



Whole-Genome Sequence of *Corynebacterium pseudotuberculosis* PA04, Isolated from the Lymph Node of a Sheep in the Amazon, Brazil

Wana L. O. Costa,^a Jorianne T. C. Alves,^a Larissa M. Dias,^a Carlos Leonardo de Aragão Araújo,^a Eziqiel Morais,^b André G. M. Silva,^b Soraya S. Andrade,^a Rommel T. J. Ramos,^a Artur Silva,^a Adriana R. C. Folador^{a*}

Center of Genomics and System Biology, Laboratory of Genomic and Bioinformatics, Federal University of Pará, Belém, Pará, Brazil^a; Federal University of Pará, Campus of Castanhal, Pará, Brazil^b

ABSTRACT This study reports the complete genome sequence of *Corynebacterium pseudotuberculosis* strain PA04, isolated from a sheep in the Amazon, Brazil. This bacterium is the etiological agent of caseous lymphadenitis. This genome contains 2,338,093 bp, 52.2% G+C content, and a total of 2,104 coding sequences (CDSs), 41 pseudogenes, 12 rRNAs, and 49 tRNAs.

Corynebacterium pseudotuberculosis is a Gram-positive bacterium which is the causative agent of caseous lymphadenitis (CLA), a chronic disease that affects small ruminants, mostly sheep and goats, although it also affects other animals, such as horses, cattle, buffalo, and, in rare cases, humans (1–3).

C. pseudotuberculosis infections are found worldwide, with higher prevalence in meat-producing countries, such as Australia, New Zealand, South Africa, the United States, Canada, and Brazil (1, 2, 4). In Brazil, most of the reported cases are from the Northeast (5). In the northern part of Brazil, according to the report of the Brazilian Institute of Geography and Statistics (IBGE) in 2015 (<http://www.ibge.gov.br/estadosat/temas.php?siglapa&temapecuaria2015>), most sheep and goat herds in the region are from the State of Pará, which contribute 50% of the goat and 36% of the sheep herd breeding in the north of Brazil. However, the lack of data related to cases of CLA underestimates the actual prevalence of this disease in the state (6). This absence of information may be explained by deficiencies in notification of this disease in many countries (7).

Whole-genome sequencing of *C. pseudotuberculosis* has contributed to the use of omics approaches to study particular characteristics of this pathogen, its ability to cause infection, and its gene expression (8), and to predict valuable proteins for drug target investigations (9, 10).

C. pseudotuberculosis strain PA04 is the third complete genome to be published from the State of Pará. It was isolated from a mandibular lymph node puncture from a male Dorper breed sheep in Pará, northern Brazil. This strain has been deposited in a Brazilian collection.

Identification was performed by biochemical and molecular methods, using the API Coryne kit (bioMérieux, USA) and PCR multiplex with *rpoB*, *16S*, and *pld* genes, respectively (11). The genome was sequenced by the Ion Torrent PGM platform using the 318 Chip, with a fragment library, where a total of 560,337,368 bp were produced, with a genomic coverage of 239×. The quality of the raw data was evaluated using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and filtering and trimming were performed using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) to re-

Received 20 February 2017 Accepted 21 February 2017 Published 20 April 2017

Citation Costa WLO, Alves JTC, Dias LM, Araújo CLDA, Morais E, Silva AGM, Andrade SS, Ramos RTJ, Silva A, Folador ARC. 2017. Whole-genome sequence of *Corynebacterium pseudotuberculosis* PA04, isolated from the lymph node of a sheep in the Amazon, Brazil. *Genome Announc* 5:e00202-17. <https://doi.org/10.1128/genomeA.00202-17>.

Copyright © 2017 Costa et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Adriana R. C. Folador, carneiroar@gmail.com.

* Present address: Adriana R. C. Folador, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brazil.

move reads with Phred quality scores below 20. The genome assembly was performed using the Mira software (12), which provided 40 contigs with an N_{50} of 221,388 bp and a total size of 2,353,386 bp. The number of contigs was reduced to four using the SeqMan Pro tool of the Lasergene 11 Core Suite (DNASTar), and the scaffold was generated by Mauve (13). Gap closure was performed by CLC Genomics Workbench (CLC bio).

This genome was automatically annotated using Rapid Annotations using Subsystems Technology (RAST) 2.0 (14). The RNAmmer 1.2 software (15) was used for the prediction of tRNAs and rRNAs. The coding sequence (CDS) correction was performed using the Artemis software (16) associated with BLASTp (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>) and UniProt (<http://www.uniprot.org>) databases.

This genome contains 2,338,093 bp, with a G+C content of 52.2% and a total of 2,104 CDSs, 41 pseudogenes, 12 rRNAs, and 49 tRNAs.

Accession number(s). This whole-genome project has been deposited at the GenBank database under the accession number [CP019587](https://www.ncbi.nlm.nih.gov/GenBank/CP019587).

ACKNOWLEDGMENTS

The present study was supported by the National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), Foundation for Supporting Research in the State of Pará (FAPESPA), and by the Genome and Proteome Network of the State of Pará (RPGP).

REFERENCES

- Soares SC, Trost E, Ramos RTJ, Carneiro AR, Santos AR, Pinto AC, Barbosa E, Aburjaile F, Ali A, Diniz CAA, Hassan SS, Fiaux K, Guimarães LC, Bakhtiar SM, Pereira U, Almeida SS, Abreu VAC, Rocha FS, Dorella FA, Miyoshi A, Silva A, Azevedo V, Tauch A. 2013. Genome sequence of *Corynebacterium pseudotuberculosis* biovar equi strain 258 and prediction of antigenic targets to improve biotechnological vaccine production. *J Biotechnol* 167:135–141. <https://doi.org/10.1016/j.jbiotec.2012.11.003>.
- Dorella FA, Pacheco LGC, Oliveira SC, Miyoshi A, Azevedo V. 2006. *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. *Vet Res* 37:201–218. <https://doi.org/10.1051/vetres:2005056>.
- D'Afonseca V. 2011. Pangenoma de *Corynebacterium pseudotuberculosis* biovar ovis e de espécies do gênero *Corynebacterium*. Ph.D. thesis. Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
- Arsenault J, Girard C, Dubreuil P, Daignault D, Galarneau JR, Boisclair J, Simard C, Bélanger D. 2003. Prevalence of and carcass condemnation from maedi-visna, paratuberculosis and caseous lymphadenitis in culled sheep from Quebec, Canada. *Prev Vet Med* 59:67–81. [https://doi.org/10.1016/S0167-5877\(03\)00060-6](https://doi.org/10.1016/S0167-5877(03)00060-6).
- Pinheiro RR, Gouveia AMG, Alves FSF, Haddad JPA. 2000. Aspectos epidemiológicos da caprinocultura cearense. *Arq Bras Med Vet Zootec* 52:534–543. <https://doi.org/10.1590/S0102-09352000000500021>.
- Alves JTC, Veras AAO, Cavalcante ALQ, de Sá PHCG, Dias LM, Guimarães LC, Morais E, Silva AGM, Azevedo V, Ramos RTJ, Silva A, Carneiro AR. 2016. Complete genome sequence of *Corynebacterium pseudotuberculosis* strain PA01, isolated from sheep in Pará, Brazil. *Genome Announc* 4(1):e01664-15. <https://doi.org/10.1128/genomeA.01664-15>.
- Çetinkaya B, Karahan M, Atil E, Kalin R, De Baere T, Vaneechoutte M. 2002. Identification of *Corynebacterium pseudotuberculosis* isolates from sheep and goats by PCR. *Vet Microbiol* 88:75–83. [https://doi.org/10.1016/S0378-1135\(02\)00089-5](https://doi.org/10.1016/S0378-1135(02)00089-5).
- Pinto AC, de Sá PH, Ramos RT, Barbosa S, Barbosa HP, Ribeiro AC, Silva WM, Rocha FS, Santana MP, de Paula Castro TL, Miyoshi A, Schneider MP, Silva A, Azevedo V. 2014. Differential transcriptional profile of *Corynebacterium pseudotuberculosis* in response to abiotic stresses. *BMC Genomics* 15:14. <https://doi.org/10.1186/1471-2164-15-14>.
- Silva WM, Carvalho RD, Soares SC, Bastos IF, Foador EL, Souza GHMF, Le Loir YL, Miyoshi A, Silva A, Azevedo V. 2014. Label-free proteomic analysis to confirm the predicted proteome of *Corynebacterium pseudotuberculosis* under nitrosative stress mediated by nitric oxide. *BMC Genomics* 15:1065. <https://doi.org/10.1186/1471-2164-15-1065>.
- Radusky LG, Hassan SS, Lanzarotti E, Tiwari S, Jamal SB, Ali J, Ali A, Ferreira RS, Barh D, Silva A, Turjanski AG, Azevedo VA. 2015. An integrated structural proteomics approach along the druggable genome of *Corynebacterium pseudotuberculosis* species for putative druggable targets. *BMC Genomics* 16(Suppl 5):S9. <https://doi.org/10.1186/1471-2164-16-S5-S9>.
- Pacheco LGC, Pena RR, Castro TLP, Dorella FA, Bahia RC, Carminati R, Frota MNL, Oliveira SC, Meyer R, Alves FSF, Miyoshi A, Azevedo V. 2007. Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. *J Med Microbiol* 56:480–486. <https://doi.org/10.1099/jmm.0.46997-0>.
- Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Müller WE, Wetter T, Suhai S. 2004. Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Res* 14:1147–1159. <https://doi.org/10.1101/gr.1917404>.
- Darling ACE, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 14:1394–1403. <https://doi.org/10.1101/gr.2289704>.
- 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, McNeil 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. <https://doi.org/10.1186/1471-2164-9-75>.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
- Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, Barrell B. 2000. Artemis: sequence visualization and annotation. *Bioinformatics* 16:944–945. <https://doi.org/10.1093/bioinformatics/16.10.944>.