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GENOME ANNOUNCEMENT

Complete Sequences of Plasmids from the Hemolytic-Uremic Syndrome-Associated *Escherichia coli* Strain HUSEC41

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The complete and annotated sequences of four plasmids from a historical enteroaggregative Shiga toxin-producing *Escherichia coli* (HUSEC) serotype O104:H4 strain, HUSEC41/01-09591, isolated in 2001 in Germany are reported.

The *Escherichia coli* serotype O104:H4 sequence type (ST) 678 strain which caused a disease outbreak in Germany in 2011 harbors three plasmids encoding a putative autotransporter serine protease, the aggregative adherence regulator *aggR*, and the extended-spectrum beta-lactamase CTX-M-15, all of which have contributed to the evolution of this virulent strain (1, 3, 6). A subsequent study of plasmids from the historical enteroaggregative Shiga toxin-producing serotype O104:H4 strain HUSEC41, isolated in 2001 from a child presenting with hemolytic-uremic syndrome, reported the detection and partial sequence of two plasmids, 95 and 75 kb (5). Our analysis of HUSEC41, however, indicated the presence of four plasmids, with sizes of 92, 74, 8, and 5 kb. This observation prompted us to determine the complete sequences of all plasmids in strain HUSEC41. These sequences provide a backdrop for the comparative analysis of the genealogy and evolution of plasmids that have contributed to the virulence properties of the HUSEC strain responsible for the recent outbreak of 2011.

Genomic DNA was isolated as described by Pitcher et al. (7). Sequencing was performed by Vertis (Germany) on a 454 GS-FLX system. Reads were assembled *de novo* with the 454 Newbler assembler, and resulting contigs were mapped against reference plasmids to determine a plasmid context. PCR-based techniques were used to close the gaps, followed by sequencing with ABI BigDye 3.0 technology (Applied Biosystems, Germany). A total of 17 contigs were assembled in four circular replicons with an average coverage of 45×. ORF calling and a first-pass automatic annotation were performed using RAST (rast.nmpdr.org) followed by manual comparative curation (4) and sequence similarity searches versus the NCBI (www.ncbi.nlm.nih.gov/BLAST), PFAM, and IS Finder (www-is.biotoul.fr) databases.

Four plasmids were detected: pHUSEC41-1, a large conjugative IncI1-type plasmid of 91,942 bp; pHUSEC41-2, a 73,564-bp nonconjugative IncF-type plasmid; and two small plasmids of 7,930 bp and 5,153 bp, designated pHUSEC41-3 and -4, respectively.

Plasmid pHUSEC41-1 displays 131 ORFs and harbors the resistance genes for streptomycin and sulfonamides. The organization comprising *trbC*, *sul2*, *strA*, bla_{TEM-1}, and *strB* is similar to p3521, an IncB plasmid (GenBank no. GU256641), and the IncQ RSF 1010 plasmid (GenBank no. M28829) (6). The transfer region of pHUSEC41-1 includes *trb*, *tra*, and *pil*, which are most related to plasmid p026vir (GenBank no. FJ386569).

The pHUSEC41-2 IncF plasmid contains 140 coding sequences (CDS) and is related to p55989 from the enteroaggregative *E. coli* strain 55989 (GenBank no. LB226692). Unlike pHUSEC41-1, it was not transferable to *E. coli* C118 by conjugation. The pHUSEC41-2 transfer region was found to exhibit deletions (e.g., *traV*) similar to those previously seen with other IncF plasmids with impaired conjugation properties (2). Plasmid pHUSEC41-3 displays 15 ORFs. Four of these are related to plasmid ColE1 mobilization proteins (MobA to -D) (GenBank no. J01566) (8).

We found 9 CDS on the smallest plasmid pHUSEC41-4, which resembles (~70% identity) plasmid ColE1 (8) minus its mobilization module. Comobilization of pHUSEC41-3 and -1 to *E. coli* CC118 occurred with a frequency of 10^{-5} per donor cell.

Nucleotide sequence accession numbers. The plasmid sequences reported here have been deposited in the EMBL database under accession numbers HE603110 (pHUSEC41-1), HE603111 (pHUSEC41-2), HE603112 (pHUSEC41-3), and HE603113 (pHUSEC41-4).

ACKNOWLEDGEMENTS

This study was supported by grants from the Federal Ministry of Education and Research (BMBF, Germany) within the framework of the RESET research network (contract no. 01KI1013G) and the Innovation Funds of the Ministry of Science and Arts of the State of Hessen to C.I. and T.C., respectively.

We thank Christina Gerstmann and Alexandra Amend-Foerster for excellent technical assistance. Furthermore, we acknowledge the German National Reference Center for Salmonellae and Other Bacterial Enteric Pathogens for providing strain HUSEC41.

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Received 16 October 2011 **Accepted** 21 October 2011

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doi:10.1128/JB.06368-11

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