



Draft Genome Sequence of *Enterobacter cloacae* 3D9 (Phylum *Proteobacteria*)

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ABSTRACT Presented here is the draft genome sequence of *Enterobacter cloacae* 3D9. This candidate seed endophyte was isolated from *Zea nicaraguensis*. The genome contains 4,653,375 bp in 28 contigs.

Endophytes are microbes that exist inside plants without causing disease (1). Endophytic species, such as *Enterobacter* spp., likely play important roles in plant microbiomes, as many have been found to act as plant growth-promoting bacteria in diverse crops, such as maize, rice, and tomatoes (2, 3). There are a variety of mechanisms by which *Enterobacter* species can promote plant growth, such as the solubilization of phosphate (4) or fixation of atmospheric nitrogen (5).

Grasses such as maize, wheat, and rice are the world's three most important food crops, but these grasses require large amounts of nitrogen fertilizer. There is incentive to find alternative ways to provide usable nitrogen to these crops, and the use of microorganisms is currently an attractive research target. Recently, a screen of 75 candidate endophytes isolated from diverse wild and domesticated maize genotypes was performed to identify strains that could increase the biomass of annual ryegrass, a maize relative, in a nitrogen-free medium (6). The candidate endophyte *Enterobacter cloacae* 3D9 consistently promoted plant biomass in a nitrogen-free medium. The growth-promoting mechanism of action is unknown, but possibilities include nitrogen fixation, recycling of nitrogen from senescing plant tissue, or scavenging of secreted nitrogen metabolites (6, 7). Here, we present the draft genome sequence of *E. cloacae* 3D9 in hopes of further understanding its mechanism of action.

E. cloacae 3D9 was isolated from surface-sterilized seeds of *Zea nicaraguensis* in 2011 (8). For genomic DNA isolation, a single colony from an LB plate was used to inoculate an overnight LB culture at 30°C with shaking at 200 rpm. A bacterial genomic DNA isolation kit (catalog number 17900; Norgen, Thorold, ON, Canada) was used to extract genomic DNA from the overnight culture. The genomic DNA (1 ng) was used to create a paired-end library using a Nextera XT DNA library prep kit (Illumina, San Diego, CA). Sequencing was carried out using an Illumina MiSeq instrument with 50 pg of 3D9 genomic DNA, which yielded 10,039,290 reads with an average length of 251 bp for a 542-fold coverage. In total, 10,032,951 reads remained after trimming low-quality reads. *De novo* assembly was performed using CLC Genomics Workbench (v.10.0.1; Qiagen) and resulted in 23 contigs (minimum length, 1,164 bp; maximum length, 935,739 bp; N_{50} , 396,804 bp). The assembled genome had 4,653,375 bp and a GC content of 56.2%.

The Rapid Annotations using Subsystems Technology (RAST) server was used to annotate the genome sequence (9). Using KmerFinder 3.0, the species was determined to be *E. cloacae* (10, 11), with 98.6% nucleotide similarity to Hoffman complex IV (12). *E. cloacae* IV 3D9 contains 4,262 predicted protein-coding genes and 74 noncoding RNAs. Neither BLAST nor RAST searches identified a nitrogen fixation operon in the genome, suggesting that the genes are located on a plasmid or that there is an alternative mechanism of plant growth promotion under nitrogen-limited conditions.

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Data availability. The whole-genome shotgun project was deposited at DDBJ/EMBL/GenBank under the accession number [QKSC0000000](https://doi.org/10.1093/ncbi/qksc0000000). The version described in this paper is version QKSC01000000. Raw Illumina reads are available in the SRA under accession number [SRR7341760](https://doi.org/10.1093/bioinformatics/btt734).

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REFERENCES

- Johnston-Monje D, Raizada MN. 2011. Plant and endophyte relationships: nutrient management. In Moo-Young M, Comprehensive biotechnology, 2nd ed. Pergamon, Oxford, United Kingdom.
- Ogbo F, Okonkwo J. 2012. Some characteristics of a plant growth promoting *Enterobacter* sp. isolated from the roots of maize. *Adv Microbiol* 2:368–374. <https://doi.org/10.4236/aim.2012.23046>.
- Mayak S, Tirosh T, Glick BR. 2001. Stimulation of the growth of tomato, pepper and mung bean plants by the plant growth-promoting bacterium *Enterobacter cloacae* CAL3. *Biol Agric Hortic* 19:261–274. <https://doi.org/10.1080/01448765.2001.9754929>.
- Shehata HR, Dumigan C, Watts S, Raizada MN. 2017. An endophytic microbe from an unusual volcanic swamp corn seeks and inhabits root hair cells to extract rock phosphate. *Sci Rep* 7:13479r. <https://doi.org/10.1038/s41598-017-14080-x>.
- Peng G, Zhang W, Luo H, Xie H, Lai W, Tan Z. 2009. *Enterobacter oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species *Oryza latifolia*. *Int J Syst Evol Microbiol* 59:1650–1655. <https://doi.org/10.1099/ijs.0.005967-0>.
- Shehata HR, Lyons EM, Raizada MN. 2017. Turfgrasses as model assay systems for high-throughput *in planta* screening of beneficial endophytes isolated from cereal crops. *Symbiosis* 76:71–76.
- Tkacz A, Poole P. 2015. Role of root microbiota in plant productivity. *J Exp Bot* 66:2167–2175. <https://doi.org/10.1093/jxb/erv157>.
- Johnston-Monje D, Raizada MN. 2011. Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One* 6:e20396.
- Aziz RK, Bartels D, Best AA, Dejongh M, Disz T, Edwards RA, Formosa K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, Mcneil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:1–15. <https://doi.org/10.1186/1471-2164-9-75>.
- Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Moller N, Aarestrup FM. 2014. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *J Clin Microbiol* 52:139–146. <https://doi.org/10.1128/JCM.02452-13>.
- Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, Sicheritz-Pontén T, Aarestrup FM, Ussery DW, Lund O. 2014. Benchmarking of methods for genomic taxonomy. *J Clin Microbiol* 52:1529–1539. <https://doi.org/10.1128/JCM.02981-13>.
- Paauw A, Caspers MPM, Schuren FHJ, Leverstein-van Hall MA, Delétoile A, Montijn RC, Verhoef J, Fluit AC. 2008. Genomic diversity within the *Enterobacter cloacae* complex. *PLoS One* 3:e3018. <https://doi.org/10.1371/journal.pone.0003018>.