Draft Genome Sequences of *Escherichia coli* O113:H21 Strains Recovered from a Major Produce Production Region in California

Beatriz Quiñones, Jaszemyn C. Yambao, Bertram G. Lee

U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Produce Safety and Microbiology Research Unit, Albany, California, USA

**ABSTRACT**

Shiga toxin-producing *Escherichia coli* is a foodborne and waterborne pathogen and is responsible for outbreaks of human gastroenteritis. This report documents the draft genome sequences of seven O113:H21 strains recovered from livestock, wildlife, and soil samples recovered from a major agricultural region for leafy greens in California, USA.

Shiga toxin-producing *Escherichia coli* (STEC) is a foodborne and waterborne pathogen and is responsible for outbreaks of human gastroenteritis with diverse clinical spectra, ranging from watery and bloody diarrhea to hemorrhagic colitis (1–3). In some cases, the infection progresses to more severe conditions such as the hemolytic uremic syndrome, and the onset of the life-threatening disease symptoms has been associated with the production of Shiga toxins (Stx). Serotype O157:H7 has been commonly associated with the development of severe disease symptoms; however, non-O157 serotypes have been implicated in human outbreaks from waterborne and foodborne sources (3–5). In particular, serotype O113:H21 has been shown to be also responsible for cases of hemolytic uremic syndrome (6, 7). Surveillance studies have indicated cattle to be the main reservoir of O113:H21 strains lacking the adhesin intimin but still harboring Stx gene subtypes, frequently implicated in human infections and severe illnesses (8, 9). The increased detection of STEC O113:H21 and its links to severe clinical cases highlight the importance of this serotype as a relevant emerging foodborne pathogen (4, 10, 11). This report documented the draft whole-genome sequences of seven *E. coli* O113:H7 strains, previously recovered from livestock, wildlife, and soil samples collected in a major agricultural region for leafy-green production in California’s Central Coast (12).

Genomic DNA of the sequenced strains was extracted from 1 mL of overnight culture using the Wizard genomic DNA purification kit (Promega Corp., Madison, WI). The purity of the DNA was assessed by fluorometric measurement using the Quant-iT PicoGreen DNA assay kit (Invitrogen, Carlsbad, CA). For the O113:H21 strain RM7806, whole-genome sequencing and 12-kb insert paired-end 454 sequencing libraries were prepared and sequenced using the GS-FLX Genome Sequencer (Roche, Indianapolis, IN). For the remaining O113:H21 strains, whole-genome sequencing was performed on an Illumina MiSeq sequencer (Illumina, Inc., San Diego, CA). DNA sequencing libraries with 575-bp to 675-bp inserts were prepared using the KAPA LTP library preparation kit (KAPA Biosystems, Wilmington, MA). The pooled amplicon libraries were loaded into a MiSeq system and sequenced using a MiSeq reagent kit v2 with 2 × 250 cycles (Illumina, Inc.). Draft genomes were assembled using Newbler assembler (version 2.6, Roche) to generate a contig graph file (13). For each sequenced O113:H21 genome, the contig with the stx gene subtype, stx2a, was identified, and Sanger DNA sequencing of
contig-bridging amplicons was performed to close the scaffold into a single contig to identify the insertion loci of the \textit{stx\_2A} encoding prophage. The \textit{stx\_2A} in all O113:H21 strains was located downstream of the antiterminator \textit{Q} gene in the late gene region of the prophage. The average genome size in the examined strains was about 5,175,000 bp with either a 50.5\% or 50.6\% G+C content. The sequencing data of these O113:H21 strains will aid in a better understanding of the pathogenic potential of STEC strains recovered from a major produce production region in the United States.

**Accession number(s).** The whole-genome sequences have been deposited at GenBank under the accession numbers listed in Table 1.

**ACKNOWLEDGMENTS**

This material is based upon work supported in part by the U.S. Department of Agriculture (USDA), Agricultural Research Service, CRIS Project No. 2030-42000-051-00D.

We gratefully thank William G. Miller for helpful discussions on data analysis as well as Emma Yee and Steven Huynh for technical assistance with Roche 454 and Illumina MiSeq sequencing platforms, respectively.

**REFERENCES**

1. Bolton DJ. 2011. Verocytotoxigenic (Shiga toxin-producing) \textit{Escherichia coli}: virulence factors and pathogenicity in the farm to fork paradigm. Foodborne Pathog Dis 8:357–365. https://doi.org/10.1089/fpd.2010.0699.

2. Gyles CL. 2007. Shiga toxin-producing \textit{Escherichia coli}: an overview. J Anim Sci 85:E45–E62. https://doi.org/10.2527/jas.2006-508.

3. Mathusa EC, Chen Y, Enache E, Hontz L. 2010. Non-O157 Shiga-toxigenic (verocytotoxigenic) \textit{Escherichia coli}; under-rated pathogens. Crit Rev Microbiol 33:67–87. https://doi.org/10.1080/10408410601172172.

4. Bettelheim KA. 2007. The non-O157 Shiga-toxigenic (verocytotoxigenic) \textit{Escherichia coli} under-recognized pathogens. Curr Rev Microbiol 33:67–87. https://doi.org/10.1080/10408410601172172.

5. Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. 2005. Non-O157 Shiga toxin-producing \textit{Escherichia coli} infections in the United States, 1983–2002. J Infect Dis 192:1422–1429. https://doi.org/10.1086/466536.

6. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing \textit{Escherichia coli}. J Infect Dis 151:775–782. https://doi.org/10.1093/infdis/151.5.775.

7. Karmali MA, Mascareras M, Shen S, Ziebell K, Johnson S, Reid-Smith R, Isaac-Renton J, Clark C, Rahn K, Kaper JB. 2003. Association of genomic O island 122 of \textit{Escherichia coli} EDL 933 with verocytotoxin-producing \textit{Escherichia coli} seropathotypes that are linked to epidemic and/or serious disease. J Clin Microbiol 41:4930–4940. https://doi.org/10.1128/JCM.41.11.4930-4940.2003.

8. Feng PCH, Delannoy S, Lacher DW, dos Santos LF, Beutin L, Fach P, Rivas M, Hartland EL, Paton AW, Guth BEC. 2014. Genetic diversity and virulence potential of Shiga toxin-producing \textit{Escherichia coli} O113:H21 strains isolated from clinical, environmental, and food sources. Appl Environ Microbiol 80:4757–4763. https://doi.org/10.1128/AEM.01824-14.

9. Feng P, Delannoy S, Lacher DW, Bosilevac JM, Fach P. 2017. Characterization and virulence potential of serogroup O113 Shiga toxin-producing \textit{Escherichia coli} strains isolated from beef and cattle in the United States. J Food Prot 80:383–391. https://doi.org/10.3151/0362-028X.JFPP.2017.04.0699.

10. Monaghan AM, Byrne B, McDowell D, Carroll AM, McNamara EB, Bolton DJ. 2012. Characterization of farm, food, and clinical Shiga toxin-producing \textit{Escherichia coli} (STEC) O113. Foodborne Pathog Dis 9:1088–1096. https://doi.org/10.1089/fpd.2012.1257.

11. Cooley MB, Jay-Russell M, Atwill ER, Carychao D, Nguyen K, Quiñones B, Patel R, Walker S, Swimley M, Pierre-Jerome E, Gordus AG, Mandrell RE. 2013. Development of a robust method for isolation of Shiga toxin-positive \textit{Escherichia coli} (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California. PLoS One 8:e65716. https://doi.org/10.1371/journal.pone.0065716.

12. Miller WG, Yee E. 2015. Complete genome sequence of \textit{Campylobacter gracilis} ATCC 33236. Genome Announc 3(5):e01087-15. https://doi.org/10.1128/genomeA.01087-15.