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Complete Genome Sequence of *Melissococcus plutonius* ATCC 35311▽

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We report the first completely annotated genome sequence of *Melissococcus plutonius* ATCC 35311. *M. plutonius* is a one-genus, one-species bacterium and the etiological agent of European foulbrood of the honeybee. The genome sequence will provide new insights into the molecular mechanisms underlying its pathogenicity.

Melissococcus plutonius, the etiological agent of European foulbrood of the honeybee (10), was first cultured and characterized in detail by Bailey (2). Although selective media have been used to cultivate *M. plutonius* (5), the relative complexity of its culture procedure makes it extremely difficult to isolate. Although it can be detected using a PCR method targeting its 16S rRNA sequence (6, 8), lack of genome information hinders further development of a rapid and reliable diagnostic method for European foulbrood. The strain selected for sequencing (*M. plutonius* ATCC 35311) was the type strain of *M. plutonius*, which was originally deposited at the National Collection of Dairy Organisms by Bailey and Collins (3).

The genome of *M. plutonius* was sequenced using the Roche genome sequencer FLX Titanium. We obtained a total of 246,722 reads, covering a total of 90,529,838 bp, or 45.1-fold coverage. Sequences were assembled into a total of 47 contigs. Gaps were filled by Sanger sequencing of PCR products by brute force amplification of the regions between each pair of contigs. Primary coding sequence (CDS) extraction and initial functional assignment were performed by the automated annotation servers RAST (1) and ISGA (7). Their results were compared to verify the annotation and were corrected manually by *in silico* molecular cloning (*In Silico* Biology, Inc., Kanagawa, Japan).

The *M. plutonius* ATCC 35311 genome consists of a single circular chromosome of 1,891,014 bp, with an average GC content of 31.4%, and a plasmid of 177,718 bp, with an average GC content of 29.2%. The chromosome contained a total of 1,773 protein-coding genes, 61 tRNA genes for all amino acids, and four *rrn* operons, while the plasmid contained a total of 150 protein-coding genes. In addition, the chromosome harbors 1 prophage-like element. Whole-chromosome comparison using the BLAST algorithm showed that the closest organism to *M. plutonius* ATCC 35311 was *Enterococcus faecalis* V583 (9), with 12% genome coverage.

M. plutonius requires potassium for efficient growth. Interestingly, we found that *M. plutonius* ATCC 35311 harbors only two genes, one encoding a potassium efflux system KefA protein and the other encoding a high-conductance mechanosensitive channel, that are putatively involved in potassium homeostasis. *M. plutonius* ATCC 35311 also lacks a tricarboxylic acid (TCA) cycle, an electron transport system, and a sugar alcohol utilization system, although it likely has a glycolysis system and a pentose phosphate pathway. *M. plutonius* ATCC 35311 harbors clustered regularly interspaced short palindromic repeats (CRISPR), which confer resistance to infection by phages (4). The plasmid of *M. plutonius* ATCC 35311 encodes a number of transporters, sugar chain-modifying enzymes, and, interestingly, an NADH dehydrogenase.

Nucleotide sequence accession numbers. Nucleotide sequences of the chromosome and plasmid of *M. plutonius* ATCC 35311 have been deposited in the DNA Database of Japan database under accession no. AP012200 and AP012201, respectively.

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