

Genome Sequence of *Escherichia coli* O157:H7 Strain 2886-75, Associated with the First Reported Case of Human Infection in the United States

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First identified in 1982 as a human pathogen, enterohemorrhagic *Escherichia coli* of the O157:H7 serotype is a major cause of food-borne acquired human infections. Here, we report the genome sequence of the first known strain of this serotype isolated in the United States.

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Since the initial report in 1982 that *Escherichia coli* O157:H7 is associated with severe human disease, the serotype O157:H7 has assumed a position of dominance among enterohemorrhagic *E. coli* (EHEC) serotypes in North America responsible for global widespread outbreaks of severe gastrointestinal disease (1, 2). This lineage of Shiga toxin-producing *E. coli* (STEC) O157:H7 is non-sorbitol fermenting and β -glucuronidase negative and has evolved from an O55:H7 progenitor (3, 4). The isolation in 1975 of this *E. coli* O157:H7 strain, designated 2886-75, from an adult with hemorrhagic colitis (HC) (5, 6) predated the 1982 Oregon and Michigan hamburger-associated *E. coli* O157:H7 outbreaks (6). Since 1982, this serogroup has emerged as the dominant cause of EHEC infections in North America. Infections typically present with symptoms of bloody diarrhea coupled with severe abdominal pain (5, 6) but can rapidly progress to life-threatening complications, such as hemolytic uremic syndrome (HUS), HC, and central nervous system failure (7–12).

Genomic DNA was subjected to Illumina sequencing using paired-end libraries with 300-bp inserts on the HiSeq platform. The draft genome was assembled with Velvet assembler (13, 14), and the IGS Annotation Engine and Manatee were used for genome annotation and visualization (15). Availability of the high-quality genome sequence enabled the determination of the pathogenome virulence state (16) and phylogenomic grouping according to established genotypic classification methods using *in-silico* and experimental assays (17–20). PCR genotyping confirmed the *stx* genotype and determined the occupancy of both the *yehV* and *wrbA* bacteriophage insertion sites (21). Strain 2886-75

has an unusual genotype. Unlike the majority of *E. coli* O157:H7 recovered from humans in the United States (22–24), this isolate is *stx*₁ positive and *stx*₂ negative. The *yehV* site is occupied by the *stx*₁ bacteriophage that is not stably integrated. Hence, the genomic architecture does not fit the emergence scenario typical of other human-pathogenic *E. coli* O157:H7 strains, and this isolate cannot be placed into clusters 1, 2, or 3 (25). However, this strain shows other typical genetic hallmarks of EHEC. Strain 2886-75 carries the lineage-specific virulence plasmid pO157 (26, 27), the T allele of the translocated intimin receptor (*tir*) (255 T>A), and a chimeric polymorphic variant of repeat region 1 (RR1) with the absence of the repeat regions RR2 and RR3, placing strain 2886-75 closest to group 8 (28). Multilocus sequence typing (MLST) (18) based on the nucleotide sequences of 15 housekeeping genes revealed that 2886-75 exhibits allele combination 23.11 (19) and belongs to the sequence type 11 (ST11) and complex/ABD group (18, 20). Strain 2886-75 is a representative of lineage I (17, 29) and clade 3.16 (30). The genome sequence presented here will be a valuable resource in studying *E. coli* O157:H7 pathogenome evolution by comparing this isolate to the extant genotypes and will aid in the development of a higher-resolution phylogenomic framework for improved molecular-guided pathogen surveillance and outbreak investigations (10, 11, 31).

Nucleotide sequence accession number. This genome sequence is deposited in GenBank under the accession number AVRR000000000. A bacterial strain culture is available from the Biodefense and Emerging Infections Research Resources Repository (<http://www.beiresources.org/>).

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REFERENCES

- Lowe RM, Baines D, Selinger LB, Thomas JE, McAllister TA, Sharma R. 2009. *Escherichia coli* O157:H7 strain origin, lineage, and Shiga toxin 2 expression affect colonization of cattle. *Appl. Environ. Microbiol.* 75: 5074–5081. <http://dx.doi.org/10.1128/AEM.00391-09>.
- Beutin L. 2006. Emerging enterohaemorrhagic *Escherichia coli*, causes and effects of the rise of a human pathogen. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 53:299–305. <http://dx.doi.org/10.1111/j.1439-0450.2006.00968.x>.
- Leopold SR, Magrini V, Holt NJ, Shaikh N, Mardis ER, Cagno J, Ogura Y, Iguchi A, Hayashi T, Mellmann A, Karch H, Besser TE, Sawyer SA, Whittam TS, Tarr PI. 2009. A precise reconstruction of the emergence and constrained radiations of *Escherichia coli* O157 portrayed by backbone concatenomic analysis. *Proc. Natl. Acad. Sci. U. S. A.* 106:8713–8718. <http://dx.doi.org/10.1073/pnas.0812949106>.
- Asadulghani M, Ogura Y, Ooka T, Itoh T, Sawaguchi A, Iguchi A, Nakayama K, Hayashi T. 2009. The defective prophage pool of *Escherichia coli* O157: prophage-prophage interactions potentiate horizontal transfer of virulence determinants. *PLoS Pathog.* 5 e1000408. <http://dx.doi.org/10.1371/journal.ppat.1000408>.
- Riley LW, Remis RS, Helgeson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681–685. <http://dx.doi.org/10.1056/NEJM198303243081203>.
- Wells JG, Davis BR, Wachsmuth IK, Riley LW, Remis RS, Sokolow R, Morris GK. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clin. Microbiol.* 18:512–520.
- Riley DG, Gray JT, Loneragan GH, Barling KS, Chase CC, Jr. 2003. *Escherichia coli* O157:H7 prevalence in fecal samples of cattle from a southeastern beef cow-calf herd. *J. Food Prot.* 66:1778–1782.
- Besser RE, Griffin PM, Slutsker L. 1999. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. *Annu. Rev. Med.* 50:355–367. <http://dx.doi.org/10.1146/annurev.med.50.1.355>.
- Cimolai N, Morrison BJ, Carter JE. 1992. Risk factors for the central nervous system manifestations of gastroenteritis-associated hemolytic-uremic syndrome. *Pediatrics* 90:616–621.
- Eppinger M, Mammel MK, Leclerc JE, Ravel J, Cebula TA. 2011. Genomic anatomy of *Escherichia coli* O157:H7 outbreaks. *Proc. Natl. Acad. Sci. U. S. A.* 108:20142–20147. <http://dx.doi.org/10.1073/pnas.1107176108>.
- Eppinger M, Mammel MK, Leclerc JE, Ravel J, Cebula TA. 2011. Genome signatures of *Escherichia coli* O157:H7 isolates from the bovine host reservoir. *Appl. Environ. Microbiol.* 77:2916–2925. <http://dx.doi.org/10.1128/AEM.02554-10>.
- Chaisri U, Nagata M, Kurazono H, Horie H, Tongtawe P, Hayashi H, Watanabe T, Tapchaisri P, Chongsa-nguan M, Chaicumpa W. 2001. Localization of Shiga toxins of enterohaemorrhagic *Escherichia coli* in kidneys of paediatric and geriatric patients with fatal haemolytic uraemic syndrome. *Microb. Pathog.* 31:59–67. <http://dx.doi.org/10.1006/mpat.2001.0447>.
- Zerbino DR. 2010. Using the Velvet *de novo* assembler for short-read sequencing technologies. *Curr. Protoc. Bioinformatics* chapter 11:Unit 1115. <http://dx.doi.org/10.1002/0471250953.bi110531>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
- Galens K, Orvis J, Daugherty S, Creasy HH, Angiuoli S, White O, Wortman J, Mahurkar A, Giglio MG. 2011. The IGS standard operating procedure for automated prokaryotic annotation. *Stand. Genomic Sci.* 4:244–251. <http://dx.doi.org/10.4056/signs.1223234>.
- Besser TE, Shaikh N, Holt NJ, Tarr PI, Konkel ME, Malik-Kale P, Walsh CW, Whittam TS, Bono JL. 2007. Greater diversity of Shiga toxin-encoding bacteriophage insertion sites among *Escherichia coli* O157:H7 isolates from cattle than in those from humans. *Appl. Environ. Microbiol.* 73:671–679. <http://dx.doi.org/10.1128/AEM.01035-06>.
- Yang Z, Kovar J, Kim J, Nietfeldt J, Smith DR, Moxley RA, Olson ME, Fey PD, Benson AK. 2004. Identification of common subpopulations of non-sorbitol-fermenting, beta-glucuronidase-negative *Escherichia coli* O157:H7 from bovine production environments and human clinical samples. *Appl. Environ. Microbiol.* 70:6846–6854. <http://dx.doi.org/10.1128/AEM.70.11.6846-6854.2004>.
- Rajkhowa S, Scaria J, Garcia DL, Musser KA, Akey BL, Chang YF. 2010. Analysis of *Escherichia coli* O157 clinical isolates by multilocus sequence typing. *BMC Res. Notes* 3:343. <http://dx.doi.org/10.1186/1756-0500-3-343>.
- Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* 60: 1136–1151. <http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x>.
- Reid SD, Herbelin CJ, Bumbaugh AC, Selander RK, Whittam TS. 2000. Parallel evolution of virulence in pathogenic *Escherichia coli*. *Nature* 406: 64–67. <http://dx.doi.org/10.1038/35017546>.
- Shaikh N, Tarr PI. 2003. *Escherichia coli* O157:H7 Shiga toxin-encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. *J. Bacteriol.* 185:3596–3605. <http://dx.doi.org/10.1128/JB.185.12.3596-3605.2003>.
- Tarr PI, Neill MA, Clausen CR, Newland JW, Neill RJ, Moseley SL. 1989. Genotypic variation in pathogenic *Escherichia coli* O157:H7 isolated from patients in Washington, 1984–1987. *J. Infect. Dis.* 159:344–347.
- Jelacic S, Wobbe CL, Boster DR, Ciol MA, Watkins SL, Tarr PI, Stapleton AE. 2002. ABO and P1 blood group antigen expression and stx genotype and outcome of childhood *Escherichia coli* O157:H7 infections. *J. Infect. Dis.* 185:214–219. <http://dx.doi.org/10.1086/338480>.
- Jelacic JK, Damrow T, Chen GS, Jelacic S, Bielaszewska M, Ciol M, Carvalho HM, Melton-Celsa AR, O'Brien AD, Tarr PI. 2003. Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. *J. Infect. Dis.* 188:719–729. <http://dx.doi.org/10.1086/376999>.
- Shaikh N, Holt NJ, Johnson JR, Tarr PI. 2007. Fim operon variation in the emergence of enterohemorrhagic *Escherichia coli*: an evolutionary and functional analysis. *FEMS Microbiol. Lett.* 273:58–63. <http://dx.doi.org/10.1111/j.1574-6968.2007.00781.x>.
- Lim JY, Yoon J, Hovde CJ. 2010. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *J. Microbiol. Biotechnol.* 20:5–14.
- Lee JE, Reed J, Shields MS, Spiegel KM, Farrell LD, Sheridan PP. 2007. Phylogenetic analysis of Shiga toxin 1 and Shiga toxin 2 genes associated with disease outbreaks. *BMC Microbiol.* 7:109. <http://dx.doi.org/10.1186/1471-2180-7-109>.
- Bono JL, Keen JE, Clawson ML, Durso LM, Heaton MP, Laegreid WW. 2007. Association of *Escherichia coli* O157:H7 *tir* polymorphisms with human infection. *BMC Infect. Dis.* 7:98. <http://dx.doi.org/10.1186/1471-2334-7-98>.
- Laing CR, Buchanan C, Taboada EN, Zhang Y, Karmali MA, Thomas JE, Gannon VP. 2009. *In silico* genomic analyses reveal three distinct lineages of *Escherichia coli* O157:H7, one of which is associated with hyper-virulence. *BMC Genomics* 10:287. <http://dx.doi.org/10.1186/1471-2164-10-287>.
- Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, Mladonicky JM, Somsel P, Rudrik JT, Dietrich SE, Zhang W, Swaminathan B, Alland D, Whittam TS. 2008. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. *Proc. Natl. Acad. Sci. U. S. A.* 105:4868–4873. <http://dx.doi.org/10.1073/pnas.0710834105>.
- Eppinger M, Daugherty S, Agrawal S, Galens K, Sengamalay N, Sadzewicz L, Tallon L, Cebula TA, Mammel MK, Feng P, Soderlund R, Tarr PI, Debroy C, Dudley EG, Fraser CM, Ravel J. 2013. Whole-genome draft sequences of 26 enterohemorrhagic *Escherichia coli* O157:H7 strains. *Genome Announc.* 1(2):e00134-12. <http://dx.doi.org/10.1128/genomeA.00134-12>.