

AMERICAN SOCIETY FOR MICROBIOLOGY

Complete Genome Sequence of *Salmonella enterica* subsp. *enterica* Serotype Derby, Associated with the Pork Sector in France

Yann Sévellec,^{a,d} Sophie A. Granier,^{a,d} Nicolas Radomski,^{a,d} Arnaud Felten,^{a,d} Simon Le Hello,^b Carole Feurer,^c Michel-Yves Mistou,^{a,d} Sabrina Cadel-Six^{a,d}

^aUniversité Paris-Est, Marne-la-Vallée, France

^bInstitut Pasteur, Centre National de Référence des *Salmonella*, Unité des Bactéries Pathogènes Entériques, Paris, France

^cFrench Institute for the Pig and Pork Industry (IFIP), Le Rheu, France ^dANSES, Laboratory for Food Safety, Maisons-Alfort, France

ABSTRACT In the European Union, *Salmonella enterica* subsp. *enterica* serovar Derby is the most abundant serotype isolated from pork. Recent studies have shown that this serotype is polyphyletic. However, one main genomic lineage, characterized by sequence type 40 (ST40), the presence of the *Salmonella* pathogenicity island 23, and showing resistance to streptomycin, sulphonamides, and tetracycline (STR-SSS-TET), is pork associated. Here, we describe the complete genome sequence of a strain from this lineage isolated in France.

n the European Union, *Salmonella enterica* subsp. *enterica* serovar Derby (*S*. Derby) was the fifth serovar reported from human cases of salmonellosis in 2016 (0.7%; 325/44,462 confirmed cases) (1). European monitoring data linked this serovar predominantly to pigs and pork meat and, to a lesser extent, turkey and cattle (1). Recent studies based on whole-genome sequencing (WGS) have shown that distinct genomic lineages of *S*. Derby exist, associated with either pork or poultry (2, 3). The main genomic lineage associated with pork is characterized by multilocus sequence typing (MLST) profile 40 (sequence type 40 [ST40]), presence of genes mediating resistance to aminoglycosides, sulfonamides, and tetracyclines (3), and presence of *Salmonella* pathogenicity island 23 (SPI-23), which was previously associated with pork enterocyte invasion (2). We present here the complete genome sequence of *S. enterica* subsp. *enterica* serovar Derby strain 2014LSAL02547, which represents this genomic lineage.

Strain 2014LSAL02547 was isolated in 2014 from a pig carcass sampled at a slaughterhouse in Brittany, France, and identified as belonging to *Salmonella* serovar Derby, according to the White-Kauffmann-Le Minor scheme (4). Its genome was sequenced using Illumina HiSeq (i.e., paired-end read sequencing, 2 × 150 bp) and PacBio (i.e., long-read sequencing) technologies. Concerning the Illumina HiSeq sequencing, genomic DNA was isolated from overnight culture at 37°C on a tryptone soy yeast extract agar plate using the Wizard genomic DNA purification kit (Promega, France) according to the manufacturer's instructions for Gram-negative organisms. The DNA concentration was measured with a Qubit fluorometer, and a gel of 0.8% agarose was used to assess the quality of the extraction (and an eventual degradation of the DNA). Library preparation and sequencing were performed by the Institut du Cerveau et de la Moelle épinière (www.icm-institute.org) using NextEra XT technology and a NextSeq 500 sequencer, respectively (Illumina). Concerning the PacBio sequencing, DNA extraction and library preparation were performed by Genoscreen (Lille, France). DNA was extracted using a Gentra Puregene

Received 30 July 2018 Accepted 2 September 2018 Published 27 September 2018

Citation Sévellec Y, Granier SA, Radomski N, Felten A, Le Hello S, Feurer C, Mistou M-Y, Cadel-Six S. 2018. Complete genome sequence of *Salmonella enterica* subsp. *enterica* serotype Derby, associated with the pork sector in France. Microbiol Resour Announc 7:e01027-18. https://doi.org/10.1128/MRA.01027-18.

Editor Iddo Friedberg, Iowa State University Copyright © 2018 Sévellec et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Sabrina Cadel-Six, sabrina.cadelsix@anses.fr.

[This article was published on 27 September 2018 with Nicolas Radomski's surname misspelled as "Randomski" in the byline. The byline was updated in the current version, posted on 28 November 2018.]

TABLE 1 Structure of the SGI-1C and SPI-23 from S. Derby strain 2014LSAL02547^a

Genomic island				
and position	CDS	Protein	Length (bp)	
SGI-1C				
1	int	Integrase	1,158	
2	s002	Helix-turn-helix domain protein	327	
3	rep	Replication protein	954	
4	S004	Hypothetical protein	228	
5	S005	Hypothetical protein	2.760	
6	5006	Hypothetical protein	534	
7	5007	Hypothetical protein	612	
8	5008	Hypothetical protein	210	
9	5000	Hypothetical protein	201	
10	S010		255	
10	traG	Pilus assembly protein TraG	3 405	
17	5012	Conjugative relayerame accessory transporen protein	1 4 25	
12	5012	Hupothetical protein	050	
13	5013	Hypothetical protein	030	
14	5014		411	
15	5015	Hypothetical protein	270	
10	5015	Hypothetical protein	213	
17	5017	Hypothetical protein	243	
18	Inti I	Phage integrase	966	
19	prokka_3715	Hypothetical protein	645	
20	apIIR	Type 2 restriction enzyme Apli	1,092	
21	taqIM	Modification methylase Taql	1,830	
22	hin	DNA-invertase hin	588	
23	intl1	Integrase Int1	1,014	
24	aadA2	Streptomycin 3"-adenylyltransferase	780	
25	qacEdelta1	Quaternary ammonium compound efflux small multidrug resistance	348	
		transporter QacE delta 1		
26	sul1	Sulfonamide-resistant dihydropteroate synthase	840	
27	уреА	Putative acetyltransferase	501	
28	intB	Transposase/IS protein	786	
29	istA	Integrase core domain IS26	1,515	
30	tniB	Bacterial TniB protein	861	
31	tnsB	Transposon Tn7 transposition protein TnsB	1,680	
32	cph2	Phytochrome-like protein cph2	708	
33	merE	Mercury resistance protein MerE	237	
34	mta	Zinc-responsive transcriptional regulator	363	
35	merA	Mercuric reductase	1,695	
36	merC	Mercuric resistance protein MerC	423	
37	merP	Mercuric transport protein periplasmic component precursor	276	
38	merT	MerT mercuric transport protein	351	
39	merR	Mercuric resistance operon regulatory protein	435	
40	prokka 3736	Hypothetical protein (putative relaxase)	243	
41	tetR	Tetracycline repressor protein class A from transposon 1721	678	
42	tetA	Tetracycline resistance protein, class C	1,200	
43	vedA	Putative inner membrane transporter YedA	783	
44	tnsB	Transposon Tn7 transposition protein TnsB	708	
45	prokka 3741	Hypothetical protein	1.035	
	pronta_07 TT		.,	
SPI-23				
1	intA3	Integrase A	1,275	
2	prokka 01810	Hypothetical protein	252	
3	prokka_01811	Abortive infection phage resistance protein	1.113	
4	prokka 01812	Bacterial shuffle protein	1,488	
5	prokka 01813	Major subunit of bundle-forming pilus precursor	558	
6	hfpA	Conjugal transfer protein TraD	306	
7	traD	Hypothetical protein	1 515	
8	prokka 01816	Hypothetical protein	735	
9	prokka_01817	Hypothetical protein	576	
10	prokka 01818	Hypothetical protein	570	
11	prokka_01810	Hypothetical protein	342	
12	$prokk_2 01920$	Hypothetical protein	Δ71	
13	prokka_01020	Hypothetical protein	552	
14	prokka_01021	Hypothetical protein	180	
15	proka_01022	Hypothetical protein	240	
16	prokka_01023		24U 1 251	
10	ргокка_01824	nypothetical protein	1,201	

(Continued on next page)

TABLE 1 (Continued)

Genomic island				
and position	CDS	Protein	Length (bp)	
17	prokka_01825	Hypothetical protein	282	
18	prokka_01826	Hypothetical protein	438	
19	prokka_01827	Hypothetical protein	558	
20	prokka_01828	Hypothetical protein	183	
21	prokka_01829	Hypothetical protein	477	
22	prokka_01830	Hypothetical protein	2,730	
23	prokka_01831	Hypothetical protein	504	
24	prokka_01832	Hypothetical protein	93	
25	prokka_01833	Hypothetical protein	870	
26	prokka_01834	Hypothetical protein	588	
27	prokka_01835	Hypothetical protein	984	
28	prokka_01836	RNA pyrophosphohydrolase	444	
29	prokka_01837	Hypothetical protein	519	
30	prokka_01838	Hypothetical protein	261	
31	prokka_01839	Hypothetical protein	735	
32	prokka_01840	Hypothetical protein	738	
33	prokka_01841	hypothetical protein	480	
34	hns_2	DNA binding protein H_NS	405	
35	prokka_01843	Hypothetical protein	549	
36	prokka_01844	Hypothetical protein	1,272	
37	prokka_01845	Hypothetical protein	297	
38	prokka_01846	Hypothetical protein	291	
39	prokka_01847	Hypothetical protein	219	
40	prokka_01848	Hypothetical protein	876	
41	prokka_01849	Hypothetical protein	525	

^aGenBank accession number CP029486. SGI-1C, Salmonella genomic island 1 type C (bases 427735 to 472096); SPI-23, Salmonella pathogenicity island 23 (bases 2369809 to 2406412).

kit (Qiagen), the DNA concentration was assessed using a Qubit fluorometer, and the DNA extract's quality was checked by agarose gel electrophoresis. Library preparation was made by DNA fragmentation and ligation of single-molecule real-time (SMRT) adaptors. Prior to sequencing, BluePippin size selection (Sage Science) was set at 15 kb in order to achieve identical sequence overlaps. PacBio sequencing was performed on one SMRT cell. Quality of the Illumina and PacBio reads was examined using FastQC v0.11.5 (5). Prinseq v0.20.4 (6) was used to select Illumina long reads of good quality (no undefined bases; Phred, >30; length, >60 kb). SMRT Analysis v2.3.0 software was used to assemble PacBio reads. In SMRT Analysis, the Hierarchical Genome Assembly Process (HGAP) v3.0 (7) was invoked to correct the subreads (length, >1,000 bases; read score, 0.8), and Celera v8.3 (8) was used for assembly (subread length, >500 bases; deep coverage, >25×). SAMtools v1.5 (9) was used to map the Illumina short pairedend reads against the PacBio assembly to correct potential assembly mistakes and to determine the depth of the final assembly. The final deep coverage obtained was $146 \times .$ A unique 4.86-Mb contig was obtained, with a GC content of 51.12%. The genome was annotated using Prokka (10). It included 4,549 coding sequences (CDS) and 88 tRNAs.

Genes mediating antimicrobial resistance phenotype STR-SSS-TET (showing resistance to streptomycin, sulfonamides, and tetracycline) are part of the *Salmonella* genomic island 1 (SGI-1) (3), described in Table 1. This SGI-1 element integrated a cluster of mercury resistance genes (*merA*, *merC*, *merP*, *merT*, and *merR*) located in a Tn7 transposon. SPI-23 from the 2014LSAL02547 genome was 36,603 bp long and was located between the *DAD50_12070* and *mftA* genes (Table 1).

Data availability. This whole-genome assembly sequence was deposited in the NCBI database under the accession number CP029486. The SRA accession numbers are SRX3643218 (Illumina reads) and SRX4523973 (PacBio reads).

ACKNOWLEDGMENTS

This study was funded by the French Ministry of Agriculture, Food and Forestry, by the *Salmonella* Network from the French Agency for Food, Environmental and Occu-

pational Health and Safety (ANSES) Laboratory for Food Safety and by INAPORC. Y.S. is the recipient of a doctoral fellowship (DGER-ANSES) cofunded by AgroParisTech and the ANSES.

REFERENCES

- European Food Safety Authority. 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2016. EFSA J 15:e05077. https://doi.org/10.2903/j .efsa.2017.5077.
- Hayward MR, AbuOun M, La Ragione RM, Tchorzewska MA, Cooley WA, Everest DJ, Petrovska L, Jansen VA, Woodward MJ. 2014. SPI-23 of S. Derby: role in adherence and invasion of porcine tissues. PLoS One 9:e107857. https://doi.org/10.1371/journal.pone.0107857.
- Sévellec Y, Vignaud M-L, Granier SA, Lailler R, Feurer C, Le Hello S, Mistou M-Y, Cadel-Six S. 2018. Polyphyletic nature of *Salmonella enterica* serotype Derby and lineage-specific host-association revealed by genomewide analysis. Front Microbiol 9:891. https://doi.org/10.3389/fmicb.2018 .00891.
- Grimont PAD, Weill FX. 2007. Antigenic formulae of the Salmonella serovars, 9th ed. WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur, Paris, France.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/ fastqc.
- 6. Schmieder R, Edwards R. 2011. Quality control and preprocessing of

metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10 .1093/bioinformatics/btr026.

- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10 .1038/nmeth.2474.
- Myers EW, Sutton GG, Delcher AL, Dew IM, Fasulo DP, Flanigan MJ, Kravitz SA, Mobarry CM, Reinert KH, Remington KA, Anson EL, Bolanos RA, Chou HH, Jordan CM, Halpern AL, Lonardi S, Beasley EM, Brandon RC, Chen L, Dunn PJ, Lai Z, Liang Y, Nusskern DR, Zhan M, Zhang Q, Zheng X, Rubin GM, Adams MD, Venter JC. 2000. A whole-genome assembly of *Drosophila*. Science 287:2196–2204. https://doi.org/10.1126/science.287 .5461.2196.
- Li H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27:2987–2993. https://doi.org/10 .1093/bioinformatics/btr509.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.