First Complete Genome Sequence of *Haematobacter massiliensis* OT1 (Chromosome and Multiple Plasmids), Isolated from Human Skin

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**ABSTRACT** *Haematobacter massiliensis* OT1 was isolated from human skin. This strain can catabolize 4-hydroxybenzoate. Here, we present the first complete whole-genome sequence of this species, which has one chromosome (2,467 kbp) and nine plasmids (total of 1,765 kbp). The analysis of the *H. massiliensis* OT1 genome indicated a potential for autotrophic growth.

*Haematobacter massiliensis* is a Gram-negative aerobic bacillus belonging to the family *Rhodobacteraceae* (1). This species has been reported to be found on human skin and as a rare opportunistic pathogen linked with endocarditis and septicemia (1). Currently, only three genome assemblies of *H. massiliensis* are publicly available in the NCBI database, each having more than 50 scaffolds. Here, we report the complete genome sequence of *H. massiliensis* OT1, an isolate from human skin.

*H. massiliensis* OT1 (KCTC 62810) was isolated from the forehead of a male college student by swab sampling and direct streaking on minimal salts basal (MSB) medium containing 0.01% yeast extract and 0.05% 4-hydroxybenzoate (2). The agar medium was incubated for 3 days under aerobic conditions at 28°C for growth. Ethics approval for subject sampling was granted by the institutional review board of Changwon National University. Total DNA was extracted using the phenol extraction method from *H. massiliensis* OT1 cells cultured in the liquid medium under the conditions described above (3). The genomic SMRTbell library with a total of 5 μg was constructed with the SMRTbell template prep kit v1.0 (part number 100-259-100), following the manufacturer’s instructions (Pacific Biosciences). Fragments of the template smaller than 20 kbp were removed using the BluePippin size selection system, and the constructed library was validated using the Agilent 2100 Bioanalyzer. The SMRTbell library was sequenced using 1 single-molecule real-time (SMRT) cell using C4 chemistry, and 240-minute movies were captured for each SMRT cell using the MagBead OneCellPerWell v1 protocol (Pacific Biosciences) of the PacBio RSII sequencing platform (DNA Link, Seoul, South Korea). *De novo* assembly was conducted according to the Hierarchical Genome Assembly Process (HGAP) workflow v2.3, including consensus polishing with Quiver (4). After filtering the subreads, a pool of 1,656,433,062 bp from 152,503 long reads with an N50 value of 2,496,671 bp were produced, yielding 233-fold genome coverage. Complete genome sequences were obtained using bioinformatics analyses as previously described (5). Gene predictions and annotations were provided by NCBI using the NCBI Prokaryotic Genome Annotation Pipeline (6). The SEED subsystem was used via Rapid Annotations using Subsystems Technology (RAST) for functional categorization of the predicted proteins (7).

The complete genome of *H. massiliensis* OT1 contains one circular chromosome and nine plasmids, named pOT1-1 to pOT1-9. The genome statistics are shown in Table 1.

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Citation Lim JY, Ganzorig M, Huang S-L, Lee K. 2019. First complete genome sequence of *Haematobacter massiliensis* OT1 (chromosome and multiple plasmids), isolated from human skin. Microbiol Resour Announc 8.e00292-19. https://doi.org/10.1128/MRA.00292-19.

Editor Catherine Putonti, Loyola University Chicago

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Received 19 March 2019
Accepted 7 April 2019
Published 2 May 2019
It is interesting to note that the plasmids constitute 42% of the genes and contain one copy of rRNA genes (5S, 16S, 23S) and six coding regions of tRNAs. The genes encoding the enzymes that catabolize 4-hydroxybenzoate through the β-ketoadipate pathway are carried on the chromosome (pcaIJF) and plasmids pOT1-1 (pcaD) and pOT1-3 (pobA and pcaCHGB) (8). The H. massiliensis OT1 genome contains genes related to the Calvin-Benson cycle and the RuBisCO genes (rbcLS) for autotrophic growth, as found in Paracoccus yeei TT13, another skin isolate (9). The H. massiliensis OT1 genome will help reveal not only the cellular and catabolic adaptation mechanisms of this bacterium to colonize human skin but also the acquisition of multiple plasmids.

Data availability. The complete genome sequences, including those of plasmids of the H. massiliensis OT1 strain, were deposited in GenBank under the accession numbers CP035506 to CP035515 (Table 1). This genome has a base modification file available under GenBank accession number CP035510. The raw sequencing data have been deposited in the SRA under accession number PRJNA517385.

ACKNOWLEDGMENTS

This research was financially supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (grant 2016R1D1A1B01007777) and by the framework of international cooperation program (grant 2013K2A1B8053138).

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TABLE 1 General genomic features of H. massiliensis OT1

| Genome   | Genome size (bp) | No. of coding sequences | G+C content (%) | No. of rRNAs | No. of tRNAs | GenBank accession no. |
|----------|------------------|--------------------------|-----------------|-------------|-------------|----------------------|
| Chromosome | 2,467,987       | 2,375                    | 64.72           | 6           | 42          | CP035510             |
| pOT1-1    | 398,426          | 348                      | 64.53           | 0           | 1           | CP035511             |
| pOT1-2    | 349,143          | 301                      | 64.63           | 3           | 4           | CP035512             |
| pOT1-3    | 285,811          | 250                      | 64.45           | 0           | 1           | CP035513             |
| pOT1-4    | 187,343          | 158                      | 65.33           | 0           | 0           | CP035514             |
| pOT1-5    | 183,422          | 172                      | 61.61           | 0           | 0           | CP035515             |
| pOT1-6    | 118,868          | 95                       | 64.90           | 0           | 0           | CP035506             |
| pOT1-7    | 103,683          | 94                       | 64.42           | 0           | 0           | CP035507             |
| pOT1-8    | 83,590           | 88                       | 62.38           | 0           | 0           | CP035508             |
| pOT1-9    | 54,639           | 53                       | 61.84           | 0           | 0           | CP035509             |
| Total     | 4,232,912        | 3,934                    | 64.48           | 9           | 48          |                      |