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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Mycoplasma bovis* Type Strain PG45 (ATCC 25523)▽

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This complete and fully assembled genome sequence of *Mycoplasma bovis* type strain PG45 is the first available for this species and offers a framework for comparison with additional pathogenic isolates. The single circular chromosome of 1,003,404 bp reveals multiple gene sets and mechanisms involved in variable expression of surface antigens and the incursion of numerous and assorted mobile elements, despite its reduced size.

Mycoplasma bovis is a major bacterial pathogen causing widespread respiratory disease, mastitis, and arthritis in cattle (1, 5). A member of the wall-less *Mollicutes* class, it displays marked genome reduction and a parasitic lifestyle lacking key metabolic pathways. It is refractory to several classes of antibiotics, and bacterins for immunological control need continued improvement. Adaptive variation through high-frequency phase variation of surface lipoproteins (LPs) is known to occur through site-specific recombination among the family of *vsp* genes (8, 9). Horizontal gene transfer (HGT) of some gene sets (13), numerous classes of insertion-like sequences (IS elements) (7), and integrative conjugative elements (ICE) (10) are reported in *M. bovis* or its close phylogenetic relative *M. agalactiae*, a pathogenic species infecting caprine hosts. Complete sequencing and assembly of the *M. bovis* genome were pursued to better reveal its content and dynamics relevant to disease control measures. In order to minimize rearrangements or mutational heterogeneity in the genome sample, template DNA was prepared from an axenic culture propagated from a single colony isolate (MU clone A2, phenotype VspO ON; available under proper regulatory controls). The genome, comprising a single circular chromosome, was sequenced to closure using the Sanger random shotgun method (3), yielding approximately 8-fold sequence coverage.

The genome has a 29.3% G+C content, contains 826 open reading frames (ORFs; including 61 pseudogenes) with an 89% coding density, and has limited sets of 6 rRNA and 34 tRNA genes, characteristic of *Mollicutes*. A large set of 54 IS elements (comprising seven distinct categories) (7) is scattered throughout the chromosome. Two ICE occur. ICEB-1 (23,271 bp, 18 ORFs) is a counterpart to ICEA, an element similarly positioned in the highly syntenous *M. agalactiae* PG2 genome (11), thereby suggesting that integration occurred in a common ancestor prior to speciation. ICEB-2 (37,408 bp, 33 ORFs) is inserted into an IS element that is implicated in the inversion of a 483-kb region of the *M. bovis* PG45 chromosome, relative to *M. agalactiae* PG2. The potential for genome plasticity among strains of *M. bovis* is underscored by these features.

The predicted LP surface proteome of *M. bovis* type strain PG45 was determined using established algorithms (2, 6) and an extended search pattern, {DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGSTKRQ]-C, to include atypical residues (underlined) at the −1 position of the lipobox, previously demonstrated in this lineage of *Mollicutes* (4, 9). Of 96 predicted LPs, 40 contain atypical lipoboxes, including 35 with K or R at the −1 position (13 of which are encoded in the known *vsp* genes). Two groups of LP genes reveal signatures denoting distinct, mutation-based mechanisms of phase variation. First, the same *vsp* genes are present but extensively rearranged compared to the cluster previously reported on cloned genomic fragments from the PG45 strain of *M. bovis* (9). Second, a newly identified set of 19 dispersed LP genes, each with a rare homopolymeric tract of 7 to 12 G or C residues in the N-terminal coding region, represent contingency loci subject to frameshift mutations, thereby predicting combinatorial patterns of expression for their translation products. Fifteen of these LP genes (annotated as authentic frameshifts) are in the OFF configuration. Finally, nine LP genes, some having these poly-G or -C tracts, encode DUF285 motifs denoting recently described *p*alindromic *a*mphipathic *r*epeat *c*oding *e*lements (PARCELs) distributed by HGT among diverse bacteria (12), including some *Mollicutes* (12, 13). Analogous systems of variation and chromosomal plasticity occur in *M. bovis* and *M. agalactiae*, predicting phenotypic and genotypic instability in populations. These properties may be important in assigning phenotypes to pathogenic field isolates and in the design of strategies to develop effective control measures.

Nucleotide sequence accession number. The complete genome sequence of *M. bovis* type strain PG45 is available in GenBank under accession number CP002188.

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