Complete Genome Sequences of Two *Melissococcus plutonius* Strains with Different Virulence Profiles, Obtained by PacBio Sequencing

Kayo Okumura,a Daisuke Takamatsu,b,c Masatoshi Okurab

aDepartment of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan
bDivision of Bacterial and Parasitic Disease, National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan
cThe United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan

**ABSTRACT** *Melissococcus plutonius* attacks honeybee larvae, causing European foulbrood. Based on their virulence toward larvae, *M. plutonius* isolates were classified into three types, highly virulent, moderately virulent, and avirulent. We herein performed whole-genome sequencing of *M. plutonius* isolates with different virulence levels to promote an understanding of the pathogenesis of this disease.

The causative agent of European foulbrood, *Melissococcus plutonius*, infects honeybee larvae, with serious impacts on bee health (1). Based on multilocus sequence typing analysis, *M. plutonius* isolates were classified into three clonal complexes (CCs), CC3, CC12, and CC13 (2). These CCs exhibited different virulence profiles toward honeybee larvae in experimental infections; CC12 and CC3 strains were extremely and moderately virulent, respectively, whereas the representative CC13 strain was avirulent (3). To clarify the genetic basis of the distinct pathological characteristics of each CC, we performed complete genome sequencing of *M. plutonius* DAT606 and DAT585, which are representative CC3 and CC13 strains, respectively. Previously, we sequenced the genomes of two *M. plutonius* strains, one type strain and one highly virulent strain belonging to CC12 (4, 5). Taken together with the previous genomic data, we have covered all virulence profiles of *M. plutonius*.

*M. plutonius* DAT606 and DAT585 were isolated from diseased European honeybee (*Apis mellifera*) larvae in Japan (6) and cultured anaerobically on brain heart infusion agar supplemented with KH2PO4 and starch (KSBHI agar) for 4 days at 35°C. Then, genomic DNA was extracted as described previously, with a slight modification (6); proteinase K treatment was not performed.

Whole-genome sequencing of *M. plutonius* DAT585 and DAT606 was performed on the PacBio (Menlo Park, CA, USA) RS II platform. The library was prepared using single-molecule real-time (SMRT) cell 8Pac V3 and the P6 DNA polymerase binding kit (PacBio), according to the manufacturer’s instructions. Reads were filtered and assembled using SMRT Analysis v2.3 (PacBio) with default settings. The DAT585 genome yielded 100,098 reads encompassing 950,202,716 bp. The mean subread length and N50 value were 9,492 bp and 13,788 bp, respectively. The DAT606 genome yielded 76,697 reads covering 615,765,788 bp. The mean subread length and N50 value were 8,028 bp and 12,056 bp, respectively. Subsequently, the filtered reads for the two genomes were assembled de novo, producing two circular contigs. As reported previously (5), virulent strains possess another plasmid, pMP19; therefore, for virulent strain DAT606, Sanger sequencing was conducted using conventional primer walking, followed by sequence assembly with Sequencher 5.2 software (Gene Codes, Ann Arbor, MI, USA). Primary coding sequence extraction and initial functional assignment were performed using the automated annotation server RASTtk (7). To verify the annotation, the data were
The chromosomes of both strains contain 60 tRNA genes for all amino acids and four rRNA operons. Additionally, both chromosomes harbor two prophages, one intact and one incomplete. The DAT606 genome contains two plasmids, pMP1 and pMP19, although pMP19 was partially sequenced because of long repeated sequences in a plasmid gene. However, the avirulent strain, DAT585, harbors the pMP1 plasmid only (Table 1).

**Data availability.** The whole-genome sequences of the chromosome and two plasmids of *M. plutonius* DAT585 and DAT606 were deposited in DDBJ/GenBank under accession numbers AP018524 to AP018528 (Table 1). The raw sequence reads were deposited in the DDBJ Sequence Read Archive (DRA)/NCBI SRA under accession numbers DRA008260 and DRA008261 (Table 1).

**ACKNOWLEDGMENTS**

This study was supported by a Grant-in-Aid for Scientific Research (C) (17K08818) from the Japan Society for the Promotion of Science.

D.T. designed the study, and K.O. and D.T. determined the sequences. K.O. deposited the data in DDBJ and GenBank. All authors contributed to data analysis and preparation of the manuscript and approved the final version.

We declare no competing interests.

**REFERENCES**

1. Bailey L. 1983. *Melissococcus pluton*, the cause of European foulbrood of honey bees (*Apis spp*). J Appl Bacteriol 55:65–69. https://doi.org/10.1111/j.1365-2672.1983.tb02648.x.

2. Budge GE, Shirley MD, Jones B, Quill E, Tomkies V, Feil EJ, Brown MA, Haynes EG. 2014. Molecular epidemiology and population structure of the honey bee brood pathogen *Melissococcus plutonius*. ISME J 8:1588–1597. https://doi.org/10.1038/ismej.2014.20.

3. Nakamura K, Yamazaki Y, Shiraiishi A, Kobayashi S, Harada M, Yoshiyama M, Osaki M, Okura M, Takamatsu D. 2016. Virulence differences among *Melissococcus plutonius* strains with different genetic backgrounds in *Apis mellifera* larvae under an improved experimental condition. Sci Rep 6:33239. https://doi.org/10.1038/srep33239.

4. Okumura K, Araki R, Okura M, Kirikae T, Takamatsu D, Osaki M, Miyoshi-Akiyama T. 2011. Complete genome sequence of *Melissococcus plutonius* ATCC 35311. J Bacteriol 193:4029–4030. https://doi.org/10.1128/JB.05151-11.

5. Okumura K, Takamatsu D, Okura M. 2018. Complete genome sequence of *Melissococcus plutonius* DAT561, a strain that shows an unusual growth profile, obtained by PacBio sequencing. Genome Announc 6:e00431-18. https://doi.org/10.1128/genomeA.00431-18.

6. Arai R, Tominaga K, Kuwata M, Okura M, Ito K, Okamura N, Onishi H, Osaki M, Sugimura Y, Yoshiyama M, Takamatsu D. 2012. Diversity of *Melissococcus plutonius* from honeybee larvae in Japan and experimental reproduction of European foulbrood with cultured atypical isolates. PLoS One 7:e33708. https://doi.org/10.1371/journal.pone.0033708.

7. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RAST: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.

8. Andri D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.

---

**TABLE 1** General features of *Melissococcus plutonius* genomes determined by PacBio sequencing in this study and previous studies

| Strain (clonal complex) | Chromosome or plasmid | Size (bp) | No. of coding sequences | No. of pseudogenes | GC content (%) | Accession no. for: |
|------------------------|-----------------------|----------|-------------------------|-------------------|----------------|-------------------|
| DAT585 (CC13)          | Chromosome            | 1,890,300| 1,481                   | 151               | 31.4           | AP018524          |
|                        | pMP1                  | 177,500  | 136                     | 13                | 29.2           | AP018525          |
| DAT606 (CC3)           | Chromosome            | 1,898,117| 1,489                   | 148               | 31.4           | AP018526          |
|                        | pMP1                  | 177,678  | 136                     | 16                | 29.2           | AP018527          |
|                        | pMP19                 | 19,989   | 24                      | 0                 | 30.3           | AP018528          |
| DAT561 (CC12)          | Chromosome            | 1,847,807| 1,531                   | 18                | 31.5           | AP018492          |
|                        | pMP1                  | 200,057  | 159                     | 3                 | 29.2           | AP018493          |
|                        | pMP19                 | 19,967   | 28                      | 0                 | 30.3           | AP018494          |