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PROKARYOTES

Complete Genome Sequences of Two Atypical Enteropathogenic *Escherichia coli* O145 Environmental Strains

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ABSTRACT *Escherichia coli* O145 strains RM14715 and RM14723 were isolated from wildlife feces near a leafy greens-growing region in Yuma, Arizona. Both strains carry a distinct genotype compared with the *E. coli* O145 strains isolated from Salinas Valley, California. Here we report complete genome sequences and annotations of RM14715 and RM14723.

Evolution of Shiga toxin-producing *Escherichia coli* (STEC) has been postulated as a model of stepwise acquisition and/or loss of virulence and phenotypic traits (1, 2). For example, *E. coli* O157:H7, a prototype of STEC, evolved from enteropathogenic *E. coli* (EPEC) O55:H7 via acquisition of Shiga toxin genes and a large virulence plasmid, followed by the O-antigen switch. We previously showed that STEC O145:H28 shares a common evolutionary lineage with STEC O157/EPEC O55 and belongs to phylogroup E (3, 4). A recent study reported that STEC O145:H25 is genetically more similar to other non-O157 STEC strains of phylogroup B1 than to O145:H28 (5), suggesting a parallel evolution of STEC O145. Strains RM14715 and RM14723, both isolated from a leafy greens-growing region in Yuma, Arizona, exhibit a distinct genotype compared with the STEC O145:H28 strains (6). To better understand the diversity of *E. coli* O145, we sequenced the genomes of RM14715 and RM14723.

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" (7), followed with template binding using P6 v2 sequencing polymerase and Magbeads. The SMRTbell sequencing libraries were prepared using 7 μg of sheared DNA and a SMRTbell template prep kit 3.0. The SMRT cells were run with a 0.1-nM on-plate concentration, P6/C4 sequencing chemistry, MagBeadOneCellPerWell v1 collection protocol, and 360-min data collection mode. A FASTQ file was generated using SMRT Analysis (v 2.3.0), and assembly was done with RS_HGAP_Assembly.3. The complete genome sequences were submitted to GenBank for annotation using the NCBI Prokaryotic Genome Annotation Pipeline.

The *E. coli* RM14715 genome is composed of a 4,825,089-bp chromosome, encoding 4,947 coding sequences (CDSs), 22 rRNAs, and 88 tRNAs. The *E. coli* RM14723 genome is composed of a 4,754,025-bp chromosome, encoding 4,826 CDSs, 22 rRNAs, and 88 tRNAs. The average GC contents for both genomes are 50.6%. The serotypes of both strains were confirmed to be O145:H34 by BLAST searches of *E. coli* O-antigen and H-antigen databases (8, 9). The sequence types (STs) of RM14715 and RM14723 are ST32 and ST620, respectively, using the Michigan scheme (6), and ST1877 and ST35, respectively, using the Warwick scheme (10). *In silico* phylotyping using the Clermont method (11) placed both strains in phylogroup B2. Multilocus sequence type (MLST)-based phylogenetic analyses grouped the two strains to a separate cluster that shares a common evolutionary lineage with EPEC strains, including E2348/69 (GenBank accession number NC_011601) and avian pathogenic *Escherichia coli* (APEC) strains, including 018 (NZ_CP006830). A total of 4,369 CDSs are conserved between the two

Received 6 April 2018 **Accepted** 7 April 2018 **Published** 10 May 2018

Citation Carter MQ, Pham A. 2018. Complete genome sequences of two atypical enteropathogenic *Escherichia coli* O145 environmental strains. Genome Announc 6:e00418-18. https://doi.org/10.1128/genomeA.00418-18.

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genomes, while 578 and 457 CDSs are specific to RM14715 and RM14723, respectively. Prophages contributed largely to the strain-specific CDSs in both genomes. Among the 15 intact prophages identified by PHASTER (12, 13) in RM14715 ($n = 7$) and RM14723 ($n = 8$), only 2 are common. Strains RM14715 and RM14723 are classified as atypical EPEC strains since they each contain a complete locus of enterocyte effacement (LEE) pathogenicity island, but lack an *E. coli* adherence factor (EAF) plasmid (14). Furthermore, several putative integration sites for Stx-converting phages are conserved in both genomes, implying their potential to evolve to STEC O145:H34.

Accession number(s). The *E. coli* O145:H34 strain RM14715 and RM14723 genome sequences were deposited in GenBank under the accession numbers CP027104 and CP027103, respectively.

ACKNOWLEDGMENTS

This research work was supported by USDA-ARS CRIS 2030-42000-050-00D.

We thank Robert Mandrell for providing the strains RM14715 and RM14723.

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