

## GENOME ANNOUNCEMENTS

### Complete Genome Sequence of *Enterobacter cloacae* subsp. *cloacae* Type Strain ATCC 13047<sup>†</sup>

Yan Ren,<sup>1,4,5</sup> Yi Ren,<sup>6,7</sup> Zhemin Zhou,<sup>1,4,5</sup> Xi Guo,<sup>1,2,3</sup> Yayue Li,<sup>6,7</sup> Lu Feng,<sup>1,3,5</sup> and Lei Wang<sup>1,3,5\*</sup>

*TEDA School of Biological Sciences and Biotechnology, Nankai University,<sup>1</sup> Tianjin Key Laboratory of Microbial Functional Genomics,<sup>2</sup> Tianjin Research Center for Functional Genomics and Biochips,<sup>3</sup> Engineering and Research Center for Microbial Functional Genomics and Detection Technology, Ministry of Education,<sup>4</sup> Key Laboratory of Molecular Microbiology and Technology, Ministry of Education,<sup>5</sup> and Tianjin Biochip Corporation,<sup>6</sup> 23 Hongda Street, TEDA, Tianjin 300457, People's Republic of China, and Tianjin University of Science and Technology, Tianjin 300457, People's Republic of China<sup>7</sup>*

Received 21 January 2010/Accepted 23 February 2010

***Enterobacter cloacae* is an important nosocomial pathogen. Here, we report the completion of the genome sequence of *E. cloacae* ATCC 13047, the type strain of *E. cloacae* subsp. *cloacae*. Multiple sets of virulence determinant and heavy-metal resistance genes have been found in the genome. To the best of our knowledge, this is the first complete genome sequence of the *E. cloacae* species.**

*Enterobacter* species are important human opportunistic pathogens, responsible for nosocomial infections such as urinary tract infections, osteomyelitis, cholecystitis, and neonatal meningitis (15). *Enterobacter cloacae*, the type species of *Enterobacter*, is a prevalent nosocomial pathogen due to high-level resistance to disinfectants and antimicrobial agents (9). *E. cloacae* ATCC 13047 was isolated from human cerebrospinal fluid by Edwin Oakes Jordan in 1890 and is the type strain of *E. cloacae* subsp. *cloacae* (8).

Whole-genome sequencing of *E. cloacae* ATCC 13047 was performed with a combined strategy using a Sanger shotgun approach (5) and 454 single-end sequencing technology (13). Genomic libraries containing 5-kb inserts were constructed, and 10,236 sequences were generated with an ABI 3730 DNA analyzer, giving 1.5-fold coverage of the genome. A total of 281,462 single-end reads, giving 19.1-fold coverage of the genome, were generated using the GS FLX system (454 Life Sciences Corporation) and assembled into 255 contigs with the 454 Newbler assembler ([www.454.com/product-solutions/analysis-tools/gs-de-novo-assembler.asp](http://www.454.com/product-solutions/analysis-tools/gs-de-novo-assembler.asp)). Newbler-generated contigs and ABI reads were assembled using the Phred/Phrap/ConSeq software package (6). Sequence gaps were filled through sequencing of PCR products. Prediction and annotation of protein-encoding genes were performed as described previously (4).

The complete *E. cloacae* ATCC 13047 genome contains a single circular chromosome of 5,314,588 bp and two circular plasmids, pECL\_A and pECL\_B, of 200,370 and 85,650 bp. The overall GC content of the chromosome is 54.79%, whereas the two plasmids have GC contents of 52.45 and 46.76%. The

chromosome contains 5,166 protein-encoding genes, 24 tRNA-encoding genes, and 8 rRNA-encoding genes. Plasmids pECL\_A and pECL\_B carry 278 and 124 protein-encoding genes, respectively.

The genome of *E. cloacae* ATCC 13047 possesses virulence properties recognized to be important in the onset of infection. On the chromosome, seven loci encoding proteins for fimbrial biosynthesis and six genes encoding adhesin/invasin-like proteins are found. This strain has two loci encoding iron-chelating compounds and three genes encoding hemolysin-like proteins. The O antigen gene cluster contains all genes necessary for the biosynthesis of pseudaminic acid, which belongs to the family of nonulosonic acid (NulO) (12). During infection, microbes displaying NulO sugar mimicry may downregulate host complement-mediated killing and be advantageous in a wide range of animal body habitats (1). The organism carries genes for 37 multidrug efflux proteins, 7 antimicrobial peptide resistance proteins, and 11 β-lactamases, suggesting its broad range of antibiotic resistance. All the above-mentioned genetic elements are important for adherence and invasion and for survival and growth during antibiotic therapy and therefore may contribute to pathogenesis (11).

The chromosome of *E. cloacae* ATCC 13047 carries seven operons involved in toxic heavy-metal resistance, including two *sil* operons (7), three *ars* operons (10), a *mer* operon (14), and a *cop* operon (2). Plasmid pECL\_A harbors a *cop* operon, two *mer* operons, a *sil* operon, an *ars* operon, and a *ter* operon (3). The presence of diverse and duplicated copies of heavy-metal resistance operons may be important for this organism to survive, especially in a heavy-metal-rich environment, such as sewage.

**Nucleotide sequence accession numbers.** The *E. cloacae* ATCC 13047 chromosome and plasmid pECL\_A and pECL\_B sequences have been deposited in GenBank under accession numbers CP001918, CP001919, and CP001920.

\* Corresponding author. Mailing address: TEDA School of Biological Sciences and Biotechnology, Nankai University, 23 Hongda Street, TEDA, Tianjin 300457, People's Republic of China. Phone: 86-22-66229588. Fax: 86-22-66229596. E-mail: wanglei@nankai.edu.cn.

† Published ahead of print on 5 March 2010.

This work was supported by the Tianjin Municipal Special Fund for Science and Technology Innovation grant 05FZZDSH00800, the National Natural Science Foundation of China (NSFC) key programs grants 30530010 and 20536040, the Chinese National Science Fund for Distinguished Young Scholars (grant 30788001), NSFC general program grants 30670038, 30870070, 30870078, and 30771175, the National 863 Program of China grants 2006AA020703 and 2006AA06Z409, the National 973 Program of China grant 2009CB522603, and the National Key Programs for Infectious Diseases of China grants 2008ZX10004-002, 2008ZX10004-009, and 2009ZX10004-108.

## REFERENCES

- Carlin, A. F., A. L. Lewis, A. Varki, and V. Nizet. 2007. Group B streptococcal capsular sialic acids interact with siglecs (immunoglobulin-like lectins) on human leukocytes. *J. Bacteriol.* **189**:1231–1237.
- Cha, J. S., and D. A. Cooksey. 1991. Copper resistance in *Pseudomonas syringae* mediated by periplasmic and outer membrane proteins. *Proc. Natl. Acad. Sci. U. S. A.* **88**:8915–8919.
- Chasteen, T. G., D. E. Fuentes, J. C. Tantalean, and C. C. Vasquez. 2009. Tellurite: history, oxidative stress, and molecular mechanisms of resistance. *FEMS Microbiol. Rev.* **33**:820–832.
- Feng, L., P. R. Reeves, R. Lan, Y. Ren, C. Gao, Z. Zhou, Y. Ren, J. Cheng, W. Wang, J. Wang, W. Qian, D. Li, and L. Wang. 2008. A recalibrated molecular clock and independent origins for the cholera pandemic clones. *PLoS One* **3**:e4053.
- Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J. F. Tomb, B. A. Dougherty, J. M. Merrick, K. McKenney, G. Sutton, W. Fitzhugh, C. Fields, J. D. Gocayne, J. Scott, R. Shirley, L. I. Liu, A. Glodek, J. M. Kelley, J. F. Weidman, C. A. Phillips, T. Spriggs, E. Hedblom, M. D. Cotton, T. R. Utterback, M. C. Hanna, D. T. Nguyen, D. M. Saudek, R. C. Brandon, L. D. Fine, J. L. Fritchman, J. L. Fuhrmann, N. S. Geoghegan, C. L. Gnehm, L. A. McDonald, K. V. Small, C. M. Fraser, H. O. Smith, and J. C. Venter. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* **269**:496–512.
- Gordon, D., C. Abajian, and P. Green. 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* **8**:195–202.
- Gupta, A., K. Matsui, J. F. Lo, and S. Silver. 1999. Molecular basis for resistance to silver cations in *Salmonella*. *Nat. Med.* **5**:183–188.
- Hoffmann, H., S. Stndl, W. Ludwig, A. Stumpf, A. Mehlen, J. Heesemann, D. Monget, K. H. Schleifer, and A. Roggenkamp. 2005. Reassignment of *Enterobacter dissolvens* to *Enterobacter cloacae* as *E. cloacae* subspecies *dissolvens* comb. nov. and emended description of *Enterobacter asburiae* and *Enterobacter kobei*. *Syst. Appl. Microbiol.* **28**:196–205.
- John, J. F., Jr., R. J. Sharbaugh, and E. R. Bannister. 1982. *Enterobacter cloacae*: bacteremia, epidemiology, and antibiotic resistance. *Rev. Infect. Dis.* **4**:13–28.
- Kaur, P., and B. P. Rosen. 1992. Plasmid-encoded resistance to arsenic and antimony. *Plasmid* **27**:29–40.
- Keller, R., M. Z. Pedroso, R. Ritchmann, and R. M. Silva. 1998. Occurrence of virulence-associated properties in *Enterobacter cloacae*. *Infect. Immun.* **66**:645–649.
- Lewis, A. L., N. Desa, E. E. Hansen, Y. A. Knirel, J. I. Gordon, P. Gagneux, V. Nizet, and A. Varki. 2009. Innovations in host and microbial sialic acid biosynthesis revealed by phylogenomic prediction of nonulosonic acid structure. *Proc. Natl. Acad. Sci. U. S. A.* **106**:13552–13557.
- Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y. J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Iرزک, S. C. Jando, M. L. Alenquer, T. P. Jarvie, K. B. Jirage, J. B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
- Misra, T. K. 1992. Bacterial resistances to inorganic mercury salts and organomercurials. *Plasmid* **27**:4–16.
- Sanders, W. E., and C. C. Sanders. 1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. *Clin. Microbiol. Rev.* **10**:220–241.