

Whole-Genome Sequencing of *Salmonella enterica* subsp. *enterica* Serovar Cubana Strains Isolated from Agricultural Sources

Faiza H. Benahmed,^a Gopal R. Gopinath,^b Hua Wang,^c Junia Jean-Gilles Beaubrun,^b Christopher Grim,^b Chong-Ming Cheng,^d Michael McClelland,^e Sherry Ayers,^a Jason Abbott,^a Prerak Desai,^e Jonathan G. Frye,^f George Weinstock,^g Thomas S. Hammack,^c Darcy E. Hanes,^b Mark A. Rasmussen,^{a*} Maureen K. Davidson^a

U.S. Food and Drug Administration, Center for Veterinary Medicine, Division of Animal and Food Microbiology, Laurel, Maryland, USA^a; U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Applied Research and Safety Assessment, Division of Virulence Assessment, Laurel, Maryland, USA^b; U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Microbiology, College Park, Maryland, USA^c; U.S. Food and Drug Administration, Pacific Regional Laboratory Southwest, Irvine, California, USA^d; University of California, Department of Microbiology and Molecular Genetics, Irvine, California, USA^e; Agricultural Research Service, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Athens, Georgia, USA^f; The Genome Institute, Washington University School of Medicine, St. Louis, Missouri, USA^g

* Present address: Mark A. Rasmussen, Leopold Center for Sustainable Agriculture, Iowa State University, Ames, Iowa, USA.

We report the draft genomes of *Salmonella enterica* subsp. *enterica* serovar Cubana strain CVM42234, isolated from chick feed in 2012, and *S. Cubana* strain 76814, isolated from swine in 2004. The genome sizes are 4,975,046 and 4,936,251 bp, respectively.

Received 9 December 2013 Accepted 13 December 2013 Published 23 January 2014

Citation Benahmed FH, Gopinath GR, Wang H, Jean-Gilles Beaubrun J, Grim C, Cheng C-M, McClelland M, Ayers S, Abbott J, Desai P, Frye JG, Weinstock G, Hammack TS, Hanes DE, Rasmussen MA, Davidson MK. 2014. Whole-genome sequencing of *Salmonella enterica* subsp. *enterica* serovar Cubana strains isolated from agricultural sources. *Genome Announc.* 2(1):e01184-13. doi:10.1128/genomeA.01184-13.

Copyright © 2014 Benahmed et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Maureen K. Davidson, Maureen.davidson@fda.hhs.gov.

The presence of bacterial pathogens, such as *Salmonella*, in animal feed is an important route of animal infections and thus is an important risk factor for the human food chain and public health. There are >2,500 serovars of *Salmonella*, and all of them are considered a potential threat to human health (1). *Salmonella enterica* subsp. *enterica* serovar Cubana has been reported in swine that received contaminated feed (2) and in human foods (3). Whole-genome sequencing of *Salmonella* serotypes will allow the discovery of new genes unique to *Salmonella* and will support outbreak investigations.

The *S. Cubana* CVM42234 strain was isolated from chick feed with the Bacteriological Analytical Manual *Salmonella* culture method (4, 5), detected by a quantitative PCR (qPCR) method (6, 7), and serotyped with PCR serotyping (8) and by traditional serological methods according to the CDC protocols (9–11). The serotype antigens are 1, 13, 23, and z29-. Pulsed-field gel electrophoresis (PFGE) was performed according to the CDC methods (12), and the PFGE pattern was JDGX01.0018. *In vitro* antimicrobial susceptibility testing was done according to the standard National Antimicrobial Resistance Monitoring System protocol using the CMV2AGNF panel of antimicrobials (13). *S. Cubana* strain 76814 was tested using a similar microbroth dilution method (Trek Biosystems, Cleveland, OH). Strain CVM42234 was susceptible to all antimicrobials tested, but strain 76814 was resistant to streptomycin, sulfamethoxazole, and tetracycline.

Strain CVM42234 DNA was extracted using the QIAcube (Qiagen, Valencia, CA), the DNA library was constructed according to the Illumina protocol with Nextera XT DNA Sample Prep kit, and it was sequenced using Illumina MiSeq (Illumina, San Diego, CA). CLC bio software version 6.0.1 (Germantown, MD) was used for the trimming and *de novo* assembly of the paired-end

reads to 100 contigs. Strain 76814 DNA was isolated with the GenElute isolation kit (Sigma-Aldrich, St. Louis, MO) for bacteria, the library was made by shearing and ligating Illumina sequencing adapters to the genomic DNA, and it was sequenced on HiSeq 2000 (Illumina) then assembled using one Button Velvet (European Bioinformatics Institute, Hinxton, Cambridgeshire United Kingdom) into 247 contigs (172 scaffolds).

Both the CVM42234 and 76814 draft genomes were annotated using RAST (14). The two *S. Cubana* genomes have an average nucleotide identity of 99.5% and reveal many common genetic features, as well as some differences. They both have a multidrug resistance *mdtABCD* cluster, a multiple antibiotic resistance (*mar*) locus, and several multidrug resistance efflux pumps. Loci for resistance to metals, such as arsenic, copper, silver, cobalt, zinc, and mercury, are present. Many flagellar motility genes also are present. CVM42234 contains two discrete clusters of phage proteins. A conjugative plasmid and a small plasmid bearing a mercury resistance locus were identified from the 76814 genome assembly. The presence of many antimicrobial resistance genes and strain-specific mobile elements indicate that *S. Cubana* may exhibit typical heterogeneity based on host and geographical factors as seen in many other pathogenic *Salmonella* serovars. The annotations of both genomes are publically available at RAST.

Nucleotide sequence accession numbers. The draft genome sequences for these two *Salmonella* serovar Cubana strains have been deposited at DDBJ/EMBL/GenBank under accession no. [AZGR000000000](https://www.ncbi.nlm.nih.gov/nuccore/AZGR000000000) and [ATEU000000000](https://www.ncbi.nlm.nih.gov/nuccore/ATEU000000000).

ACKNOWLEDGMENTS

We thank Steffen Porwollik and Pui Cheng for their expert assistance.

M.M. was supported in part by NIH grants no. AI039557, AI052237,

AI073971, AI075093, AI077645, and AI083646, USDA grants no. 2009-03579 and 2011-67017-30127, the Binational Agricultural Research and Development Fund, and a grant from the Center for Produce Safety.

We thank Wen Lin for providing the Applied Biosystems 7500 Fast instrument during this study, Venkatakrishna Shyamala for reviewing the manuscript, and Bill Klimki of the National Library of Medicine/National Center for Biotechnology Information for assistance in submitting the metadata associated with this sequencing project.

REFERENCES

1. World Health Organization (WHO). 2005. Drug-resistant *Salmonella*. Fact sheet no. 139. World Health Organization, Geneva, Switzerland. <http://www.who.int/mediacentre/factsheets/fs139/en/print.html>.
2. Osterberg J, Vågsholm I, Boqvist S, Lewerin SS. 2006. Feed-borne outbreak of *Salmonella* Cubana in Swedish pig farms: risk factors and factors affecting the restriction period in infected farms. *Acta Vet. Scand.* 47:13–22. <http://dx.doi.org/10.1186/1751-0147-47-13>.
3. Taormina PJ, Beuchat LR, Slutsker L. 1999. Infections associated with eating seed sprouts: an international concern. *Emerg. Infect. Dis.* 5:626–634.
4. Cheng CM, Lin W, Van KT, Phan L, Tran NN, Farmer D. 2008. Rapid detection of *Salmonella* in foods using real-time PCR. *J. Food Prot.* 71: 2436–2441.
5. Cheng C-M, Van KT, Lin W, Ruby RM. 2009. Inter-laboratory validation of a real-time PCR 24-hour rapid method for detection of *Salmonella* in foods. *J. Food Prot.* 72:945–951.
6. Andrews WH, Hammack T. 2007. Chapter 5, *Salmonella*. In *Bacteriological analytical manual online*. U.S. Food and Drug Administration, Silver Spring, MD. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>.
7. Andrews WH, Hammack TS. 2003. Chapter 1, Food sampling and preparation of sample homogenate. In *Bacteriological analytical manual online*. U.S. Food and Drug Administration, Silver Spring, MD. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063335.htm>.
8. Jean-Gilles Beaubrun J, Cheng CM, Chen KS, Ewing L, Wang H, Agpaoa MC, Huang MC, Dickey E, Du JM, Williams-Hill DM, Hamilton B, Micallef SA, Rosenberg Goldstein RE, George A, Joseph SW, Sapkota AR, Jacobson AP, Tall BD, Kothary MH, Dudley K, Hanes DE. 2012. The evaluation of a PCR-based method for identification of *Salmonella enterica* serotypes from environmental samples and various food matrices. *Food Microbiol.* 31:199–209.
9. Grimont PAD, Weill FX. 2007. Antigenic formulae of the *Salmonella* serovars, 9th ed. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institute Pasteur, Paris, France.
10. Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemühl J, Grimont PA, Weill FX. 2010. Supplement 2003–2007 (no. 47) to the White–Kauffmann–Le Minor scheme. *Res. Microbiol.* 161:26–29. <http://dx.doi.org/10.1016/j.resmic.2009.10.002>.
11. Centers for Disease Control and Prevention. 1988. Identification and serotyping of *Salmonella*. Frances W. Brenner Alma C. McWhorter-Murlin. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Bacterial and Mycotic Diseases, Foodborne and Diarrheal Diseases Branch, Foodborne and Diarrheal Diseases Laboratory Section, National *Salmonella* Reference Laboratory. CDC, Atlanta, GA.
12. Centers for Disease Control and Prevention. 2002. Standardized molecular subtyping of foodborne bacterial pathogens by pulsed-field gel electrophoresis. Centers for Disease Control and Prevention, Atlanta, GA.
13. U.S. Food and Drug Administration. 2011. National Antimicrobial Resistance Monitoring System 2011 retail meat report. U.S. FDA, Silver Spring, MD. <http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM334834.pdf>.
14. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNell LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.