



# Fecal Metagenome Sequences from Lactating Dairy Cows Shedding *Escherichia coli* O157:H7

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**ABSTRACT** Cattle are primary reservoirs of *Escherichia coli* O157:H7, a causative agent of severe human infections. To facilitate analyses of the communities in which this pathogen is found, we sequenced the fecal metagenomes of 10 dairy cows shedding *E. coli* O157:H7 and added them to the public domain.

Shiga toxin-producing *Escherichia coli* (STEC) species are major zoonotic bacterial pathogens responsible for foodborne infections worldwide, and *Escherichia coli* O157:H7 is the most common etiological agent of hemolytic uremic syndrome in the United States. (1). Cattle can harbor this pathogen in their intestines without symptoms of infection and intermittently shed it in their feces. Human exposure may occur through consumption of contaminated products (milk and meat) and through cross-contamination of produce, water, wildlife, and the farm environment (2–5). In addition to several contributing factors, such as seasonal variation, nutritional condition, and host physiology, cattle gastrointestinal microbiota may have direct or indirect effects on the carriage and shedding (and super shedding) of *E. coli* O157:H7 (3, 5–8). These effects may be exerted by competitive exclusion (from mucosal adhesion sites), release of secondary metabolites, bioavailability of nutrients, or functional synergy. Understanding the ecological dynamics of *E. coli* O157:H7 in the ruminant intestine in relation to the common gut microflora is important to develop strategies for reducing shedding and mitigating human outbreaks.

During a longitudinal study of an individual commercial dairy farm and its associated offsite heifer raising operation in Pennsylvania (USA), *E. coli* O157:H7 isolates were sporadically recovered from fecal grab, manure composite, milk filter, and environmental (fly, trough water) samples (9, 10). Ten fecal samples collected from the lactating cows that were shedding *E. coli* O157:H7 were stored at  $-80^{\circ}\text{C}$  (Table 1), and the samples were later thawed for metagenome sequencing after DNA extraction using a QIAamp fast DNA stool minikit (Qiagen, Waltham, MA). Nextera DNA library preparation kits (Illumina, San Diego, CA) were used to prepare sequencing libraries of the 10 samples. Paired-end sequencing ( $2 \times 151$  bp) was conducted on an Illumina NextSeq 500 sequencing platform with a NextSeq 500/550 v2 high-output flow cell. Raw reads were demultiplexed using the BCL2FastQ program and cleaned using DeconSeq (11) and Trimmomatic v0.36 (12).

Cleaned and curated reads were used to identify bacterial, archaeal, eukaryotic, and mitochondrial small subunits (12S, 16S, and 18S) rRNA sequences with MeTaxa v2 (13). Assigned taxonomic profiles of the fecal metagenomes included *Archaea* (*Crenarchaeota* and *Euryarchaeota*), *Bacteria* (mainly *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*), and eukaryotes (Fungi, Excavata, Metazoa, Viridiplantae, and others). The metagenomic data will be used to investigate the phylogenetic and functional diversity of the fecal microflora, metabolic pathways, microbial stress and virulence factor-related gene profiles, and other potential biomarkers that may be associated with *E. coli*

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**TABLE 1** Metadata and accession numbers of deposited metagenome sequences

Sample identifier <sup>a</sup>	Sampling date (mo/day/yr)	Total no. of reads	MeTaxa2 analysis <sup>b</sup>			NCBI accession no.
			Bacteria	Archaea	Eukaryotes	
B-27-100	6/14/2010	20,884,502	20,113	474	205	SRX4547096
B-S104-6	6/30/2010	11,094,746	10,998	73	49	SRX4547097
B-27-102	6/14/2010	43,291,458	51,593	1,238	72	SRX4547098
B-S104-5	6/30/2010	17,717,456	22,118	335	18	SRX4547099
B-27-111	6/14/2010	8,684,666	10,070	261	13	SRX4547086
B-S104-4	6/30/2010	73,242,474	52,393	2,075	694	SRX4547087
B-27-118	6/14/2010	105,817,744	75,028	1,618	249	SRX4547094
B-S104-3	6/30/2010	32,199,304	19,687	853	46	SRX4547095
B-27-142	6/14/2010	93,199,932	72,797	2,297	108	SRX4547091
B-S104-7	6/30/2010	23,886,090	15,971	455	37	SRX4547088

<sup>a</sup>Fecal samples from individual animals.

<sup>b</sup>Data are numbers of reads assigned to specific taxa.

O157:H7 carriage and shedding in ruminants. A detailed report of the results of comparative analyses of these metagenomes will be published elsewhere.

**Data availability.** The metagenomes of these 10 fecal samples have been deposited in GenBank under the accession numbers listed in Table 1.

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We declare no competing interests. The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

## REFERENCES

- Centers for Disease Control and Prevention. 2017. Summary of notifiable infectious diseases and conditions—United States, 2015. *MMWR Morb Mortal Wkly Rep* 64:1–143. <https://doi.org/10.15585/mmwr.mm6453a1>.
- Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of *Escherichia coli* O157: H7 outbreaks, United States, 1982–2002. *Emerg Infect Dis* 11:603. <https://doi.org/10.3201/eid1104.040739>.
- Chase-Topping M, Gally D, Low C, Matthews L, Woolhouse M. 2008. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nat Rev Microbiol* 6:904. <https://doi.org/10.1038/nrmicro2029>.
- Ferens WA, Hovde CJ. 2011. *Escherichia coli* O157: H7: animal reservoir and sources of human infection. *Foodborne Pathog Dis* 8:465–487. <https://doi.org/10.1089/fpd.2010.0673>.
- Stenkamp-Strahm C, McConnel C, Magzamen S, Abdo Z, Reynolds S. 2018. Associations between *Escherichia coli* O157 shedding and the faecal microbiota of dairy cows. *J Appl Microbiol* 124:881–898. <https://doi.org/10.1111/jam.13679>.
- Stenkamp-Strahm C, McConnel C, Rao S, Magnuson R, Hyatt DR, Linke L. 2017. Climate, lactation, and treatment factors influence faecal shedding of *Escherichia coli* O157 pathotypes in dairy cows. *Epidemiol Infect* 145:115–125. <https://doi.org/10.1017/S0950268816001928>.
- Venegas-Vargas C, Henderson S, Khare A, Mosci RE, Lehnert JD, Singh P, Ouellette L, Norby B, Funk JA, Rust S, Bartlett P. 2016. Factors associated with Shiga toxin-producing *Escherichia coli* shedding in dairy and beef cattle. *Appl Environ Microbiol* 82:5049–5056. <https://doi.org/10.1128/AEM.00829-16>.
- Xu Y, Dugat-Bony E, Zaheer R, Selinger L, Barbieri R, Munns K, McAllister TA, Selinger LB. 2014. *Escherichia coli* O157: H7 super-shedder and non-shedder feedlot steers harbour distinct fecal bacterial communities. *PLoS One* 9:e98115. <https://doi.org/10.1371/journal.pone.0098115>.
- Kim SW, Karns JS, Jas VK, Haley BJ. 2017. Genome sequences of 30 *Escherichia coli* O157: H7 isolates recovered from a single dairy farm and its associated off-site heifer-raising facility. *Genome Announc* 5:e00814-17.
- Pradhan AK, Van Kessel JS, Karns JS, Wolfgang DR, Hovingh E, Nelen KA, Smith JM, Whitlock RH, Fyock T, Ladely S, Fedorka-Cray PJ, Schukken YH. 2009. Dynamics of endemic infectious diseases of animal and human importance on three dairy herds in the northeastern United States. *J Dairy Sci* 92:1811–1825. <https://doi.org/10.3168/jds.2008-1486>.
- Schmieder R, Edwards R. 2011. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. *PLoS One* 6:e17288. <https://doi.org/10.1371/journal.pone.0017288>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bengtsson J, Eriksson KM, Hartmann M, Wang Z, Shenoy BD, Grelet GA, Abarenkov K, Petri A, Rosenblad MA, Nilsson RH. 2011. Metaxa: a software tool for automated detection and discrimination among ribosomal small subunit (12S/16S/18S) sequences of archaea, bacteria, eukaryotes, mitochondria, and chloroplasts in metagenomes and environmental sequencing datasets. *Antonie Van Leeuwenhoek* 100:471. <https://doi.org/10.1007/s10482-011-9598-6>.