AMERICAN SOCIETY FOR MICROBIOLOGY
Microbiology® Resource Announcements
GENOME SEQUENCES

Draft Genome Sequence of *Salmonella enterica* subsp. *enterica* Serovar Enteritidis ODA 99-30581-13, a Heat-Resistant Strain Isolated from Shell Eggs

Yumin Xu,[a] Ahmed G. Abdelhamid,[a] Ahmed E. Yousef[a,b]

[a]Department of Food Science and Technology, The Ohio State University, Columbus, Ohio, USA
[b]Department of Microbiology, The Ohio State University, Columbus, Ohio, USA

ABSTRACT *Salmonella enterica* serovar Enteritidis ODA 99-30581-13 is a relatively heat-resistant strain isolated from shell eggs. The strain has a 4,777,965-bp genome sequence (52.1% GC content) that was predicted to encode 4,455 proteins, including heat stress response proteins and stress response regulators; these may be involved in its heat resistance.

Salmonella enterica serovar Enteritidis has caused numerous salmonellosis outbreaks linked to egg consumption (1–4). Thermal pasteurization methods were developed to combat *Salmonella* contamination in shell eggs. Considering its relative heat resistance, *Salmonella* Enteritidis ODA 99-30581-13 was used as the target pathogen of shell egg pasteurization (5, 6). The current work presents the genome sequence of strain ODA 99-30581-13 and the genes likely linked to its heat resistance.

Strain ODA 99-30581-13 was streaked from frozen stock onto tryptic soy agar medium (BD, Sparks, MD), followed by culturing in tryptic soy broth (BD); incubation in both transfers was done at 37°C for 24 h. Genomic DNA (gDNA) was isolated from 1 ml of the overnight culture (10^9 CFU/ml) using a QIAamp DNA minikit (Qiagen, Germantown, MD) according to the manufacturer's protocol. The purity and concentration of the gDNA were evaluated by the NanoVue Plus spectrophotometer (Biochrom USA, Holliston, MA). DNA libraries of the gDNA were prepared using the Nextera DNA Flex library preparation kit (Illumina, Madison, WI) and barcoded using the Nextera DNA CD index library indexing kit (Illumina) according to the manufacturer's instructions. The DNA libraries were quantified using the Qubit fluorimeter (Invitrogen, Waltham, MA), and their sizes were measured using the 2100 Bioanalyzer system (Agilent Technologies, CA). The genome was sequenced using the Illumina MiSeq platform (Food Microbiology Laboratory, The Ohio State University, Columbus, OH). The platform generated 478.5 Mb of data, including 1,910,230 raw reads (paired-end, 2 × 300-bp format) with 214.3× coverage and a quality score of 37, calculated by FastQC v0.11.9 software (7). Using Illumina's "Generate FASTQ analysis" module to trim adaptor sequences and SPAdes v3.10.1 (8) for *de novo* assembly resulted in 30 contigs (N_{50}, 491,928 bp), which were ordered against the reference genome of *Salmonella enterica* serovar Typhimurium LT2 by progressiveMauve v2.4.0 (9). Annotation of the *de novo*-assembled genome sequence was realized through the NCBI Prokaryotic Genome Annotation Pipeline (10). Default settings were applied for the bioinformatics software that was used in this study.

Analysis of the *Salmonella* Enteritidis ODA 99-30581-13 genome sequence showed that it has 4,777,965 bp (GC content, 52.1%) and encodes 4,455 proteins, 75 tRNAs, 15 rRNAs, and 15 noncoding RNAs (ncRNAs). Strain ODA 99-30581-13 belongs to sequence type 11 and serotype Enteritidis, as determined by MLST v2.0 (11) and SeqSero v1.2 (12), respectively. The genome of ODA 99-30581-13 harbors genes

Citation Xu Y, Abdelhamid AG, Yousef AE. 2021. Draft genome sequence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis ODA 99-30581-13, a heat-resistant strain isolated from shell eggs. Microbiol Resour Announc 10:e01461-20. https://doi.org/10.1128/MRA.01461-20.

Editor Vincent Bruno, University of Maryland School of Medicine

Address correspondence to Ahmed E. Yousef, yousef.1@osu.edu.

Received 18 December 2020
Accepted 10 February 2021
Published 4 March 2021

involved in heat shock response (*dnaK*, *dnaJ*, *grpE*, *clpP*, *hscAB*, *htrA*, *hsiJ*, *hspQ*, *hsp15*, *hsp20*, *ibpA*, *ibpB*, and *surA*) and heat stress response regulators (*rpoS* and *rpoH*) (13). Additionally, the general stress protein (NCBI Protein accession number WP_000807638.1; locus_tag IAF69_RS06740), which promotes heat resistance specific to *Salmonella* Enteritidis (14), was encoded in the ODA 99-30581-13 annotated genome sequence. The presence of these heat stress response-related genes might explain the relative heat resistance of strain ODA 99-30581-13.

Data availability. The genome sequence of *Salmonella* Enteritidis ODA 99-30581-13 was deposited at GenBank under accession number JACTGY000000000.1, BioProject accession number PRJNA659787, BioSample accession number SAMN15925966, and SRA accession number SRX9029726.

ACKNOWLEDGMENT

The study was supported by the Ohio Agricultural Research and Development Center (OARDC) equipment fund.

REFERENCES

1. Centers for Disease Control and Prevention (CDC). 2010. Multistate outbreak of human *Salmonella* Enteritidis infections associated with shell eggs (final update). https://www.cdc.gov/salmonella/2010/shell-eggs-12-2-10.html.
2. CDC. 2016. Multistate outbreak of *Salmonella* Oranienburg infections linked to Good Earth Egg Company shell eggs (final update). https://www.cdc.gov/salmonella/oranienburg-10-16/index.html.
3. Food and Drug Administration. 2018. FDA investigated multistate outbreak of *Salmonella* Braenderup linked to shell eggs from Rose Acre Farms. https://www.fda.gov/food/outbreaks-foodborne-illness/fda-investigated-multistate-outbreak-salmonella-braenderup-linked-shell-eggs-rose-acre-farms.
4. CDC. 2018. Outbreak of *Salmonella* infections linked to Gravel Ridge Farms shell eggs—final update. https://www.cdc.gov/salmonella/enteritidis-09-18/index.html.
5. Perry JJ. 2010. Ozone based treatments for inactivation of *Salmonella enterica* serovar Enteritidis in shell eggs. PhD dissertation. The Ohio State University, Columbus, OH.
6. Perry JJ, Yousef AE. 2013. Factors affecting thermal resistance of *Salmonella enterica* serovar Enteritidis ODA 99-30581-13 in shell egg contents and use of heat-ozone combinations for egg pasteurization. J Food Prot 76:213–219. https://doi.org/10.4315/0362-028X.JFP-12-324.
7. Andrews S. 2015. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics. https://www.bioinformatics.babraham.ac.uk/projects/fastqc.
8. 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.
9. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
10. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
11. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Ponten T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11.
12. Zhang S, Yin Y, Jones MB, Zhang Z, Deatherage Kaiser BL, Dinsmore BA, Fitzgerald C, Fields PI, Deng X. 2015. *Salmonella* serotype determination utilizing high-throughput genome sequencing data. J Clin Microbiol 53:1685–1692. https://doi.org/10.1128/JCM.00323-15.
13. Dawoud TM, Davis ML, Park SH, Kim SA, Kwon YM, Jarvis N, O'Bryan CA, Shi Z, Crandall PG, Ricke SC. 2017. The potential link between thermal resistance and virulence in *Salmonella*: a review. Front Vet Sci 4:93. https://doi.org/10.3389/fvets.2017.00093.
14. Arunima A, Swain SK, Ray S, Prusty BK, Suar M. 2020. RpoS-regulated *SEN1538* gene promotes resistance to stress and influences *Salmonella enterica* serovar enteritidis virulence. Virulence 11:295–314. https://doi.org/10.1080/21505594.2020.1743540.