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Draft Genome Sequence of *Enterobacter* sp. Strain EA-1, an Electrochemically Active Microorganism Isolated from Tropical Sediment

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ABSTRACT *Enterobacter* sp. strain EA-1 is an electrochemically active bacterium isolated from tropical sediment in Singapore. Here, the annotated draft genome assembly of the bacterium is reported. Whole-genome comparison indicates that *Enterobacter* sp. EA-1, along with a previously sequenced *Enterobacter* isolate from East Asia, forms a distinct clade within the *Enterobacter* genus.

Members of the genus *Enterobacter* belong to the class *Gammaproteobacteria* and are rod-shaped, Gram-negative, motile, facultative anaerobes. They are commonly found in soil, water, sewage, and the intestines of animals and humans and have been associated with nosocomial infections (1). *Enterobacter* sp. strain EA-1 was isolated from tropical sediment in Singapore during an electrochemical enrichment and was found to be capable of extracellular electron transfer (2).

Genomic DNA was purified from 1 ml of an overnight culture grown in Luria-Bertani (LB) broth at room temperature and with shaking at 150 rpm. The Wizard genomic DNA purification kit (Promega, USA) was used according to the protocol for Gram-negative bacteria supplied by the manufacturer.

The draft genome sequence was determined by shotgun sequencing on a MinION Mk1B sequencer (Oxford Nanopore, UK) using R9 chemistry and library protocol SQK-LSK108. Raw sequencing data were assembled using Canu version 1.3 (3), resulting in a single contig which was further refined using nanopolish version 0.7.1 (4), manually trimmed for overlaps at the ends, and automatically annotated using the NCBI Prokaryotic Genome Annotation Pipeline (5).

The draft genome of EA-1 is composed of 5,145,523 bp, with an average G+C content of 54.56%, and it contains 81 tRNAs, 22 rRNAs organized in 7 operons, and 1 transfer-messenger RNA (tmRNA). A comparison of the 16S rRNA gene sequence revealed >98% identity to the 16S rRNA genes of *Enterobacter cloacae* ATCC 13047, *Enterobacter cloacae* SBP-8, and *Enterobacter* sp. strain FY-07. However, a whole-genome comparison indicated an average nucleotide identity (6) of 78.12% compared to *E. cloacae* ATCC 13047 (NCBI accession number CP001918), 78.09% compared to *E. cloacae* SBP-8 (NCBI accession number CP016906), and 93.38% compared to FY-07 (NCBI accession number CP012487), suggesting that EA-1 forms, together with FY-07, a distinct clade within the *Enterobacter* genus related to but separate from *E. cloacae*.

In-detail genome alignments between EA-1 and FY-07 using the Artemis Comparison Tool (7) showed that the genes associated with bacterial cellulose (BC) in FY-07, a highly effective producer of the polysaccharide (8), are also present in strain EA-1, with

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open reading frame (ORF) nucleotide identities ranging from 82 to 95%. Pairwise global alignment was conducted at the EMBL-EBI website (http://www.ebi.ac.uk/Tools/psa/) using the EMBOSS Needle tool (Needleman-Wunsch algorithm). In FY-07, these genes are arranged in three BC-producing operons: *bcsI*, *bcsII*, and *bcsIII* (9). In *bcsI*, the FY-07 locus tags are as follows, with the corresponding EA-1 locus tags in parentheses: AKI40_0196 (CWS02_01210), AKI40_0197 (CWS02_01215), AKI40_0198 (CWS02_01220), AKI40_0199 (CWS02_01225), AKI40_0200 (CWS02_01230), AKI40_0201 (CWS02_01235), and AKI40_0202 (CWS02_01240). For *bcsII*, the FY-07 and corresponding EA-1 locus tags (in parentheses) are AKI40_0206 (CWS02_01260), AKI40_0207 (CWS02_01265), AKI40_0208 (CWS02_01270, CWS02_01275, and CWS02_01280), AKI40_0209 (CWS02_01285), AKI40_0210 (CWS02_01290), and AKI40_0211 (CWS02_01295). In the case of the EA-1 locus tag corresponding to FY-07's AKI40_0208, multiple ORFs were stitched together during nucleotide alignment in order to identify the complete homologous gene sequence which was interrupted due to frameshifting errors associated with the sequencing technology used. For *bcsIII*, the FY-07 and corresponding EA-1 locus tags (in parentheses) are AKI40_0894 (CWS02_05060), AKI40_0893 (CWS02_05055), AKI40_0892 (CWS02_05050), and AKI40_0891 (CWS02_05045). For other BC-related genes (not operon-associated), the FY-07 and corresponding EA-1 locus tags are AKI40_0203 (CWS02_01245), AKI40_0204 (CWS02_01250), and AKI40_0205 (CWS02_01255).

Accession number(s). The whole-genome shotgun project was deposited in NCBI under the accession number CP025776.

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REFERENCES

1. Grimont PAD, Grimont F. 2015. *Enterobacter*, p 1–17. *In* Whitman WB (ed), Bergey's manual of systematics of archaea and bacteria John Wiley & Sons, Ltd., New York, NY.
2. Doyle LE, Yung PY, Mitra SD, Wuertz S, Williams RBH, Lauro FM, Marsili E. 2017. Electrochemical and genomic analysis of novel electroactive isolates obtained via potentiostatic enrichment from tropical sediment. J Power Sources 356:539–548. https://doi.org/10.1016/j.jpowsour.2017.03.147.
3. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
4. Loman NJ, Quick J, Simpson JT. 2015. A complete bacterial genome assembled *de novo* using only nanopore sequencing data. Nat Methods 12:733–735. https://doi.org/10.1038/nmeth.3444.
5. 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.
6. Richter M, Rossello-Mora R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106:19126–19131. https://doi.org/10.1073/pnas.0906412106.
7. Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. 2005. ACT: the Artemis Comparison Tool. Bioinformatics 21:3422–3423. https://doi.org/10.1093/bioinformatics/bti553.
8. Ma T, Ji K, Wang W, Wang J, Li Z, Ran H, Liu B, Li G. 2012. Cellulose synthesized by *Enterobacter* sp. FY-07 under aerobic and anaerobic conditions. Bioresour Technol 126:18–23. https://doi.org/10.1016/j.biortech.2012.09.040.
9. Ji KH, Wang W, Zeng B, Chen SB, Zhao QQ, Chen YQ, Li GQ, Ma T. 2016. Bacterial cellulose synthesis mechanism of facultative anaerobe *Enterobacter* sp. FY-07. Sci Rep 6:21863. https://doi.org/10.1038/srep21863.