Whole-Genome Sequences of Two *Salmonella enterica* Serovar Dublin Strains That Harbor the *viaA*, *viaB*, and *ompB* Loci of the Vi Antigen

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ABSTRACT   Here, we report the genome sequences of two *Salmonella enterica* serovar Dublin strains, 03EB8736SAL and 03EB8994SAL, isolated from raw-milk cheese and milk filtrate, respectively. Analysis of the draft genomes of the two isolates reveals the presence of the *viaA*, *viaB*, and *ompB* loci of the Vi capsular polysaccharide antigen (Vi antigen).

*S*almonella enterica serovar Dublin is adapted to cattle and can be transmitted to humans via the consumption of contaminated raw milk and raw-milk cheeses (1, 2). Human infection by *S*. Dublin is primarily characterized by self-limiting gastrointestinal illness; however, a high proportion of *S*. Dublin cases are associated with systemic illness (3, 4). Fatal human outbreaks of *S*. Dublin were recently reported in France due to the consumption of raw-milk cheese (5).

Vi capsular polysaccharide antigen (Vi antigen) is commonly found in strains of human-adapted typhoidal *Salmonella enterica* serovars Typhi and Paratyphi (6). It contributes to bacterial virulence and pathogenesis (7, 8). The presence of Vi antigen within nontyphoidal *S*. Dublin strains might contribute to bacterial virulence and invasiveness. Although the Vi antigen was detected in some strains of *S*. Dublin (3, 9), it is not very common (4, 10). Expression of the Vi antigen is controlled by 3 chromosomal loci, *viaA*, *viaB*, and *ompB*. Both *viaA* and *viaB* loci are harbored by *Salmonella* pathogenicity island 7 (SPI-7) (11). The *viaA* locus contains genes that are present not only in Vi-expressing strains of *Salmonella* but also in *Escherichia coli* and *Citrobacter* species (12). The *viaB* locus is composed of 11 genes, including 5 genes for Vi biosynthesis (*tivA*, *tivB*, *tivC*, *tivD*, and *tivE*), 5 genes for Vi antigen export (*vexA*, *vexB*, *vexC*, *vexD*, and *vexE*), and open reading frame 11 (ORF11). The *ompB* locus contains two regulatory systems (*rscB*-*rscC* and *ompR*-*envz*), and it controls the regulation of Vi polysaccharide synthesis (8). Here, we report the draft genome sequences of two *S*. Dublin strains, 03EB8736SAL and 03EB8994SAL, isolated from raw-milk cheese and milk filtrate, respectively, that harbor the three chromosomal loci of the Vi antigen.

The two strains, 03EB8736SAL and 03EB8994SAL, were identified as belonging to *Salmonella* Dublin according to the White-Kauffmann-Le Minor scheme (https://www.pasteur.fr/sites/default/files/veng_0.pdf). Genomic DNA was extracted using the QIAamp DNA minikit (Qiagen, UK). The quality of DNA was checked using gel electrophoresis, and DNA quantity was determined using the Qubit quantification platform (Invitrogen, USA). Genomic DNA libraries were prepared using the Nextera XT library preparation kit (Illumina, San Diego, CA, USA), following the manufacturer's protocol.

TABLE 1 Assembly data for *Salmonella* Dublin strains sequenced in this study

Parameter	Value or result for strain:	
	03EB8736SAL	03EB8994SAL
No. of raw reads	1,092,502	480,292
Mean coverage ($\times$)	91.95	38.60
Assembly parameters		
No. of contigs	30	32
Size of draft genome (bp)	4,993,782	4,994,000
GC content (%)	52.18	52.18
N_{50} value	432,097	458,013
Vi antigen and plasmids, accession no. (% identity)		
viaA, viaB, and *ompB* of Vi antigen, AL513382	Present (99)	Present (99)
pSE81-1705, NZ_CP018654	Present (99.83)	Present (99.83)
pOU1113, NC_007208	Present (99.61)	Present (99.61)
pSPUV, NC_019112	Present (99.80)	Present (99.80)
pCFSAN000725_01, NZ_CP012348	Present (99.74)	Present (99.74)
pQJDsal2, NZ_CP022965	Present (99.38)	Present (99.38)
pSG, HG970001	Present (99.72)	Present (99.72)
Plasmid 3, NZ_LN868945	Present (98.72)	Present (98.72)

Whole-genome sequencing (WGS) of multiplexed libraries was carried out on the Illumina HiSeq platform using a 250-bp paired-end protocol. The total number of reads for each *S.* Dublin isolate is provided in Table 1.

The quality of Illumina sequencing data was evaluated using FastQC toolkit version 0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter sequences were removed using ea-utils package version 1.04.807 (https://expressionanalysis.github.io/ea-utils/). Sequencing data were *de novo* assembled using SPAdes version 3.11 (13) (Fig. 1). SPAdes was run using different assembly parameters looking for best assembly with the highest N_{50} value and largest contig size. The quality of multiple assemblies were compared using Quality Assessment Tool for Genome Assemblies (QUAST) (http://quast.bioinf.spbau.ru/). The assembly data (size of draft genome, number of contigs, GC content, and N_{50} value) for each *S.* Dublin isolate are provided in Table 1. The virulence genes involved with the *viaB* locus of the Vi antigen were determined using BLASTn version 2.2.25 (14) with above 90% similarity. BLAST Ring Image Generator (BRIG) version 0.95-dev.0004 (15) was used to illustrate the presence of Vi chromosomal loci within the draft genomes of the two *S.* Dublin strains, and *Salmonella enterica* serovar Typhi strain CT18 harboring Vi antigen (GenBank accession number AL513382) was used as a reference. Moreover, analysis of harbored plasmid/s within the draft genome of the two *S.* Dublin isolates, 03EB8736SAL and 03EB8994SAL, was performed using PLSDB with search strategy Mash screen, and the default values were a maximum *P* value of 0.1 and minimum identity of 0.99 (https://ccb-microbe.cs.uni-saarland.de/plsdb/) (16). Interestingly, seven plasmids that were heterogeneous in size (33 to 147 kb) were detected within the draft genomes, as shown in Table 1. Plasmids can harbor virulence genes required to trigger systemic illness (17).

The presence of Vi chromosomal loci within nontyphoidal *S.* Dublin strains might play an important role in the ability of bacteria to cause invasive illness in humans. Further studies will be carried out to evaluate the expression of Vi antigen and determine the exact role of Vi antigen in *S.* Dublin virulence and pathogenesis.

Data availability. The raw sequence reads and draft assemblies of the two *S.* Dublin isolates, 03EB8736SAL and 03EB8994SAL, have been deposited in the European Nucleotide Archive (ENA) under project number PRJEB30372. The raw sequence reads are available under accession numbers ERS3015385 and ERS3015387, and the
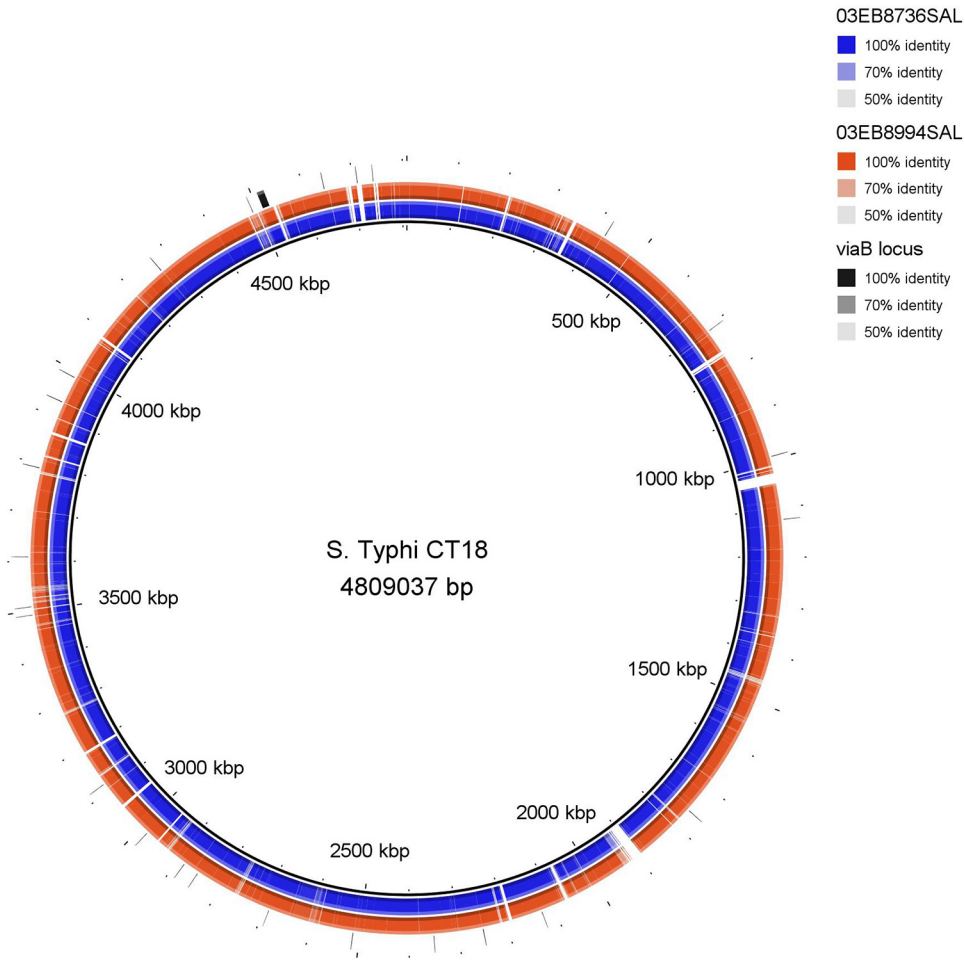
FIG 1 Complete genome alignment of the two *Salmonella* Dublin strains, 03EB8736SAL and 03EB8994SAL, generated using BRIG (15). The three chromosomal loci of the Vi antigen, including the *viaB* locus, are present in the two *Salmonella* Dublin strains as well as in the reference strain of *Salmonella* Typhi strain CT18 (GenBank accession number AL513382).

draft genome sequences are available under accession numbers ERS3015643 and ERS3015644, respectively.

REFERENCES

1. Maguire H, Cowden J, Jacob M, Rowe B, Roberts D, Bruce J, Mitchell E. 1992. An outbreak of *Salmonella* Dublin infection in England and Wales associated with a soft unpasteurized cows' milk cheese. Epidemiol Infect 109:389–396. https://doi.org/10.1017/S0950268800050378.
2. Cummings KJ, Virkler PD, Wagner B, Lussier EA, Thompson BS. 2018. Herd-level prevalence of *Salmonella* Dublin among New York dairy farms based on antibody testing of bulk tank milk. Zoonoses Public Health 65:1003–1007. https://doi.org/10.1111/zph.12523.
3. Mohammed M, Le Hello S, Leekitcharoenphon P, Hendriksen R. 2017. The invasome of *Salmonella* Dublin as revealed by whole genome sequencing. BMC Infect Dis 17:544. https://doi.org/10.1186/s12879-017-2628-x.
4. Mohammed M, Cormican M. 2016. Whole genome sequencing provides insights into the genetic determinants of invasiveness in *Salmonella* Dublin. Epidemiol Infect 144:2430–2439. https://doi.org/10.1017/S0950268816000492.
5. Cadel-Six S, Vignaud M-L, Mohammed M. 2019. Draft genome sequences of *Salmonella enterica* subsp. *enterica* serovar Dublin strains from St. Nectaire and Morbier cheeses characterized by multilocus variable-number tandem-repeat analysis profiles associated with two fatal outbreaks in France. Microbiol Resour Announc 8:e01361-18. https://doi.org/10.1128/MRA.01361-18.
6. Johnson R, Mylona E, Frankel G. 2018. Typhoidal *Salmonella*: distinctive virulence factors and pathogenesis. Cell Microbiol 20:e12939. https://doi.org/10.1111/cmi.12939.
7. Keitel WA, Bond NL, Zahradnik JM, Cramton TA, Robbins JB. 1994. Clinical and serological responses following primary and booster immunization with *Salmonella typhi* Vi capsular polysaccharide vaccines. Vaccine 12:195–199. https://doi.org/10.1016/0264-410X(94)90194-5.
8. Hu X, Chen Z, Xiong K, Wang J, Rao X, Cong Y. 2017. Vi capsular polysaccharide: synthesis, virulence, and application. Crit Rev Microbiol 43:440–452. https://doi.org/10.1080/1040841X.2016.1249335.

9. Morris C, Tam CKP, Wallis TS, Jones PW, Hackett J. 2003. *Salmonella enterica* serovar Dublin strains which are Vi antigen-positive use type IVB pili for bacterial self-association and human intestinal cell entry. Microb Pathog 35:279–284. https://doi.org/10.1016/j.micpath.2003.08.001.

10. Mohammed M, Delappe N, O'Connor J, McKeown P, Garvey P, Cormican M. 2016. Whole genome sequencing provides an unambiguous link between *Salmonella* Dublin outbreak strain and a historical isolate. Epidemiol Infect 144:576–581. https://doi.org/10.1017/S0950268815001636.

11. Seth-Smith HMB. 2008. SPI-7: Salmonella's Vi-encoding pathogenicity island. J Infect Dev Ctries 2:267–271. https://doi.org/10.3855/jidc.220.

12. Johnson EM, Baron LS. 1969. Genetic transfer of the Vi antigen from *Salmonella typhosa* to *Escherichia coli*. J Bacteriol 99:358–359.

13. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

14. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.

15. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402.

16. Galata V, Fehlmann T, Backes C, Keller A. 2018. PLSDB: a resource of complete bacterial plasmids. Nucleic Acids Res 8:D195–D202. https://doi.org/10.1093/nar/gky1050.

17. Libby SJ, Adams LG, Ficht TA, Allen C, Whitford HA, Buchmeier NA, Bossie S, Guiney DG. 1997. The spv genes on the *Salmonella* Dublin virulence plasmid are required for severe enteritis and systemic infection in the natural host. Infect Immun 65:1786–1792.