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GENOME SEQUENCES

Complete Genome Sequences of Three Shiga Toxin-Producing *Escherichia coli* O111:H8 Strains Exhibiting an Aggregation Phenotype

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ABSTRACT Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains are a common source of foodborne illness. STEC O111 is among the most prevalent non-O157 STEC serogroups. Few completed genomes of STEC O111 strains have been reported to date. We report here the complete genomic sequences of three O111:H8 strains that display a distinct aggregation phenotype.

Foodborne infections with Shiga toxin-producing *Escherichia coli* (STEC) strains are a global burden, being responsible worldwide for nearly 3 million cases of acute illness annually (1). Non-O157 STEC infections cause nearly 113,000 illnesses yearly in the United States (2), with STEC O111 infections accounting for about 19% of these illness cases between 2000 and 2010 (3). Many STEC O111 infections in the United States are sporadic; however, there have been 15 well-documented STEC O111 outbreaks in the United States since 1999 (4, 5), several of which were at least partially associated with produce (6–9). This announcement describes the complete genome sequences of three STEC O111 strains. Strains RM9131 and RM9975 were isolated in a major agricultural region for vegetable production in California from the intestinal contents of a cow and an American crow, respectively. Strain SD134209 was isolated from a patient with bloody diarrhea during an outbreak at a daycare facility in South Dakota (10). These three strains exhibited a strong aggregative phenotype characterized by the settlement of large bacterial assemblages in broth culture with agitation, a behavior commonly exhibited by environmental and outbreak STEC O111 isolates (11).

A single colony for each STEC O111 strain was grown overnight on LB agar plates at 37°C, and genomic DNA was extracted using a sucrose-Tris method, followed by phenol-chloroform cleanup, as described previously (12). Sequencing was performed using a Pacific Biosciences RS II platform (Menlo Park, CA), with 20-kb SMRTbell libraries, and assembled using PacBio HGAP version 3.0 in the SMRT Analysis package (version 2.3.0), as described previously (13). Additionally, sequencing was performed on a MiSeq platform (Illumina, Inc., San Diego, CA) with an LTP library preparation kit (Kapa Biosystems, Wilmington, MA), a MiSeq reagent kit version 2 (Illumina, Inc.), and 2 × 250-bp cycles, as described previously (13). A final base call validation of the PacBio contigs was performed using Illumina MiSeq reads trimmed using a quality score threshold of 20 or higher (≥Q20) and the reference assembler within Geneious (version 11.1; Biomatters, Ltd., Auckland, New Zealand). Protein-, rRNA-, and tRNA-coding genes were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (14).

These three STEC O111:H8 strains exhibiting an aggregation phenotype were sequenced and shown to possess wild-type *rpoS* genes and curli operons, which are required for their aggregative behavior (15). The plasmid pAA and aggregative adher-

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TABLE 1 Sequencing metrics and genomic data for *Escherichia coli* strains RM9131, RM9975, and SD134209

Feature	RM9131	RM9975	SD134209
Source	Cattle feces	American crow	Human
BioProject no.	PRJNA448153	PRJNA448155	PRJNA473579
SRA accession no.	SRP161702	SRP161706	SRP161696
Sequencing metrics			
Illumina MiSeq platform			
No. of reads	2,672,184	2,762,958	4,365,584
Avg length (bases)	223	224	200
Coverage (×)	113	119	154
PacBio platform			
No. of reads	133,152	117,271	255,006
Avg length (bases)	7,246	7,335[a]	6,881
Coverage (×)	178	159	318
Genomic data			
Size (bp)[a]			
Chromosome	5,238,870	5,233,637	5,359,785
plasmid1	117,123	120,253	188,342
plasmid2	76,698	78,526	78,329
GenBank accession no.			
Chromosome	CP028429	CP028432	CP029692
plasmid1	CP028430	CP028433	CP029690
plasmid2	CP028431	CP028434	CP029691
G+C content (%)	50.6	50.6	50.6
No. of CDS[a]	5,306	5,313	5,557
Plasmid-carried genes			
plasmid1	Phage genes	Phage genes	*aph6 str tet*(B)[b]
plasmid2	Hemolysin	Hemolysin	Hemolysin

[a] Numbers do not include pseudogenes; CDS, coding sequences.
[b] *aph6*, APH(6)-I family aminoglycoside *O*-phosphotransferase gene; *str*, streptomycin phosphotransferase gene; *tet*(B), tetracycline efflux transporter gene.

ence fimbriae (AAF)-encoding genes, characteristic of enteroaggregative *E. coli* (16), were absent from all three STEC O111:H8 genomes. Each strain possessed a plasmid containing a hemolysin gene similar to that in other STEC strains (17). All three strains harbored a stx_1-containing bacteriophage located adjacent to the transfer-messenger RNA (tmRNA) gene *ssrA*. The clinical isolate SD134209 also possessed a stx_2-containing bacteriophage adjacent to the $tRNA^{Arg}$ gene *argW*, while neither RM9131 nor RM9975 carried any bacteriophage adjacent to *argW* or stx_2 within the genome. The sequencing data of these STEC strains will add to our understanding of STEC O111 genomics, biology, and epidemiology and provide references for other STEC O111 sequencing projects.

Data availability. The chromosomal and plasmid sequences for each strain have been deposited in GenBank, and the accession numbers are listed in Table 1.

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REFERENCES

1. 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.
2. Batz MB, Hoffmann S, Morris JG, Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J Food Prot 75:1278–1291. https://doi.org/10.4315/0362-028X.JFP-11-418.
3. Gould LH, Mody RK, Ong KL, Clogher P, Cronquist AB, Garman KN, Lathrop S, Medus C, Spina NL, Webb TH, White PL, Wymore K, Gierke RE, Mahon BE, Griffin PM, Emerging Infections Program FoodNet Working Group. 2013. Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. Foodborne Pathog Dis 10:453–460. https://doi.org/10.1089/fpd.2012.1401.
4. Hlavsa MC, Roberts VA, Kahler AM, Hilborn ED, Mecher TR, Beach MJ, Wade TJ, Yoder JS. 2015. Outbreaks of illness associated with recreational water—United States, 2011–2012. MMWR Morb Mortal Wkly Rep 64:668–672.
5. Luna-Gierke RE, Griffin PM, Gould LH, Herman K, Bopp CA, Strockbine N,

Mody RK. 2014. Outbreaks of non-O157 Shiga toxin-producing *Escherichia coli* infection: USA. Epidemiol Infect 142:2270–2280. https://doi.org/10.1017/S0950268813003233.
6. California Department of Public Health. 2016. Environmental investigation of an Escherichia coli O111:NM outbreak in October 2015 associated with unpasteurized apple cider. California Department of Public Health, Sacramento, CA. https://www.cdph.ca.gov/Programs/CEH/DFDCS/CDPH%20Document%20Library/FDB/FoodSafetyProgram/EnvInvReports/fdbEIRHH2015.pdf.
7. Brooks JT, Bergmire-Sweat D, Kennedy M, Hendricks K, Garcia M, Marengo L, Wells J, Ying M, Bibb W, Griffin PM, Hoekstra RM, Friedman CR. 2004. Outbreak of Shiga toxin-producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis 38:190–198. https://doi.org/10.1086/380634.
8. Meyer S. 2015. *E. coli* O111 outbreak associated with green cabbage served at restaurants in Minnesota and Wisconsin—July 2014. Annual Meeting of the Association of Public Health Laboratories, 18 to 21 May 2015, Indianapolis, IN. https://www.aphl.org/conferences/proceedings/Documents/2015/InFORM/039-MeyerS.pdf.
9. Schaffzin JK, Coronado F, Dumas NB, Root TP, Halse TA, Schoonmaker-Bopp DJ, Lurie MM, Nicholas D, Gerzonich B, Johnson GS, Wallace BJ, Musser KA. 2012. Public health approach to detection of non-O157 Shiga toxin-producing *Escherichia coli*: summary of two outbreaks and laboratory procedures. Epidemiol Infect 140:283–289. https://doi.org/10.1017/S0950268811000719.
10. Carlson C. 2002. Investigation of an Escherichia coli O111:NM outbreak in a daycare in South Dakota, session 22, board 80. International Conference on Emerging Infectious Diseases, 24 to 27 March 2002, Atlanta, GA.
11. Diodati ME, Bates AH, Cooley MB, Walker S, Mandrell RE, Brandl MT. 2015. High genotypic and phenotypic similarity among Shiga toxin-producing *Escherichia coli* O111 environmental and outbreak strains. Foodborne Pathog Dis 12:235–243. https://doi.org/10.1089/fpd.2014.1887.
12. Miller WG, On SL, Wang G, Fontanoz S, Lastovica AJ, Mandrell RE. 2005. Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*. J Clin Microbiol 43:2315–2329. https://doi.org/10.1128/JCM.43.5.2315-2329.2005.
13. Parker CT, Cooper KK, Huynh S, Smith TP, Bono JL, Cooley M. 2018. Genome sequences of eight Shiga toxin-producing *Escherichia coli* strains isolated from a produce-growing region in California. Microbiol Resour Announc 7:e00807-18. https://doi.org/10.1128/MRA.00807-18.
14. 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.
15. Diodati ME, Bates AH, Miller WG, Carter MQ, Zhou Y, Brandl MT. 2016. The polymorphic aggregative phenotype of Shiga toxin-producing *Escherichia coli* O111 depends on RpoS and curli. Appl Environ Microbiol 82:1475–1485. https://doi.org/10.1128/AEM.03935-15.
16. Nataro JP. 2005. Enteroaggregative *Escherichia coli* pathogenesis. Curr Opin Gastroenterol 21:4–8.
17. Ogura Y, Ooka T, Iguchi A, Toh H, Asadulghani M, Oshima K, Kodama T, Abe H, Nakayama K, Kurokawa K, Tobe T, Hattori M, Hayashi T. 2009. Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. Proc Natl Acad Sci U S A 106:17939–17944. https://doi.org/10.1073/pnas.0903585106.