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## Complete Genome Sequence of a Natural *Escherichia coli* 0145:H11 Isolate That Belongs to Phylogroup A

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**ABSTRACT** Escherichia coli O145:H11 strain RM14721 was originally isolated from wildlife feces near a leafy greens-growing region in Yuma, AZ. This strain was initially positive for  $stx_1$ ; however, in subsequent cultures,  $stx_1$  was not detected by PCR. Here, we report the complete genome sequence and annotation of RM14721.

**T** he *Escherichia coli* species comprises commensal strains residing naturally in intestinal tracts of their mammalian hosts and pathogenic strains causing diverse intestinal and extraintestinal infections in humans and animals (1, 2). Shiga toxinproducing *Escherichia coli* (STEC) serotype O145, one of the major non-O157 serotypes associated with severe human disease (3, 4), has evolved independently via multiple lineages. Strains linked to the 2010 romaine lettuce-associated outbreak of O145 infections (5) and to the 2007 ice-cream-associated outbreak of O145 and O26 coinfections (6, 7) have a *fli*CH28 antigen, share an evolutionary lineage with STEC O157:H7, and belong to phylogroup E (8, 9). However, STEC O145:H25 is genetically more similar to other non-O157 STEC strains, including O111 strain 11128 and O26 strain 11368, which are both in phylogroup B1, than to O145:H28 (10). Strain RM14721 was isolated from wildlife feces near a leafy greens-growing region in Yuma, AZ (11). This strain was initially positive for *stx*<sub>1</sub> genes by PCR; however, in the subsequent cultures, *stx*<sub>1</sub> genes were not detected.

Single-molecule real-time (SMRT) sequencing was performed on a PacBio RS II instrument using the protocol "Procedure & Checklist—Greater Than 10 kb Template Preparation Using AMPure PB Beads" (12), followed with template binding using P6v2 sequencing polymerase and MagBeads. The SMRTbell sequencing libraries were prepared using 8  $\mu$ g of sheared DNA and the SMRTbell template prep kit 3.0. The SMRT cells were run with a 0.1 nM on-plate concentration, P6/C4 sequencing chemistry, the MB1percv1 collection protocol, and a 360-min data collection mode. A FASTQ file was generated using SMRT Analysis (version 2.3.0), and assembly was performed with RS\_HGAP\_Assembly.3. The complete genome sequence was submitted to GenBank for annotation using the Prokaryotic Genome Annotation Pipeline.

The genome of *E. coli* strain RM14721 is composed of a 4,620,018-bp chromosome and a 106,432-bp plasmid, encoding 4,870 coding sequences (CDSs), 22 rRNAs, and 86 tRNAs. The average GC contents of the chromosome and plasmid are 50.9% and 49.9%, respectively. The serotype of strain RM14721 was confirmed to be O145:H11 by a BLAST search of *E. coli* O-antigen and H-antigen databases (13, 14). The sequence type (ST) was determined to be ST1155 using the Michigan scheme (http://www.shigatox.net/ecmlst/cgi-bin/scheme) and ST48 using the Warwick scheme (15). *In silico* phylotyping using the Clermont method (16) placed this strain in phylogroup A. Consistently, the multilocus sequence typing (MLST)-based phylogenetic analysis clustered strain RM14721 with other *E. coli* A strains, including REL606 and enterotoxigenic *E. coli* (ETEC) strain H10407 when more than 400 *E. coli* strains with a complete genome available in GenBank as of January 2018 were included in the analysis. No homologs of genes

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This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Michelle Qiu Carter, michelle.carter@ars.usda.gov. encoding the heat-labile (LT) or heat-stabile (ST) enterotoxin were identified in RM14721. PHASTER (17, 18) analysis detected three intact prophages on the chromosome, spanning chromosomal positions 484362 to 507958 (23.5 kb), 1617891 to 1653895 (36.0 kb), and 2334672 to 2375071 (40.4 kb). Although no Shiga toxin genes were identified in the genome of RM14721, several putative integration sites for Stx-converting phages were identified, including AU5Stx1 (GenBank accession number KU977419), PA2 (Stx2a) (NCBI reference sequence NC\_028449), and 1717 (Stx2c) (NCBI reference sequence NC\_011357), indicating the potential of strain RM14721 to evolve into an STEC strain.

Accession number(s). The genome sequence of *E. coli* O145:H11 strain RM14721 was deposited in GenBank under the accession numbers CP027105 (chromosome) and CP027106 (plasmid).

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## REFERENCES

- Tenaillon O, Skurnik D, Picard B, Denamur E. 2010. The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol 8:207–217. https://doi.org/10.1038/nrmicro2298.
- 2. Nataro JP, Kaper JB. 1998. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11:142–201.
- Mathusa EC, Chen Y, Enache E, Hontz L. 2010. Non-O157 Shiga toxinproducing *Escherichia coli* in foods. J Food Prot 73:1721–1736. https:// doi.org/10.4315/0362-028X-73.9.1721.
- Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis 192:1422–1429. https://doi.org/10.1086/466536.
- Taylor EV, Nguyen TA, Machesky KD, Koch E, Sotir MJ, Bohm SR, Folster JP, Bokanyi R, Kupper A, Bidol SA, Emanuel A, Arends KD, Johnson SA, Dunn J, Stroika S, Patel MK, Williams I. 2013. Multistate outbreak of *Escherichia coli* O145 infections associated with romaine lettuce consumption, 2010. J Food Prot 76:939–944. https://doi.org/10.4315/0362 -028X.JFP-12-503.
- Buvens G, Possé B, De Schrijver K, De Zutter L, Lauwers S, Piérard D. 2011. Virulence profiling and quantification of verocytotoxin-producing *Escherichia coli* 0145:H28 and O26:H11 isolated during an ice creamrelated hemolytic uremic syndrome outbreak. Foodborne Pathog Dis 8:421–426. https://doi.org/10.1089/fpd.2010.0693.
- De Schrijver K, Buvens G, Posse B, Van den Branden D, Oosterlynck O, De Zutter L, Eilers K, Pierard D, Dierick K, Van Damme-Lombaerts R, Lauwers C, Jacobs R. 2008. Outbreak of verocytotoxin-producing *E. coli* 0145 and 026 infections associated with the consumption of ice cream produced at a farm, Belgium, 2007. Euro Surveill 13:8041. https://doi.org/10.2807/ ese.13.07.08041-en.
- 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. https://doi.org/10.1186/1471-2164-15-17.
- Cooper KK, Mandrell RE, Louie JW, Korlach J, Clark TA, Parker CT, Huynh S, Chain PS, Ahmed S, Carter MQ. 2014. Complete genome sequences of two *Escherichia coli* 0145:H28 outbreak strains of food origin. Genome Announc 2:e00482-14. https://doi.org/10.1128/genomeA.00482-14.

- Lorenz SC, Gonzalez-Escalona N, Kotewicz ML, Fischer M, Kase JA. 2017. Genome sequencing and comparative genomics of enterohemorrhagic *Escherichia coli* 0145:H25 and 0145:H28 reveal distinct evolutionary paths and marked variations in traits associated with virulence & colonization. BMC Microbiol 17:183. https://doi.org/10 .1186/s12866-017-1094-3.
- Carter MQ, Quinones B, He X, Zhong W, Louie JW, Lee BG, Yambao JC, Mandrell RE, Cooley MB. 2015. An environmental Shiga toxin-producing *Escherichia coli* O145 clonal population exhibits high-level phenotypic variation that includes virulence traits. Appl Environ Microbiol 82: 1090–1101. https://doi.org/10.1128/AEM.03172-15.
- Pacific Biosciences. 2018. Procedure & checklist—greater than 10 kb template preparation using AMPure PB beads. Pacific Biosciences, Menlo Park, CA. https://www.pacb.com/wp-content/uploads/Procedure-Checklist -Greater-Than-10-kb-Template-Preparation-Using-AMPure-PB-Beads-1.pdf.
- Iguchi A, Iyoda S, Kikuchi T, Ogura Y, Katsura K, Ohnishi M, Hayashi T, Thomson NR. 2015. A complete view of the genetic diversity of the *Escherichia coli* O-antigen biosynthesis gene cluster. DNA Res 22: 101–107. https://doi.org/10.1093/dnares/dsu043.
- Wang L, Rothemund D, Curd H, Reeves PR. 2003. Species-wide variation in the *Escherichia coli* flagellin (H-antigen) gene. J Bacteriol 185: 2936–2943. https://doi.org/10.1128/JB.185.9.2936-2943.2003.
- Clermont O, Gordon D, Denamur E. 2015. Guide to the various phylogenetic classification schemes for *Escherichia coli* and the correspondence among schemes. Microbiology 161:980–988. https://doi.org/10 .1099/mic.0.000063.
- Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58–65. https://doi.org/10.1111/1758-2229.12019.
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.
- Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39:W347–W352. https://doi.org/10 .1093/nar/gkr485.