

Original citation:

Araújo, Carlos Leonardo de A., Dias, Larissa M., Veras, Adonney A. O., Alves, Jorianne T. C., Cavalcante, Ana Lídia Q., Dowson, Christopher G., Azevedo, Vasco, Ramos, Rommel T. J., Silva, Artur and Carneiro, Adriana R.. (2016) Whole-genome sequence of Corynebacterium pseudotuberculosis 262 biovarequi isolated from cow milk. Genome Announcements, 4 (2). e00176.

Permanent WRAP URL:

http://wrap.warwick.ac.uk/86096

Copyright and reuse:

The Warwick Research Archive Portal (WRAP) makes this work of researchers of the University of Warwick available open access under the following conditions.

This article is made available under the Creative Commons Attribution 4.0 International license (CC BY 4.0) and may be reused according to the conditions of the license. For more details see: <u>http://creativecommons.org/licenses/by/4.0/</u>

A note on versions:

The version presented in WRAP is the published version, or, version of record, and may be cited as it appears here.

For more information, please contact the WRAP Team at: wrap@warwick.ac.uk





Whole-Genome Sequence of *Corynebacterium pseudotuberculosis* 262 Biovar *equi* Isolated from Cow Milk

Carlos Leonardo de A. Araújo,^a Larissa M. Dias,^a Adonney A. O. Veras,^a Jorianne T. C. Alves,^a Ana Lídia Q. Cavalcante,^a Christopher G. Dowson,^c Vasco Azevedo,^b Rommel T. J. Ramos,^a Artur Silva,^a Adriana R. Carneiro^{a*}

Center of Genomics and System Biology, Laboratory of Genomic and Bioinformatics, Federal University of Pará, Belém, Pará, Brazila³; Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil^b; School of Life Sciences, University of Warwick, Coventry, United Kingdom^c

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

We report the complete genome sequence of *Corynebacterium pseudotuberculosis* 262, isolated from a bovine host. *C. pseudotuberculosis* is an etiological agent of diseases with medical and veterinary relevance. The genome contains 2,325,749 bp, 52.8% G+C content, 2,022 coding sequences (CDS), 50 pseudogenes, 48 tRNAs, and 12 rRNAs.

Received 5 February 2016 Accepted 6 February 2016 Published 24 March 2016

Citation Araújo CLDA, Dias LM, Veras AAO, Alves JTC, Cavalcante ALQ, Dowson CG, Azevedo V, Ramos RTJ, Silva A, Carneiro AR. 2016. Whole-genome sequence of

Corynebacterium pseudotuberculosis 262 biovar equi isolated from cow milk. Genome Announc 4(2):e00176-16. doi:10.1128/genomeA.00176-16.

Copyright © 2016 Araújo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Adriana R. Carneiro, adrianarc@ufpa.br.

orynebacterium pseudotuberculosis is a Gram-positive, facultative intracellular, pleomorphic, nonsporulating, noncapsulated, nonmotile bacterium that is the etiological agent of caseous lymphadenitis (CLA) in small ruminants and pyogranulomatus reactions, ulcerative lymphangitis, and mastitic, necrotic, and ulcerative dermatitis in cattle, all of which are diseases with medical and veterinary relevance. C. pseudotuberculosis affects several species, including sheep, goat, horse, cattle, llama, alpaca, buffalo, and human. This organism has various survival mechanisms and uses many strategies to adapt to its environment. After infection, the bacteria become encapsulated within walled-off lesions from which they evade immune system-mediated destruction, giving rise to a state of persistence (1-3). The molecular determinants of *C. pseudotuberculosis* virulence have been described and enable the search for potential targets for the development of new vaccine candidates by "omics" methodologies (4-7).

According to their capability for nitrate reduction, the strains of *C. pseudotuberculosis* are divided into two biovars. The organisms that perform the reduction of nitrate are classified into biovar *equi*, most of which have been isolated from horses and cattle. Bacteria that cannot perform the reduction of nitrate belong to biovar *ovis*, frequently isolated from sheep and goat (8). However, in cattle there are reports of infection by both biovars (9).

Here, we report the genome sequencing of *Corynebacterium pseudotuberculosis* 262, the first strain belonging to biovar *equi* isolated from a bovine host. This strain has been deposited in a collection in Belgium.

C. pseudotuberculosis strain 262 was isolated from cow milk, and the genome sequencing was performed with an Ion Torrent PGM platform chip 318, with a fragment library. A total of 388,943,492 bp were produced, with 166× genomic coverage. Subsequently, the tool FastQC 0.11.4 (http://www.bioinformatics .babraham.ac.uk/projects/fastqc/) was used to evaluate the raw data, and FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) was used to remove the reads with quality below Phred 20. The

genome assembly was performed by Mira 4.0.2 (http://mira -assembler.sourceforge.net), which produced 29 contigs with an N_{50} of 333,604 bp. The manual curation was performed through CLC Genomics Workbench 8 and Artemis 16.0.0 software (10). Automatic genome annotation was performed using Rapid Annotations using Subsystem Technology 2.0 (RAST) (11), and manual curation was performed with Artemis software and the nonredundant protein databases Uniprot (http://www.uniprot.org/) and the National Center for Biotechnology Information (NCBI) (http: //www.ncbi.nlm.nih.gov/). tRNAs and rRNAs were predicted using the software tRNAScan-SE 1.21 (12) and RNAmmer 1.2 (13), respectively. The plasticity of pathogenicity islands (PAIs) was assessed with the Pathogenicity Island Prediction Software 1.1 (PIPS) (14), using C. glutamicum strain ATCC 21831 (CP007722.1) as the reference genome, which identified 10 pathogenicity islands.

The *C. pseudotuberculosis* strain 262 genome contains 2,325,749 bp, a G+C content of 52.8%, 2,022 coding sequences (CDS), 50 pseudogenes, 48 tRNAs, and 12 rRNAs.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited at GenBank under accession number CP012022.

FUNDING INFORMATION

This work, including the efforts of Adriana Ribeiro Carneiro, was funded by MCTI | Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). This work, including the efforts of Adriana Ribeiro Carneiro, was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This work, including the efforts of Artur Silva, was funded by Rede Paraense de Genômica e Proteômica.

REFERENCES

- Dorella FA, Pacheco LG, Oliveira SC, Miyoshi A, Azevedo V. 2006. Corynebacterium pseudotuberculosis microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Vet Res 37:201–218.
- 2. Baird GJ, Fontaine MC. 2007. Corynebacterium pseudotuberculosis and its

role in ovine caseous lymphadenitis. J Comp Pathol 137:179–210. http://dx.doi.org/10.1016/j.jcpa.2007.07.002.

- 3. Yeruham I, Elad D, Friedman S, Perl S. 2003. *Corynebacterium pseudo-tuberculosis* infection in Israeli dairy cattle. Epidemiol Infect 131:947–955. http://dx.doi.org/10.1017/S095026880300894X.
- D'Afonseca V, Moraes PM, Dorella FA, Pacheco LG, Meyer R, Portela RW, Miyoshi A, Azevedo V. 2008. A description of genes of *Corynebacterium pseudotuberculosis* useful in diagnostics and vaccine applications. Genet Mol Res 7:252–260. http://dx.doi.org/10.4238/vol7-1gmr438.
- 5. Dorella FA, Gala-Garcia A, Pinto AC, Sarrouh B, Antunes CA, Ribeiro D, Aburjaile FF, Fiaux KK, Guimarães LC. Seyffert N, El-Aouar RA, Silva R, Hassan SS, Castro TL, Marques WS, Ramos R, Carneiro A, de Sá P, Miyoshi A, Azevedo V, Silva A. 2013. Progression of 'OMICS' methodologies for understanding the pathogenicity of *Corynebacterium pseudotuberculosis*: the Brazilian experience. Comput Struct Biotechnol J 6:1–7. http://dx.doi.org/10.5936/csbj.201303013.
- Seyffert N, Silva RF, Jardin J, Silva WM, de Paula Castro TL, Tartaglia NR, de Oliveira SKT, Portela RW, Silva A, Miyoshi A, Le Loir Y, Azevedo V. 2014. Serological proteome analysis of *Corynebacterium pseudotuberculosis is*olated from different hosts reveals novel candidates for prophylactics to control caseous lymphadenitis. Vet Microbiol 174: 255–260. http://dx.doi.org/10.1016/j.vetmic.2014.08.024.
- Radusky LG, Hassan S, Lanzarotti E, Tiwari S, Jamal S, Ali J, Ali A, Ferreira R, 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. http://dx.doi.org/10.1186/1471-2164-16 -S5-S9.
- Hassan SS, Guimarães LC, Pereira Ude P, Islam A, Ali A, Bakhtiar SM, Ribeiro D, Rodrigues Dos Santos A, Soares Sde C, de C, Dorella F, Pinto AC, Schneider MP, Barbosa MS, Almeida S, Abreu V, Aburjaile

F, Carneiro AR, Cerdeira LT, Fiaux K, Barbosa E, Diniz C, Rocha FS, Ramos RT, Jain N, Tiwari S, Barh D, Miyoshi A, Müller B, Silva A, Azevedo V. 2012. Complete genome sequence of *Corynebacterium pseudotuberculosis* biovar *ovis* strain p54b96 isolated from antelope in South Africa obtained by rapid next generation sequencing technology. Stand Genomic Sci 7:189–199. http://dx.doi.org/10.4056/sigs.3066455.

- Yeruham I, Braverman Y, Shpigel NY, Chizov-Ginzburg A, Saran A, Winkler M. 1996. Mastitis in dairy cattle caused by *Corynebacterium pseudotuberculosis* and the feasibility of transmission by houseflies I. Vet Q 18:87–89. http://dx.doi.org/10.1080/01652176.1996.9694623.
- Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, Barrell B. 2000. Artemis: sequence visualization and annotation. BioInformatics 16:944–945. http://dx.doi.org/10.1093/bioinformatics/ 16.10.944.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 42: D206–D214. http://dx.doi.org/10.1093/nar/gkt1226.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
- 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. http://dx.doi.org/10.1093/ nar/gkm160.
- 14. Soares SC, Abreu VAC, Ramos RTJ, Cerdeira L, Silva A, Baumbach J, Trost E, Tauch A, Hirata R, Jr, Mattos-Guaraldi AL, Miyoshi A, Azevedo V. 2012. PIPS: pathogenicity island prediction software. PLoS One 7:e30848. http://dx.doi.org/10.1371/journal.pone.0030848.