AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# Draft Genome Sequences of Tetracycline-Resistant Shiga Toxin-Producing *Escherichia coli* Isolates from Food

**Khulud Alotaibi,[a,d,e] Lijun Hu,[b] Javier Revollo,[c] Jing Han,[a] Guodong Zhang,[b] Ashraf A. Khan[a]**

[a]Division of Microbiology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas, USA
[b]Center for Food Safety and Applied Nutrition, Division of Microbiology, Office of Regulatory Science, U.S. Food and Drug Administration, College Park, Maryland, USA
[c]Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas, USA
[d]University of Arkansas at Little Rock, Little Rock, Arkansas, USA
[e]Department of Biology, Jazan University, Jazan, Saudi Arabia

**ABSTRACT** Shiga toxin-producing *Escherichia coli* (STEC) is a foodborne pathogen transmitted from animal to humans through contaminated food. Here, we report the draft genome sequences of six STEC isolates (six serotypes) from food (cheese, coriander, and pea protein pellets) in different countries; these isolates were resistant to tetracycline, with MIC values ranging from $<$1.5 to 256 $\mu$g/mL.

Shiga toxin-producing *Escherichia coli* (STEC) can cause bloody diarrhea, hemolytic uremic syndrome (HUS), and hemorrhagic and even fatal colitis in human. It causes around 2.5 million acute illnesses each year globally, and the consumption of contaminated foods (such as meat, beef, milk, fruits, and vegetables) is the primary source of STEC transmission, contributing 50% of all STEC infections (1, 2). Although *E. coli* O157:H7 is the most important STEC serotype posing a public health threat, non-O157:H7 STEC serotypes, such as O26, O111, O103, and O145, have frequently been involved in sporadic cases and outbreaks in the United States and other countries (3). In addition, more than 400 serotypes of non-O157:H7 STEC have been reported, and there were at least 42 STEC outbreaks in the United States from 2006 to 2021 (4, 5). Recently, STEC has been elevated to a major global public health concern because of the growing number of infections and major outbreaks with multidrug-resistant (MDR) strains.

Here, we announce six draft genome sequences of STEC isolates sourced from cheese, coriander, and pea protein pellets (Table 1). They were obtained following the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) (6). All bacterial isolates were grown from frozen stocks, streaked onto MacConkey agar (FDA BAM media M91), and incubated overnight at 37 $\pm$ 1°C. The isolates were serotyped as O2:H1, O146:H21, NEG:+, O6:H10, O8:H30, and O103:H2 at the *E. coli* Reference Center (Department of Food Science) at the Pennsylvania State University (PA, USA), following the traditional agglutination-based method (7). The MICs were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) (8). The MIC data revealed that these strains were resistant to tetracycline; two isolates, 872416 and 886340, were categorized as highly resistant to tetracycline, with MIC values of 64 and 256 $\mu$g/mL, respectively (Table 1).

A single STEC colony isolated from a MacConkey agar plate was inoculated into LB (Luria-Bertani) broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37 $\pm$ 1°C overnight for genomic DNA preparation. Genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA libraries were constructed using a Nextera XT DNA library kit (Illumina, San Diego, CA, USA). The library concentration was assessed using a high-sensitivity (HS) kit (Agilent Technologies, Santa Clara, CA, USA) with a Qubit 4.0 fluorometer (Life Technologies, Carlsbad, CA, USA). The quality of the libraries was analyzed using a 2100

**Editor** David A. Baltrus, University of Arizona

Address correspondence to Ashraf A. Khan, Ashraf.Khan@fda.hhs.gov.
The authors declare no conflict of interest.

**Received** 8 March 2022
**Accepted** 27 May 2022
**Published** 13 June 2022

**TABLE 1** Genome features of Shiga toxin-producing *Escherichia coli* isolates sequenced in this project

| Isolate | Source | Serotype | MIC of tetracycline ($\mu$g/mL) | No. of reads | No. of contigs | Contig $N_{50}$ (bp) | Coverage (×) | G+C content (%) | NCBI accession no. | | | Illumina sequencer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | BioSample | SRA | GenBank | |
| 879916 | Cheddar cheese, USA | O2:H1 | 2 | 4,803,343 | 63 | 200,856 | 50 | 50.59 | SAMN16454162 | SRR12904588 | JADDRX000000000 | NextSeq 500 |
| 875221 | Coriander, Mexico | O146:H21 | <1.5 | 5,071,407 | 187 | 103,993 | 50 | 50.58 | SAMN16454163 | SRR12904587 | JADDRW000000000 | NextSeq 500 |
| 927095 | Blue cheese, France | NEG:+ | <1.5 | 4,680,969 | 121 | 114,771 | 50 | 50.57 | SAMN16454164 | SRR12904586 | JADDRV000000000 | NextSeq 500 |
| 872416 | Roquefort cheese, France | O6:H10 | 64 | 4,955,756 | 257 | 61,625 | 50 | 50.68 | SAMN16454165 | SRR12904585 | JADDRU000000000 | NextSeq 500 |
| 886340 | Roquefort cheese, France | O8:H30 | 256 | 5,349,033 | 247 | 65,023 | 50 | 50.55 | SAMN16454166 | SRR12904584 | JADDRT000000000 | MiSeq |
| 932783 | Pea protein pellets, Norway | O103:H2 | <2 | 5,099,373 | 310 | 90,199 | 50 | 50.51 | SAMN16454167 | SRR12904583 | JADDRS000000000 | NextSeq 500 |

Bioanalyzer instrument (Agilent Technologies). The pooled libraries for all isolates were loaded onto a flow cell and sequenced using the Illumina NextSeq 500 platform (Illumina) with a NextSeq reagent kit v2.5 (2 × 75-bp paired-end reads), except for strain 886340, which was sequenced using the Illumina MiSeq platform with a MiSeq reagent kit v2 (2 × 250-bp paired-end reads) (9). The trimming and assembly were performed using the CLC Genomics Workbench v11 (Qiagen, Germantown, MD, USA), and the quality of the genome assembly and the validity of the final genome were assessed using the quality control tool (QC report) under CLC Workbench. The draft genomes were annotated initially using Pathosystem Resources Integration Center (PATRIC) software v3.6.12, and the data were submitted to the NCBI for final annotation using the Prokaryotic Genome Annotation Pipeline (PGAP) under the accession numbers shown in Table 1 (10). Default parameters were used for all software unless otherwise specified. The average G+C content of these strains was approximately about 50.5% as estimated by the PATRIC database, which was used for annotation with default parameters. The genome sizes for these six strains ranged from 4,680,969 to 5,349,033 bp, the number of contigs ranged from 63 to 310, the $N_{50}$ values ranged from 61,625 to 200,856 bp, and the coverages were all 50× (Table 1).

**Data availability.** All sequenced genome data have been deposited at DDBJ/ENA/GenBank under BioProject accession number PRJNA545531 and BioSample accession numbers SAMN16454162, SAMN16454163, SAMN16454164, SAMN16454165, SAMN16454166, and SAMN16454167.

## ACKNOWLEDGMENTS

We thank John Sutherland, Bruce Erickson, and Huizhong Chen for critical review of the manuscript. We gratefully acknowledge the technical support provided by Bijay Khajanchi.

The opinions expressed in this paper are solely the responsibility of the authors and do not necessarily represent the official views and policy of the U.S. Food and Drug Administration (FDA) or the Department of Health and Human Services (HHS). Reference to any commercial material, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the FDA.

This project was supported by the internal National Center for Toxicological Research/FDA U.S. Food and Drug Administration (FDA) (protocol E0768401).

## REFERENCES

1. Hald T, Aspinall W, Devleesschauwer B, Cooke R, Corrigan T, Havelaar AH, Gibb HJ, Torgerson PR, Kirk MD, Angulo FJ, Lake RJ, Speybroeck N, Hoffmann S. 2016. World Health Organization estimates of the relative contributions of food to the burden of disease due to selected foodborne hazards: a structured expert elicitation. PLoS One 11:e0145839. https://doi.org/10.1371/journal.pone.0145839.
2. Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, Yeung DH, Kirk MD. 2014. Global incidence of human Shiga toxin–producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis 11:447–455. https://doi.org/10.1089/fpd.2013.1704.
3. Mellor GE, Fegan N, Duffy LL, McMillan KE, Jordan D, Barlow RS. 2016. National survey of Shiga toxin–producing *Escherichia coli* serotypes O26, O45, O103, O111, O121, O145, and O157 in Australian beef cattle feces. J Food Prot 79:1868–1874. https://doi.org/10.4315/0362-028X.JFP-15-507.
4. Center for Disease Control and Prevention (CDC). 2022. Reports of selected E. coli outbreak investigations. https://www.cdc.gov/ecoli/outbreaks.html. Accessed 10 February 2022.
5. Gyles C. 2007. Shiga toxin-producing *Escherichia coli*: an overview. J Anim Sci 85:E45–E62. https://doi.org/10.2527/jas.2006-508.
6. Feng P, Weagant SD, Jinneman K. 2011. Diarrheagenic *Escherichia coli*. *In* Food and Drug Administration (ed), Bacteriological analytical manual. FDA, Silver Spring, MD. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4a-diarrheagenic-Escherichia-coli. Accessed 18 May 2022.
7. Orskov I, Orskov F, Jann B, Jann K. 1977. Serology, chemistry, and genetics of O and K antigens of Escherichia coli. Bacteriol Rev 41:667–710. https://doi.org/10.1128/br.41.3.667-710.1977.
8. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard, 3rd ed. CLSI document M31-A3. Clinical and Laboratory Standards Institute, Wayne, PA. https://www.dbt.univr.it/documenti/Occorrenzalns/matdid/matdid485539.pdf. Accessed 22 February 2022.
9. Khan AA, Khajanchi BK, Khan SA, Elkins CA, Foley SL. 2017. Draft genome sequences of ciprofloxacin-resistant *Salmonella enterica* strains with multiple-antibiotic resistance, isolated from imported foods. Genome Announc 5:e01222-17. https://doi.org/10.1128/genomeA.01222-17.
10. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.