JOURNAL OF BACTERIOLOGY, May 2010, p. 2645–2646 Vol. 192, No. 10
0021-9193/10/$12.00 doi:10.1128/JB.00159-10

Complete Genome Sequence and Annotation of the *Treponema pallidum* subsp. *pallidum* Chicago Strain▽

Lorenzo Giacani,[1]†* Brendan M. Jeffrey,[2]† Barbara J. Molini,[1] HoaVan T. Le,[1] Sheila A. Lukehart,[1,3] Arturo Centurion-Lara,[1] and Daniel D. Rockey[2]

Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle, Washington 98195[1]; Molecular and Cellular Biology Program and Department of Biomedical Sciences, Oregon State University, Corvallis, Oregon 97331[2]; and Department of Global Health, University of Washington, Seattle, Washington 98195[3]

Received 15 February 2010/Accepted 15 March 2010

In syphilis research, the Nichols strain of *Treponema pallidum*, isolated in 1912, has been the most widely studied. Recently, important differences among *T. pallidum* strains emerged; therefore, we sequenced and annotated the Chicago strain genome to facilitate and encourage the use of this strain in studying the pathogenesis of syphilis.

Syphilis continues to be a common and serious disease, affecting at least 25 million adults worldwide (4). It is a recognized cofactor in the transmission and acquisition of HIV (2, 6) and is a major cause of stillbirth and perinatal morbidity in the developing world (11, 12). The peculiar biology of the causative agent of syphilis, *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), along with the inability to continuously grow this pathogen *in vitro*, has hindered progress in this field. However, syphilis research has greatly benefited from the completion of the *T. pallidum* Nichols strain genome sequence, published in 1998 (3). The Nichols strain of *T. pallidum* was isolated in 1912 from the cerebrospinal fluid (CSF) of a patient with secondary syphilis, has become the laboratory reference strain for the study of syphilis, and has consequently been passed in rabbits for nearly a century. Our growing understanding of the mechanisms behind *T. pallidum*'s ability to evade the host immune response to persist in the host, however, would not be possible without access to strains that have not undergone continual animal passage. The Chicago strain (14), first isolated in 1951, has not been continually passed in rabbits and has been critical to our understanding of fundamental aspects of the pathogenesis of syphilis, such as antigenic variation, immune escape, and persistence of the pathogen in the host (1, 5, 7). We sequenced and annotated the Chicago genome to facilitate and encourage the use of this strain in addressing questions concerning the pathogenesis of syphilis.

Genomic DNA isolated from the *T. pallidum* Chicago strain, initially obtained from Paul Hardy and Ellen Nell, was prepared for Illumina-based sequencing using the Paired End DNA sample preparation kit (Illumina Inc., San Diego, CA) following the provided protocol. Genome sequencing was performed at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University (Corvallis, OR) using a Genome Analyzer *IIx*System (Illumina Inc.). A first draft of the Chicago strain genome was assembled using the reference-guided assembly program Maq (8) with the *T. pallidum* Nichols strain genome (3) as reference. Regions in the reference-guided assembled genome where Maq could not resolve sequence were then compared to contiguous sequences assembled through the use of the *de novo* assembly software VCAKE, and a single contiguous draft sequence was then produced (13). Nucleotide changes between matched pairs were located using the Diffseq program from the Emboss software suite. For annotation, the Chicago strain genome sequence was submitted to the J. Craig Venter Institute (JCVI) Annotation Service (http://www.jcvi.org/cgi-bin/annotation/service/submit/annengine.cgi).

The Chicago genome is 1,139,281 bases long and encodes 1,081 open reading frames (ORFs). Forty-four nucleotide substitutions, 21 deletions (involving 30 nucleotides [nt]), and 75 insertions (involving 1,303 nt) were found by Diffseq software compared to the Nichols strain genome.

The Chicago genome will provide further insight into the poorly understood subject of genetic variability among syphilis strains (3, 9). Genetic variability among *T. pallidum* strains is likely one of the factors influencing the protean manifestations of syphilis infection which are seen both clinically and experimentally (10), possibly reflecting differential virulence among strains or niche differences.

Nucleotide sequence accession number. The complete genome sequence of *Treponema pallidum* subsp. *pallidum* Chicago was deposited in the GenBank database and assigned accession number CP001752.

We are thankful to Elizabeth Miko Robertson for helping with manuscript preparation and submission.

This work was supported by NIH grants AI42143 and AI63940 (to S.A.L. and A.C.-L.).

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* Corresponding author. Mailing address: University of Washington, Harborview Medical Center, 325 9th Ave., Seattle, WA 98104. Phone: (206) 897-5402. Fax: (206) 897-5363. E-mail: giacal@u.washington.edu.
† L.G. and B.M.J. contributed equally to this work.
▽ Published ahead of print on 26 March 2010.

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