Whole-Genome Sequence of Corynebacterium pseudotuberculosis PAT10 Strain Isolated from Sheep in Patagonia, Argentina

Louise Teixeira Cerdeira,¹ Anne Cybelle Pinto,² Maria Paula Cruz Schneider,¹ Sintia Silva de Almeida,² Anderson Rodrigues dos Santos,² Eudes Guilherme Vieira Barbosa,² Amjad Ali,² Maria Silvanira Barbosa,¹ Adriana Ribeiro Carneiro,¹ Rommel Thiago Jucá Ramos,¹ Rodrigo Santos de Oliveira,¹ Debmalya Barh,⁴ Neha Barve,⁴ Vasudeo Zambare,^{4,5} Silvia Estevão Belchior,³ Luis Carlos Guimarães,² Siomar de Castro Soares,² Fernanda Alves Dorella,² Flavia Souza Rocha,² Vinicius Augusto Carvalho de Abreu,² Andreas Tauch,⁶ Eva Trost,⁶

Anderson Miyoshi,² Vasco Azevedo,² and Artur Silva^{1*}

Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Brazil¹; Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil²; Universidad Nacional de la Patagonia San Juan Bosco, Chubut, Argentina³; Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakuri, Purba Medinipur, West Bengal, India⁴; Center for Bioprocessing Research and Development (CBRD),

South Dakota School of Mines and Technology, Rapid City, South Dakota⁵; and CeBiTec,

University of Bielefeld, 33594 Bielefeld, Germany⁶

Received 20 August 2011/Accepted 26 August 2011

In this work, we report the complete genome sequence of a Corynebacterium pseudotuberculosis PAT10 isolate, collected from a lung abscess in an Argentine sheep in Patagonia, whose pathogen also required an investigation of its pathogenesis. Thus, the analysis of the genome sequence offers a means to better understanding of the molecular and genetic basis of virulence of this bacterium.

The incidence of caseous lymphadenitis (CLA) is high in many regions of the world, resulting in huge and significant economic losses in agribusinesses, as it is responsible for a decrease in wool production and carcass quality (3). The disease is endemic in flocks in the provinces of Chubut and Santa Cruz in the Southern Patagonia region of Argentina, thereby leading to an outrageous prevalence rate of about 70% within individual flocks in Patagonia (4). Diseases caused by Corynebacterium pseudotuberculosis present in various clinical forms, and sheep and goats are affected by CLA (1). Analysis of the genome sequence improves our understanding of the molecular and genetic basis of the virulence of the bacterium. We hereby report the whole-genome sequence of C. pseudotuberculosis PAT10 as determined using the SOLiD platform. In total, we generated 27,858,221 mate-paired short reads (25 bp) of usable sequences (296-fold coverage). Furthermore, a hybrid de novo assembly approach was applied using 16,885,903 short (25-bp) mate-paired SOLiD filtered reads; that strategy allowed close gaps without a bench work time cost (2). The automatic and manual annotations were done using several algorithms in a multistep process.

For structural annotation, the following software was used: FgenesB (gene predictor); RNAmmer (rRNA predictor) (5); tRNA-scan-SE (tRNA predictor) (6); and Tandem Repeat Finder (repetitive DNA predictor) (http://tandem.bu.edu/trf /trf.html). Functional annotation was performed using similarity analyses and public databases and by InterProScan analysis (8). Manual annotation was performed using Artemis software (7). Identification and confirmation of pseudogenes in the genome were carried out using CLCBio Workbench 4.0.2 software. Manual analysis was performed based on the Phred quality of each base combined with analysis of the depth of coverage of the frameshift region. That analysis allowed the identification of false-positive pseudogene results. The genome of the PAT10 strain consists of a 2,335,323-bp circular chromosome, and the average G+C content of the chromosome is 52.19%. The genome was predicted to contain 2,079 coding sequences (CDS), four rRNA operons, 49 tRNAs, and 61 pseudogenes.

The characterization of the PAT10 genome should identify and unravel the mechanisms of virulence of this pathogen through comparative analyses performed with other sequenced genomes of the genus and the same species, thereby allowing the development of new diagnostics kits and vaccines.

Nucleotide sequence accession number. The genome sequence obtained in this study has been deposited in the GenBank database under accession number CP002924 (chromosome).

This work is part of the Rede Paraense de Genômica e Proteômica supported by FAPESPA CNPq, CAPES, and FAPEMIG.

REFERENCES

- 1. Barakat, A. A., S. A. Sekim, A. Atef, M. S. Saber, and E. K. Nafie. 1984. Two serotypes of Corynebacterium pseudotuberculosis isolated from different animal species. Rev. Sci. Tech. Off. Int. Epiz.: 3:151-163.
- Cerdeira, L. T., et al. 2011. Rapid hybrid de novo assembly of a microbial 2. genome using only short reads: Corynebacterium pseudotuberculosis I19 as a case study. J. Microbiol. Methods 86:218-223.
- 3. Dorella, F. A., L. G. C. Pacheco, S. C. Oliveira, A. Miyoshi, and V. Azevedo. 2006. Corynebacterium pseudotuberculosis: microbiology, biochemical

^{*} Corresponding author. Mailing address: Instituto de Ciências Biológicas, Universidade Federal do Pará, Av. Augusto Corrêa 01, Guamá, CEP 66075-110, Belém, PA, Brazil. Phone and fax: 55 91 3201-8426. E-mail: asilva@ufpa.br.

properties, pathogenesis and molecular studies of virulence. Vet. Res. 37:201-218.

- Estevao-Belchior, S., A. Gallardo, A. Abalos, N. Jodor, and D. Jensen. 2006. Actualización sobre linfoadenitis caseosa: el agente etiológico y la enfermedad. Ve. Argent. 23:258–278.
- Lagesen, K., et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.
- Lowe, T. M., and S. R. Eddy. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25:955–964.
- Rutherford, K., et al. 2000. Artemis: sequence visualization and annotation. Bioinformatics 16:944–945.
- Zdobnov, E. M., and R. Apweiler. 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. Bioinformatics 17:847–848.