

# Complete Genome Sequence of the Endophytic *Enterobacter cloacae* subsp. *cloacae* Strain ENHKU01

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***Enterobacter cloacae* subsp. *cloacae* strain ENHKU01 is a Gram-negative endophyte isolated from a diseased pepper (*Capsicum annuum*) plant in Hong Kong. This is the first complete genome sequence report of a plant-endophytic strain of *E. cloacae* subsp. *cloacae*.**

An unknown bacterium, named ENHKU01, was isolated from a diseased pepper plant infected with the plant pathogen *Ralstonia solanacearum* in Hong Kong in 2010. The endophytic but nonpathogenic character of ENHKU01 was confirmed (data not shown). Preliminary sequencing and BLAST analysis of partial 16S rRNA and housekeeping genes indicated that the isolate is most closely related to *Enterobacter*, a genus of Gram-negative proteobacteria in the *Enterobacteriaceae* family; the genus consists of an extremely diverse group of bacteria that are found associated with soil, plants, and humans.

*De novo* shotgun paired-end pyrosequencing and Sanger sequencing strategies were used to produce the genomic sequence of ENHKU01. Genomic DNA was extracted from overnight liquid cultures of ENHKU01 using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's recommended procedures. A total of 141,802 reads, representing 11× coverage of the ENHKU01 genome, were generated by the 454 GS Junior platform (454 Life Sciences, Branford, CT) (6) and assembled into 307 contigs using Newbler Assembler (454 Life Sciences). Two extra paired-end sequencing runs were carried out by using an 8-kb library to produce a draft genome with one scaffold containing 36 contigs. To complete the whole genome sequence of ENHKU01, possible misassemblies were corrected and 36 sequence gaps were filled by PCR, primer walking, and Sanger sequencing. Lasergene (DNASTAR, Madison, WI) was used for final sequence assembly and sequence manipulation.

The genome of ENHKU01 consists of a single chromosome of 4,726,582 bp with a GC content of 55.1%. Gene annotation and analysis were performed using the NCBI Prokaryotic Genome Annotation Pipeline (1). A combination of coding sequence (CDS) prediction programs provided by GLIMMER and GeneMark was used for gene prediction (4). Annotation was performed using BLASTP, and the protein sequences encoded by predicted genes were searched against all proteins from complete microbial genomes and aligned with the best BLAST hit (7). Further annotation was obtained using the SEED-based automated annotation system provided by the RAST server (2). A total of 4,338 CDSs were predicted, with 87% assigned to clusters of orthologous groups. Eighty-two tRNAs and 25 rRNAs were identified. Among the publicly available and completed genome sequences of *Enterobacter* species, i.e., *Enterobacter cloacae* subsp. *cloacae* ATCC 13047 (8), *E. cloacae* subsp. *dissolvens* SDM (11), *E. lignolyticus* SCF1 (3), *Enterobacter* sp. strain 638 (10), *E. aerogenes* KCTC2190 (9), *E. cloacae* EcWSU1 (5), and *E. asburiae* LF7a, ENHKU01 is most closely related to *E. cloacae* subsp. *cloacae* ATCC 13047, an opportunistic human pathogen (8). This is the first complete-genome sequence report of a plant-associated strain of *E. cloacae* subsp. *cloacae*.

The genome sequence of *E. cloacae* subsp. *cloacae* ENHKU01 provides an opportunity to gain insights into the differences between the diverse group of *E. cloacae* involving endophytic plant- and human-pathogenic strains through comparative genome analysis. Furthermore, the functional role of ENHKU01 associated with its host plants and the pathogen *R. solanacearum* has yet to be revealed.

**Nucleotide sequence accession number.** The *E. cloacae* subsp. *cloacae* ENHKU01 chromosome sequence has been deposited in GenBank under accession number [CP003737](https://www.ncbi.nlm.nih.gov/nuccore/CP003737).

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