:e4392. https://doi.org/10.3791/4392.
2. Mukherjee K, Raju R, Fischer R, Vilcinskas A. 2013. *Galleria mellonella* as a model host to study gut microbe homeostasis and brain infection by the human pathogen *Listeria monocytogenes*. Adv Biochem Eng Biotechnol 135:27–39. https://doi.org/10.1007/10_2013_203.
3. Bombelli P, Howe CJ, Bertocchini F. 2017. Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*. Curr Biol 27:R292–R293. https://doi.org/10.1016/j.cub.2017.02.060.
4. Lee SW, Lee DW, Choo HY. 2007. Development of economical artificial diets for greater wax moth, *Galleria mellonella* (L.). Korean J Appl Entomol 46:385–392. https://doi.org/10.5656/KSAE.2007.46.3.385.
5. Abbott SL, Janda JM. 1997. *Enterobacter cancerogenus* ("*Enterobacter taylorae*") infections associated with severe trauma or crush injuries. Am J Clin Pathol 107:359–361. https://doi.org/10.1093/ajcp/107.3.359.
6. Rolland A. 2002. The etiology of urinary tract infection: traditional and emerging pathogens. Am J Med 113:14–19.
7. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489:220–230. https://doi.org/10.1038/nature11550.
8. Chen B, The B-S, Sun C, Hu S, Lu X, Boland W, Shao Y. 2016. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. Sci Rep 6:29505. https://doi.org/10.1038/srep29505.
9. Ramya SL, Venkatesan T, Murthy KS, Jalali SK, Verghese A. 2016. Detection of carboxylesterase and esterase activity in culturable gut bacterial flora isolated from diamondback moth, *Plutella xylostella* (Linnaeus), from India and its possible role in indoxacarb degradation. Environ Microbiol 47:327–336. https://doi.org/10.1016/j.bjm.2016.01.012.
10. Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol 16:294. https://doi.org/10.1186/s13059-015-0849-0.
11. Lee I, Kim YO, Park SC, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760.
12. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226.
13. Bertelli C, Laird MR, Williams KP, Simon Fraser University Research Computing Group, Lau BY, Hoad G, Winsor GL, Brinkman FS. 2017. IslandViewer 4: Expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Res 45:W30–W35. https://doi.org/10.1093/nar/gkx343.
14. Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. https://doi.org/10.1093/nar/gkv437.