AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Complete Genome Sequences of Four Enterohemolysin-Positive (*ehxA*) Enterocyte Effacement-Negative Shiga Toxin-Producing *Escherichia coli* Strains

Sandra C. Lorenz,[a,b] Michael L. Kotewicz,[c] Maria Hoffmann,[a] Narjol Gonzalez-Escalona,[a] Markus Fischer,[b] Julie A. Kase[a]

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Microbiology, College Park, Maryland, USA[a]; University of Hamburg, Hamburg School of Food Science, Institute of Food Chemistry, Hamburg, Germany[b]; U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Molecular Biology, Laurel, Maryland, USA[c]

Shiga toxin-producing *Escherichia coli* (STEC) strains are important foodborne pathogens associated with human disease. Most disease-associated STEC strains carry the locus of enterocyte effacement (LEE); however, regularly LEE-negative STEC strains are recovered from ill patients. Few reference sequences are available for these isolate types. Here, we report here the complete genome sequences for four LEE-negative STEC strains.

Received 22 June 2016 **Accepted** 6 July 2016 **Published** 1 September 2016

Citation Lorenz SC, Kotewicz ML, Hoffmann M, Gonzalez-Escalona N, Fischer M, Kase JA. 2016. Complete genome sequences of four enterohemolysin-positive (*ehxA*) enterocyte effacement-negative Shiga toxin-producing *Escherichia coli* strains. Genome Announc 4(5):e00846-16. doi:10.1128/genomeA.00846-16.

Address correspondence to Sandra C. Lorenz, sanlorenz@gmx.de.

Shiga toxin-producing *Escherichia coli* (STEC) strains are major foodborne pathogens that can cause mild/bloody diarrhea and life-threatening hemolytic-uremic syndrome (HUS). To date, hundreds of STEC serotypes have been implicated in human disease, with O157:H7 being the most predominant (1). While many STEC strains associated with severe disease possess the locus of enterocyte effacement (LEE) pathogenicity island and often harbor a large EHEC virulence plasmid carrying the enterohemolysin-encoding gene, *ehxA* (2–4), sporadically, LEE-negative STEC strains are recovered from severely ill individuals (5, 6). However, only a few complete reference sequences are available for those STEC strains; thus, we sequenced the complete genomes of four *ehxA*-positive LEE-negative STEC strains isolated from foods.

Genomic DNA from each strain was isolated from overnight cultures, according to the DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA) instructions, and the DNA templates were sheared to ≥10 kbp utilizing g-TUBEs (Covaris, Inc., Woburn, MA). Genomic libraries were prepared according to the PacBio 10-kbp insert library protocol using the DNA template prep kit 1.0 and were additionally size-selected with the BluePippin size selection system (Sage Science, Inc., Beverly, MA). Libraries were sequenced on the Pacific Biosciences RS II sequencer (PacBio, Menlo Park, CA) using a P4-C2 chemistry kit on ≥3 single-molecule real-time (SMRT) cells with a 180-min collection protocol. Sequencing reads were *de novo* assembled with the PacBio Hierarchical Genome Assembly Process 3 (HGAP3.0)/Quiver software package. The resulting assemblies were confirmed with optical maps generated with 30-fold coverage on the Argus Mapping Station, according to the OpGen protocol (OpGen, Inc, Gaithersburg, MD). The closed genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/), and Ridom SeqSphere+ (Ridom GmbH, Münster, Germany) was used for *in silico* multilocus sequence type (MLST) analysis and to determine the presence of virulence genes.

TABLE 1 Metadata of STEC strains and presence of virulence genes, as identified by *in silico* analysis

Strain	Serotype	Source	Size (bp)	G+C content (%)	ST	Virulence factors and other genetic features[a]											Accession no.
						stx	*eae*	*ehxA*	*iha*	*sta1*	*astA*	K88	*iss*	*gad*	*lpfA*	*stcE*	
CFSAN004178	O36:H14	Alfalfa sprouts	5,498,453	50.6	1176	2g	−		−		+		−	+	+		CP015229
			213,847	45.8				+	−	+	−	−				−	CP012498
CFSAN004179	O136:H16	Bagged lettuce	5,213,998	50.8	329	1a	−		+		−		−	+	+		CP013662
			242,187	47.0				+	−	+	+	+				+	CP012501
CFSAN004180	O168:H8[b]	Lettuce	5,286,558	50.7	718	2a	−		+		−		+	+	+		CP015228
			225,292	46.3				+	−	+	+	+				−	CP012500
CFSAN004181	O168:H8	Ground beef	5,233,459	50.8	718	2a	−		−		−		+	+	+		CP013663
			223,952	46.3				+	−	+	+	+				−	CP012499

[a] *stx*, Shiga toxin variant; *eae*, intimin; *iha*, IrgA homologue adhesin; *sta1*, heat-stable enterotoxin; *astA*, enteroaggregative heat-stable enterotoxin; K88, fimbriae; *iss*, increased serum survival; *gad*, glutamate decarboxylase; *lpfA*, long polar fimbriae; *stcE*, metalloprotease.
[b] H-type identified *in silico*, initially reported as O168:HNT.

The closed chromosomes of these STEC strains varied in size from 5.2 to 5.5 Mb, with an average G+C content of 50.7%, similar to those results found in other STEC strains (7). These STEC strains belonged to three sequence types (ST), and all carried one exceptionally large >200-kb virulence plasmid (8) (Table 1). Although these strains were isolated from foods and belong to serogroups that are rarely implicated in human disease (1), *in silico* analysis identified the enterotoxin-encoding genes *sta1* and *astA*, both associated with the development of diarrhea (9). Furthermore, two strains carried the highly HUS-associated stx_{2a} variant, one carried stx_{1a}, and one carried stx_{2g}; the role of stx2g in human pathogenicity remains to be elucidated (10). Additionally, these STEC strains carry genes encoding K88 fimbriae, long polar fimbriae (Lpf), the Iha adhesin, and/or the metalloprotease StcE (Table 1), which presumably enable these STEC strains to colonize the human gut (11–13). Moreover, all carry the glutamate decarboxylase gene *gad*, enabling these organisms to survive gastric acidity (14).

Trace-back analysis is crucial during investigations of foodborne illness outbreaks. The data provided can aid in future efforts to identify the source of infection when matching clinical, food, and environmental isolates. The availability of complete genome sequences will further contribute to the ongoing investigation of genetic differences among various pathogenic *E. coli* strains.

Accession number(s). The closed and annotated chromosome and plasmid sequences were deposited in GenBank and are listed in Table 1.

ACKNOWLEDGMENTS

This project was supported by an appointment (to S.C.L.) to the Research Fellowship Program for the Center for Food Safety and Applied Nutrition administered by the Oak Ridge Associated Universities through a contract with the FDA. This project is further part of the PhD program (S.C.L.) at the University of Hamburg, Germany, under the supervision of Markus Fischer.

REFERENCES

1. **Bettelheim KA**. 2007. The non-O157 Shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. Crit Rev Microbiol **33:**67–87. http://dx.doi.org/10.1080/10408410601172172.
2. **McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB**. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc Natl Acad Sci U S A **92:**1664–1668. http://dx.doi.org/10.1073/pnas.92.5.1664.
3. **Beutin L, Aleksic S, Zimmermann S, Gleier K**. 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. Med Microbiol Immunol **183:**13–21. http://dx.doi.org/10.1007/BF00193627.
4. **Johnson TJ, Nolan LK**. 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. Microbiol Mol Biol Rev **73:**750–774. http://dx.doi.org/10.1128/MMBR.00015-09.
5. **Beutin L, Martin A**. 2012. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. J Food Prot **75:**408–418. http://dx.doi.org/10.4315/0362-028X.JFP-11-452.
6. **Paton AW, Woodrow MC, Doyle RM, Lanser JA, Paton JC**. 1999. Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic-uremic syndrome. J Clin Microbiol **37:**3357–3361.
7. **Cooper KK, Mandrell RE, Louie JW, Korlach J, Clark TA, Parker CT, Huynh S, Chain PS, Ahmed S, Carter MQ**. 2014. Comparative genomics of enterohemorrhagic *Escherichia coli* O145:H28 demonstrates a common evolutionary lineage with *Escherichia coli* O157:H7. BMC Genomics **15:** 17. http://dx.doi.org/10.1186/1471-2164-15-17.
8. **Lorenz SC, Monday SR, Hoffmann M, Fischer M, Kase JA**. 2016. Plasmids from Shiga toxin-producing *Escherichia coli* strains with rare enterohemolysin gene (ehxA) subtypes reveal pathogenicity potential and display a novel evolutionary path. Appl Environ Microbiol, in press.
9. **Weiglmeier PR, Rösch P, Berkner H**. 2010. Cure and curse: *E. coli* heat-stable enterotoxin and its receptor guanylyl cyclase C. Toxins **2:**2213–2229. http://dx.doi.org/10.3390/toxins2092213.
10. **Persson S, Olsen KE, Ethelberg S, Scheutz F**. 2007. Subtyping method for *Escherichia coli* Shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. J Clin Microbiol **45:**2020–2024. http://dx.doi.org/10.1128/JCM.02591-06.
11. **Doughty S, Sloan J, Bennett-Wood V, Robertson M, Robins-Browne RM, Hartland EL**. 2002. Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. Infect Immun **70:** 6761–6769. http://dx.doi.org/10.1128/IAI.70.12.6761-6769.2002.
12. **Deneke CF, McGowan K, Larson AD, Gorbach SL**. 1984. Attachment of human and pig (K88) enterotoxigenic *Escherichia coli* strains to either human or porcine small intestinal cells. Infect Immun **45:**522–524.
13. **Grys TE, Siegel MB, Lathem WW, Welch RA**. 2005. The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells. Infect Immun **73:**1295–1303. http://dx.doi.org/10.1128/IAI.73.3.1295-1303.2005.
14. **Castanie-Cornet M-P, Penfound TA, Smith D, Elliott JF, Foster JW**. 1999. Control of acid resistance in *Escherichia coli*. J Bacteriol **181:** 3525–3535.