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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Melissococcus plutonius* DAT561, a Strain That Shows an Unusual Growth Profile and Is Representative of an Endemic Cluster in Japan

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We report the complete genome sequence of *Melissococcus plutonius* DAT561, which is a causative agent of European foulbrood. *M. plutonius* DAT561 is a representative of nonfastidious strains isolated in Japan. The addition of potassium phosphate was not required for normal growth, unlike for typical *M. plutonius* strain/isolates.

Melissococcus plutonius is the etiological agent of European foulbrood (EFB) of honeybees (3). We recently reported the completely annotated genome sequence of the type strain of *M. plutonius* (ATCC 35311) (4). Although *M. plutonius* is believed to be remarkably homologous, *M. plutonius*-like organisms, with characteristics seemingly different from those of typical *M. plutonius* strains, have often been isolated from diseased larvae with clinical signs of EFB in Japan. We characterized the *M. plutonius*-like organisms and found they were taxonomically identical to *M. plutonius* (1). However, unlike for typical *M. plutonius* strains/isolates, the addition of potassium phosphate was not required for normal growth of atypical (*M. plutonius*-like) isolates. In addition, atypical *M. plutonius* isolates, but not typical *M. plutonius* strains/isolates, grew anaerobically on sodium phosphate-supplemented medium and aerobically on some potassium salt-supplemented media. Although *M. plutonius* is known to lose virulence quickly when cultured artificially, experimental infection of representative isolates showed that atypical *M. plutonius* strains maintained the ability to cause EFB in honeybee larvae even after repeated subculture *in vitro* in laboratory media (1). The complete genome sequence of *M. plutonius* DAT561, a representative atypical isolate, in addition to the genome sequence of the typical strain *M. plutonius* ATCC 35311, should provide valuable insight into EFB research.

The genome of *M. plutonius* was sequenced by using a Roche Genome Sequencer FLX Titanium. We obtained a total of 133,622 reads, covering a total of 48,795,366 bp, or 23.9-fold coverage. Sequences were assembled into a total of 62 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 server RAST (2). The result was compared with the annotated genome sequence of *M. plutonius* ATCC 35311 to verify annotation and corrected manually by *in silico* molecular cloning (*In Silico* Biology, Inc., Kanagawa, Japan).

The *M. plutonius* DAT561 genome consists of a single circular chromosome of 1,846,178 bp, with an average GC content of 31.5%, and a plasmid of 199,075 bp, with an average GC content of 29.1%. The chromosome contained a total of 1,567 protein-coding genes, 55 tRNA genes for all amino acids, and four *rrn* operons, while the plasmid contained a total of 156 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* DAT561 was *M. plutonius* ATCC 35311, as expected from our DNA-DNA hybridization study (1) with 99% genome coverage.

Nucleotide sequence accession numbers. Nucleotide sequences of the chromosome and plasmid of *M. plutonius* DAT561 have been deposited in the DNA Database of Japan under accession numbers AP012282 and AP012283, respectively.

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