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Genome Sequencing of Non-Pathogenic E. coli Approved as Pathogen Surrogates

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Objectives

For this study, the objectives were to produce whole genome sequence (WGS) data, and conduct sequence alignment, assembly, and analyses from DNA of non-pathogenic *E. coli* recognized as useful surrogates for STEC by the USDA-FSIS. In addition, a group of 3 rifampicin-resistant (RifR; 100 mg/L) mutants generated from parent isolates via a process of natural selection were also analyzed alongside the parent surrogate isolates.

Materials and Methods

Working stocks of the *E. coli* surrogates (5 wild-type isolates and 3 Rif^R mutants) were revived from –80°C cryo-storage, prepared on nutrient agar slants, and layered with mineral oil to prevent oxidative stress during storage at 5°C. Each bacterium was streaked onto MacConkey agar (MAC) and MacConkey agar + rifampicin (100.0 mg/L; MACR) to verify that isolates exhibited *E. coli*-typical appearances on media surfaces, that antimicrobial resistance was detected in RifR mutants, and to isolate bacterial colonies for DNA extraction to submit for WGS. Following this the *E. coli* surrogates and RifR mutants were grown in Luria-Bertani (LB) broth (24 h, 35°C) and then streaked onto LB agar and incubated, where an individual colony was isolated and grown for DNA extraction. A phenol/chloroform DNA extraction protocol was used to extract and purify the bacterial genomic DNA for WGS. After bacterial DNA was purified, the samples were submitted to the Texas A&M University (TAMU) Institute for Genome Sciences and Society (TIGSS) laboratory to undergo WGS via the Illumina MiSeq platform and the Oxford Nanopore MinION genomic sequencers. Once samples were sequenced they were analyzed via the TAMU Center for Phage Technology (CPT) GALAXY program.

Results

Upon sequencing, *E. coli* sequence data were analyzed via a constructed online database using the TAMU CPT GALAXY program, comparing obtained sequences to a combination of previously formed database of sequences of known bacterial virulence factors and antibiotic resistance. Pathogen surrogates will be verified to determine whether or not they in fact possess key STEC pathogenesis elements, including the locus of enterocyte effacement (LEE), Shiga toxins, hemolysin, as well as other virulence factors previously identified in members of the STEC.

Conclusion

The move by the USDA-FSIS to initiate WGS analysis of obtained isolates, particularly isolates obtained during outbreak or recall investigations, will produce a need for regulatory officers to be capable of differentiating WGS datasets from non-pathogenic organisms from disease agents. Data collected here will supplement existing or new WGS datasets by providing sequence data of *E. coli* useful for process validation and verification, allowing processors and regulatory officers to differentiate these organisms from pathogenic STEC.

