GENOME SEQUENCES





Draft Genome Sequence of *Clostridium perfringens* Strain TAMU, Which Causes Necrotic Enteritis in Broiler Chickens

Catherine Ausland,^a Adil Sabr Al-Ogaili,^{b,c} Juan D. Latorre,^b Guillermo Tellez-Isaias,^b Billy M. Hargis,^b Young Min Kwon,^b Margarita A. Arreguin-Nava,^d Pallavi Singh^a

^aDepartment of Biological Sciences, Northern Illinois University, DeKalb, Illinois, USA ^bDepartment of Poultry Science, University of Arkansas, Fayetteville, Arkansas, USA ^cDepartment of Medical Laboratory Techniques, Kut Tech Institute, Middle Technical University, Baghdad, Iraq ^dEco-Bio, LLC, Fayetteville, Arkansas, USA

ABSTRACT *Clostridium perfringens* causes severe gastrointestinal diseases, which include necrotic enteritis (NE) in chickens, a deadly disease worldwide. We report here the draft genome sequence of *Clostridium perfringens* strain TAMU, which was used in developing an NE chicken challenge model. This *C. perfringens* TAMU genome sequence will aid in advancing potential intervention strategies to reduce NE pathogenesis.

Clostridium perfringens, a Gram-positive spore-forming bacterium, is the causative agent of an array of diseases in humans and agricultural animals due to diverse toxins on its conjugative plasmids (1–4). One of these diseases is necrotic enteritis (NE), which causes intestinal inflammation and necrotic regions in broiler chickens, leading to high morbidity and mortality. *C. perfringens* has also been isolated from commercial turkeys with cellulitis (5). NE is increasingly a significant burden on the poultry industry, particularly with the banning of prophylactic antibiotic use (6). Here, we present the draft genome sequence of *C. perfringens* strain TAMU, which was isolated in 2004 using a brucella blood agar plate from the gut of a broiler chicken from Texas presenting NE (7, 8). Since then, our group has successfully used the *C. perfringens* strain to reproduce important aspects of NE pathogenesis using an *in vitro* digestive model (9) and *in vivo* chicken challenge models (8, 10, 11).

C. perfringens TAMU was cultured overnight in tryptic soy broth with thioglycolate, and DNA extraction was performed with a DNeasy UltraClean microbial kit (Qiagen LLC, Germantown, MD). Genomic DNA was prepared for shotgun metagenome sequencing using a Nextera XT DNA library preparation kit (Illumina, Inc., San Diego, CA) according to the manufacturer's instructions. Sequencing was performed at the University of Illinois at Chicago Sequencing Core (UICSQC) using a NextSeq 500 instrument (Illumina, Inc.) with 150-bp paired-end sequencing. In total, approximately 6.1 million reads were generated. Trimming was performed in the software package CLC Genomics Workbench v11.0.1 (Qiagen). Trimming was performed using default parameters with a threshold of Q20. Sequences demultiplexed in the BaseSpace cloud computing environment provided by the UICSQC resulted in a 3,672,352-bp draft genome assembly using SPAdes v3.11.1 (12) with 318 contigs more than 200 bp in length, an average coverage of $205 \times$, an N_{50} value of 46,751 bp, and a G+C content of 28.11%. Genome assembly quality was determined by the QUAST quality assessment tool (13).

The draft genome was annotated with the Prokaryotic Genome Annotation Pipeline from NCBI (14). Annotated features include 3,397 genes with 3,309 coding sequences (CDS), 12 rRNAs (including 5S, 16S, and 23S rRNAs), 75 tRNAs, and 4 noncoding RNAs (ncRNAs). Functional annotation with the Virulence Factors Database (VFDB) (15)

Citation Ausland C, Al-Ogaili AS, Latorre JD, Tellez-Isaias G, Hargis BM, Kwon YM, Arreguin-Nava MA, Singh P. 2020. Draft genome sequence of *Clostridium perfringens* strain TAMU, which causes necrotic enteritis in broiler chickens. Microbiol Resour Announc 9:e01357-19. https://doi.org/10.1128/MRA.01357-19.

Editor David Rasko, University of Maryland School of Medicine

Copyright © 2020 Ausland et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Pallavi Singh, psingh1@niu.edu.

Received 10 November 2019 Accepted 26 December 2019 Published 23 January 2020 predicted 25 open reading frames (ORFs) associated with virulence, including several encoding enterotoxins (n = 3), hemolysins (n = 4), and adherence factors (n = 2), as well as alpha-toxin (n = 1) and β 2-toxin (n = 1). These data have been made available on figshare (https://doi.org/10.6084/m9.figshare.11337245.v3). The Resistance Gene Identifier in the Comprehensive Antibiotic Resistance Database (16) identified 3 genes conferring resistance to peptide (n = 1) and tetracycline (n = 2) antibiotics based on protein homology models of these genes sharing 97% or greater similarity to query sequences (https://doi.org/10.6084/m9.figshare.11337311.v1). Default parameters were used for all software unless otherwise specified. In conclusion, this *C. perfringens* TAMU draft genome sequence will faciliate functional genomic analysis of virulence factors associated with NE pathogenesis.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number VOVJ00000000. The version described in this paper is version VOVJ01000000. The project data have been submitted under BioProject accession number PRJNA558493 and raw sequences under SRA accession number SRP218148.

ACKNOWLEDGMENTS

This study was supported by Eco-Bio, LLC, Northern Illinois University, and the Iraqi Ministry of Higher Education and Scientific Research.

The *C. perfringens* TAMU strain was kindly donated by Jack L. McReynolds, USDA-ARS, College Station, TX.

Margarita A. Arreguin-Nava is employed by Eco-Bio, LLC. The remaining authors declare the absence of commercial or financial relationships that may be potential conflict of interests.

REFERENCES

- Rood JI, Adams V, Lacey J, Lyras D, McClane BA, Melville SB, Moore RJ, Popoff MR, Sarker MR, Songer JG, Uzal FA, Van Immerseel F. 2018. Expansion of the *Clostridium perfringens* toxin-based typing scheme. Anaerobe 53:5–10. https://doi.org/10.1016/j.anaerobe.2018.04.011.
- Shrestha A, Uzal FA, McClane BA. 2018. Enterotoxic clostridia: Clostridium perfringens enteric diseases. Microbiol Spectr 6. https://doi.org/10.1128/ microbiolspec.GPP3-0003-2017.
- Lacey JA, Keyburn AL, Ford ME, Portela RW, Johanesen PA, Lyras D, Moore RJ. 2017. Conjugation-mediated horizontal gene transfer of *Clostridium perfringens* plasmids in the chicken gastrointestinal tract results in the formation of new virulent strains. Appl Environ Microbiol 83: e01814-17. https://doi.org/10.1128/AEM.01814-17.
- Revitt-Mills SA, Rood JI, Adams V. 2015. Clostridium perfringens extracellular toxins and enzymes: 20 and counting. Microbiol Aust 36:114–117.
- Tellez G, Pumford NR, Morgan MJ, Wolfenden AD, Hargis BM. 2009. Evidence for *Clostridium septicum* as a primary cause of cellulitis in commercial turkeys. J Vet Diagn Invest 21:374–377. https://doi.org/10 .1177/104063870902100313.
- Casewell M, Friis C, Marco E, McMullin P, Phillips I. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 52:159–161. https:// doi.org/10.1093/jac/dkg313.
- McReynolds JL, Byrd JA, Anderson RC, Moore RW, Edrington TS, Genovese KJ, Poole TL, Kubena LF, Nisbet DJ. 2004. Evaluation of immunosuppressants and dietary mechanisms in an experimental disease model for necrotic enteritis. Poult Sci 83:1948–1952. https://doi.org/10.1093/ ps/83.12.1948.
- Shivaramaiah S, Wolfenden RE, Barta JR, Morgan MJ, Wolfenden AD, Hargis BM, Téllez G. 2011. The role of an early *Salmonella* Typhimurium infection as a predisposing factor for necrotic enteritis in a laboratory challenge model. Avian Dis 55:319–323. https://doi.org/10.1637/9604 -112910-ResNote.1.
- Latorre JD, Hernandez-Velasco X, Kuttappan VA, Wolfenden RE, Vicente JL, Wolfenden AD, Bielke LR, Prado-Rebolledo OF, Morales E, Hargis BM, Tellez G. 2015. Selection of *Bacillus* spp. for cellulase and xylanase production as direct-fed microbials to reduce digesta viscosity and *Clostridium perfringens* proliferation using an *in vitro* digestive model in

different poultry diets. Front Vet Sci 2:25. https://doi.org/10.3389/fvets .2015.00025.

- Latorre JD, Adhikari B, Park SH, Teague KD, Graham LE, Mahaffey BD, Baxter MFA, Hernandez-Velasco X, Kwon YM, Ricke SC, Bielke LR, Hargis BM, Tellez G. 2018. Evaluation of the epithelial barrier function and ileal microbiome in an established necrotic enteritis challenge model in broiler chickens. Front Vet Sci 5:199. https://doi.org/10.3389/fvets.2018 .00199.
- Hernandez-Patlan D, Solis-Cruz B, Pontin KP, Hernandez-Velasco X, Merino-Guzman R, Adhikari B, Tellez-Isaias G, Latorre JD. 2019. Impact of a *Bacillus* direct-fed microbial on growth performance, intestinal barrier integrity, necrotic enteritis lesions, and ileal microbiota in broiler chickens using a laboratory challenge model. Front Vet Sci 6:108. https://doi .org/10.3389/fvets.2019.00108.
- 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.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- 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.
- Liu B, Zheng D, Jin Q, Chen L, Yang J. 2019. VFDB 2019: a comparative pathogenomic platform with an interactive Web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/qky1080.
- 16. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45:D566–D573. https://doi.org/10.1093/nar/gkw1004.