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Genome Sequence of *Mycobacterium bovis* BCG Moreau, the Brazilian Vaccine Strain against Tuberculosis

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***Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is the only vaccine available against tuberculosis, and the strains used worldwide represent a family of daughter strains with distinct genotypic characteristics. Here we report the complete genome sequence of *M. bovis* BCG Moreau, the strain in continuous use in Brazil for vaccine production since the 1920s.**

Mycobacterium bovis bacillus Calmette-Guérin (BCG) remains the only vaccine available against tuberculosis (TB). The attenuated strain was derived from an *M. bovis* isolate after 230 serial passages *in vitro* by Albert Calmette and Camille Guérin at Institut Pasteur in the early 20th century. It was distributed to laboratories worldwide and maintained in culture until the 1960s, when seed lots were established (13). BCG arrived in Brazil in the 1920s (2). Preliminary assays done at Instituto Oswaldo Cruz demonstrated the safety of BCG preparations in animal models; in 1927, Carlos Chagas, director of the Institute, together with Instituto Vital Brazil (BCG producer until 1930) and the Brazilian League against Tuberculosis (currently known as Fundação Ataulpho de Paiva, producer of BCG since 1930), decided to extend vaccination to infants (5). The strain used in Brazil is known as BCG Moreau. The vaccine is part of the National Immunization Program and is given shortly after birth, with over 99% coverage in a population close to 200 million (19). Despite its importance, little effort has been made to characterize BCG Moreau at the molecular level (3).

The different "BCG vaccines" produced worldwide comprise a heterogeneous family of daughter strains (1, 12). The complete genomes of strains Pasteur (4) and Tokyo (18) have been reported, as well as draft sequences for strains China, Denmark, Russia, and Tice (16). Here we report the complete, annotated genome sequence of *M. bovis* BCG Moreau. The sequence was obtained from the shotgun sequencing of approximately 20,640 templates from 2 independent pBluescript libraries (average inserts of 2 kb and a 5- to 10-kb range), yielding over 50,000 reads. Sequencing was performed in an ABI 3730 DNA sequencer (Fiocruz/PDTIS sequencing platform; Applied Biosystems) (14). The reads were assembled using Phrap (http://www.phrap.org/), and gaps were closed by direct sequencing of PCR templates with primers designed using Consed (8). Approximately 38 million bp were included in the assembly, yielding 6× high-quality (Q, >20) genome coverage. We used a preliminary version of RATT (15) to transfer the annotation from the BCG Pasteur (4) genome. Manual improvement of the annotation was done with Artemis (17), by further comparison to the genomes of *M. tuberculosis* H37Rv (6) and *M. bovis* AF2122/97 (7). The curated and annotated genome sequence of *M. bovis* BCG Moreau comprises a circular chromosome of 4,340,116 bp, with an average G+C content of 65.64% and a total of 3,945 predicted protein coding regions. As in *M. tuberculosis* H37Rv, the genome has a single copy of rRNA genes and 45 predicted tRNA genes. Consistent with previous studies (4, 11), we confirmed the presence of tandem duplication DU2-I, a Moreau-specific deletion in *fadD26-ppsA* (976 bp), and region of difference RD16 (a 7,608-bp deletion in BCG Moreau compared to *M. tuberculosis* H37Rv). We also confirm the in-frame deletion found in *rv3887c* (*eccD2*), but its length differs from that previously reported (876 bp in our study compared to 1,128 bp [11]). Studies have shown that "early" derived strains, such as BCG Moreau, are more immunogenic (10) and may confer better protection against tuberculosis (9). Detailed ongoing functional evaluation of selected nonsynonymous single-nucleotide polymorphisms and frameshifts caused by small indels, allowing correlation of comparative genomics with the physiology of BCG strains, will contribute to a better understanding of vaccine efficacy.

Nucleotide sequence accession number. The genome sequence of *M. bovis* BCG Moreau has been deposited in GenBank/EMBL under accession no. AM412059.

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