Complete Genome Sequence of *Enterobacter roggenkampii* Strain KQ-01, Isolated from Bacterial Wilt-Resistant Mulberry Cultivar YS283

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Abstract

An *Enterobacter roggenkampii* strain, named KQ-01, was isolated for the first time from the diseased roots of the bacterial wilt-resistant mulberry (*Morus atropurpurea*) cultivar YS283 in the western part of Guangxi Zhuang Autonomous Region, China. *E. roggenkampii* KQ-01 was characterized by a single, circular chromosome of 4,667,006 bp in size, with a 56.16% GC content. In total, 4,396 genes were annotated, of which 4,281 were assigned as protein-coding genes. In addition, 25 ribosomal RNA, 84 transfer RNA, and 6 noncoding RNA genes as well as 34 pseudogenes were predicted. The resource herein presented will help to explore the pathogenetic mechanisms of mulberry wilt disease caused by genus *Enterobacter*.

Keywords

bacterial wilt, complete genome, *Enterobacter roggenkampii* KQ-01, mulberry cultivar YS283

Mulberry is a perennial woody plant widespread in Asia, Africa, and Europe, of high economic value (Dhanyalakshmi and Nataraja 2018; Rodrigues et al. 2019). In 2008, bacterial wilt of mulberry caused by the *Enterobacter cloacae* complex (ECC) was first reported in China (Wang et al. 2008). Later, the genome sequence of *E. mori* LMG 25706 was published (Zhu et al. 2011). Although ECC represents a serious threat for most mulberry varieties, the bacterial wilt-resistant *M. atropurpurea* 'YS283' is believed to be resistant to bacterial wilt disease. Since 1993, *M. atropurpurea* 'YS283' has been selected and bred in Guangdong, China (Huang 1993), and successfully commercialized in the country due to its excellent resistance to many pathogenic bacteria such as *Ralstonia solanacearum* and related species (Dai et al. 2016). However, wilting symptoms in *M. atropurpurea* 'YS283' have been increasingly observed in Guangxi, South China, which represents the largest sericulture production site in China. The wilting symptoms of the mulberry, which appeared to be caused by a bacterial infection, were confirmed in our laboratory. To boost research on etiology and pathogenesis of such bacterial infection, we isolated one of the pathogenic bacteria from the infected mulberry (namely, *E. roggenkampii* KQ-01) and sequenced and assembled its genome. *E. roggenkampii* KQ-01 culture has been deposited in the International Depository Authority (Guangdong Microbial Culture Collection Center, Guangzhou, China) under accession number GDMCC 1.6104.

During summer 2017, *E. roggenkampii* strain KQ-01 was isolated from the infected roots of bacterial wilt-resistant mulberry (*M. atropurpurea*) cultivar YS283, found in Guangxi Zhuang Autonomous Region, China (109°26'13"E, 24°52'85"N). To isolate the pathogenic bacteria, the infected roots were washed with sterilized water prior to cutting them into 50-mm-long sections. Sections were soaked in 75% ethyl alcohol for 15 s and incubated with sterilized water in a rotary shaker at 130 rpm for 30 min at 25°C. After a gradient dilution, the supernatant was placed on a cultural plate with triphenyl tetrazolium chloride (TTC) medium and incubated at 30°C and 75% humidity for 72 h. Single colonies were picked up and

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Table 1. Genome of *Enterobacter roggenkampii* strain KQ-01

Features ^a	Numbers
Genes	4,396
Protein-coding genes	4,281
Transfer RNAs	84
Ribosomal RNAs	25
Noncoding RNAs	6
Pseudogenes	34
Hypothetical proteins	429
Proteins with COG assignments	3,903
Proteins with GO assignments	1,018
Proteins with KEGG assignments	885
Predicted GIs	36

^a COG = cluster of orthologous groups, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, and GIs = genomic islands.

streaked in TTC medium. Monoclonal colonies were selected and transferred onto a new plate with TTC medium for pure culture.

For quality assurance, genomic DNA was extracted and purified from a pure culture of a single bacterial isolate of *E. roggenkampii* KQ-01, using the Bacteria Genomic DNA Kit (CoWin Biosciences, Beijing, China). The quantity and quality of the DNA were assessed by a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Prior to DNA sequencing, the 16S ribosomal DNA (rDNA) sequence obtained was compared with those deposited in NCBI GenBank using the NCBI BLAST algorithm. For further study of the genetic background, the complete genome sequence was obtained and analyzed. For paired-end sequencing, whole-genome DNA was used to prepare a DNA library with a 350-bp insert. Deep sequencing (depth = 440×) was conducted with manufactured 2 × 150-bp alignment reads on Illumina Hiseq4000 System by Science Corporation of Gene Technology Co., Ltd. (Guangzhou, China) and, in total, 6,846,906 paired-end reads were obtained. Genome sequences were assembled using SPAdes, version 3.5.0 (Bankevich et al. 2012). The genome sequence data were uploaded to Similar Genome Finder Server (Davis et al. 2020) to determine the accurate and reliable taxonomic position. Phylogenomic analysis was performed by average nucleotide identity based on BLAST+ (ANIb) (Richter et al. 2016). The genome was automatically annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Haft et al. 2018) and the RAST tool kit (Overbeek et al. 2014). Functional analysis was done by using clusters of orthologous groups (COGs) of proteins (Galperin et al. 2015), Gene Ontology (GO) (Ashburner et al. 2000), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa et al. 2016) annotation. Identification of genomic islands (GIs) was performed using the IslandViewer 4 platform and the SIGI-HMM, IslandPick, IslandPath-DIMOB, and Islander prediction methods (Bertelli et al. 2017).

E. roggenkampii KQ-01 was first identified as ECC based on the 16S rDNA sequence. In addition, the genome sequence was highly identical (99.10%) to *E. roggenkampii* SMART_269.v1 (NCBI accession number GCA_001471995) isolated from *Homo sapiens* based on ANIb analysis. The complete genome of *E. roggenkampii* KQ-01 was composed of a single, continuous, circular chromosome of 4,667,006 bp in size, with a 56.16% GC content and no plasmids. Moreover, in total, 4,396 genes were annotated in the *E. roggenkampii* KQ-01 strain genome, of which 4,281 genes were assigned as putative protein-coding genes. In addition, 115 RNA-encoding genes, including 25 ribosomal RNA, 84 transfer RNA, and 6 noncoding RNA genes as well as 34 pseudogenes were predicted. By using functional protein alignments, 3,903 proteins were assigned to the COG database, 1,018 proteins were assigned to GO terms, and 885 proteins were mapped to KEGG pathways. By using the IslandViewer 4 platform, 36 GIs were identified. The annotated features are summarized in Table 1.

To the best of our knowledge, this is the first reported genome of *E. roggenkampii* KQ-01 isolated from mulberry. Our data provide a starting platform for comparative genomics of other *Enterobacter* spp., which are responsible for mulberry wilt disease in the main region of sericulture, and clarify the pathogenesis of *E. roggenkampii* KQ-01 in mulberry.

Data availability. The genome sequence of *E. roggkampii* strain KQ-01 has been deposited in NCBI GenBank in BioProject number PRJNA644454 under accession number CP058637. The original data have been deposited in the NCBI Sequence Read Archive under accession number SRX8696658.

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