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GENOME SEQUENCES

Genome Sequence of a *Blattabacterium* Strain Isolated from the Viviparous Cockroach, *Diploptera punctata*

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ABSTRACT Here, we report the genome sequence and characterization for a *Blattabacterium* strain isolated from the viviparous cockroach, *Diploptera punctata*, which provides amino acids critical for intrauterine embryo development. The genome was assembled by sequencing of the cockroach fat body, which is the location of this obligate symbiont.

The Pacific beetle mimic cockroach, *Diploptera punctata*, reproduces by matrotrophic viviparity. *D. punctata* embryos develop inside the brood sac, a unique organ that functions as both a uterus and a pseudoplacenta; embryos are provided with nutrients by a secretion of milk-like components (1–5). The *D. punctata* milk is deficient in two essential amino acids, tryptophan and methionine (4, 6). It has been hypothesized that endosymbiont metabolism remediates this dietary deficiency; previous research suggests that blattabacteria are the exclusive component of the embryonic microbiome (7). We present a genome analysis of a *Blattabacterium* strain derived from *D. punctata* (*Blattabacterium* sp. strain DPU) to determine the potential role that this endosymbiont has during embryonic development of *D. punctata*.

Bacterial DNA was collected from fat body tissue dissected from a female *D. punctata* cockroach using a modified version of previously described protocols (8, 9) with the use of a Qiagen DNeasy Blood & Tissue kit. Samples were homogenized in 200 μl of sterile 1× phosphate-buffered saline. This extract was passed through a 20-μm glass syringe filter (Millipore) and centrifuged for 10 min at 8,000 × *g* at 4°C. The resulting pellet was resuspended in the extraction kit lysis buffer, and DNA was extracted following the manufacturer's protocol. Illumina Nextera library preparation and HiSeq paired-end sequencing produced 6,778,349 paired-end reads of 75 bp and 4,444,306 reads of 125 bp. Less than 1% of reads were lost during quality control using Trimmomatic (10). metaSPAdes (v.1.2.2, with default settings) implemented in KBase (11, 12) generated 187 contigs with an N_{50} value of 625,590 bp, which is the length of the largest contig. BLASTn comparison of these contigs to those of the German cockroach *Blattabacterium* sp. strain Bbge genome (9) identified this largest contig as a candidate genome sequence (E value of $<$0.0001). Supported by subsequent BLASTn analyses against other *Blattabacterium* strains (E value of $<$0.0001), this contig was selected to be utilized as the genome sequence, and other contigs were discarded. BLASTn comparison of all metaSPAdes contigs to the Bbge plasmid (9) revealed that a 2,852-bp plasmid had been assembled as part of the genome; this sequence was removed from the contig for further analyses, producing a 623,008-bp contig with a GC content of 28.03% and 32.997× coverage. Coverage of the genome was assessed by mapping the paired-end reads to the extracted contig using Bowtie 2 (v.2.3.2) with default settings (13).

Prokka (14) identified 618 open reading frames, including 580 coding sequences, 34 tRNAs, 3 rRNAs, and 1 transfer-messenger RNA, using *Blattabacterium* with a similarity E value cutoff value of $<$0.0001. Almost all genes required for DNA replication, RNA

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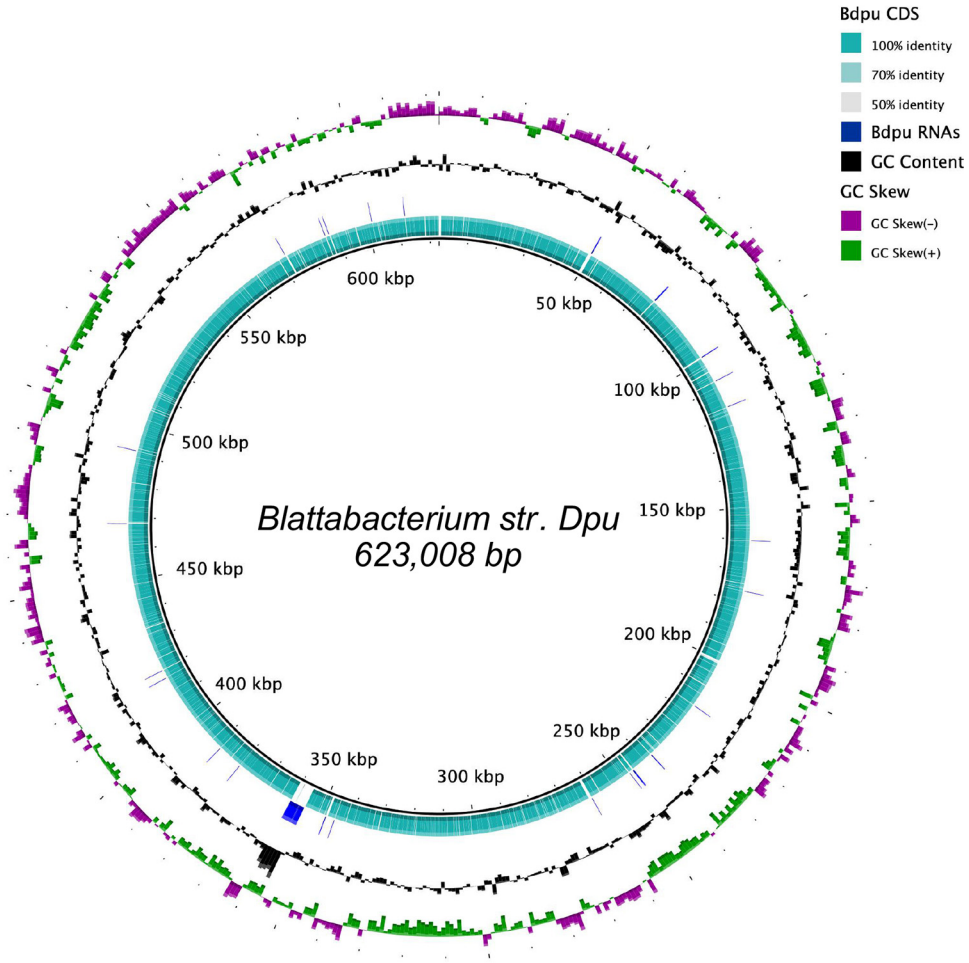

FIG 1 Genome presentation of *Blattabacterium* sp. strain DPU. Outer to inner rings represent GC skew (purple and green bars indicate negative and positive skew, respectively), GC content of each strand, RNA genes, including tRNA, rRNA, and transfer-messenger RNA genes (blue), and predicted coding sequences (teal). CDS, coding DNA sequence.

transcription, and mRNA translational machinery were identified in the assembly (Fig. 1). dUTP nucleotidohydrolase, ribonucleoside diphosphate reductase subunit β, and two hypothetical proteins were identified in the plasmid. Orthology analysis using eggNOG-mapper (15) with the full available database revealed that most coding genes serve in translation and ribosome formation. The next most prominent known genome functions are amino acid metabolism and transport, followed by energy production and conversion. In addition to enzymes for central carbohydrate metabolism and nitrogen salvage, metabolic pathway prediction using the KEGG module mapper (16) identified complete biosynthetic pathways for nearly all essential amino acids. The traditional biosynthetic pathway for methionine is incomplete, however. Genes for all enzymatic reactions to produce methionine are present except for *metA*, which facilitates the conversion of homoserine and succinyl-coenzyme A to *O*-succinylhomoserine, and the alternative *metX*, which produces *O*-acetylhomoserine. An alternative methionine pathway has been suggested in other cockroaches (8), or shared synthesis could occur with the cockroach host based on genes identified in recent transcriptomic studies (5). However, the ability to synthesize selenomethionine is retained. *Blattabacterium* sp. strain DPU also has the ability to synthesize the nonessential amino acids alanine, arginine, cysteine, glutamate, and glycine.

Data availability. Illumina raw sequence reads and genome sequences have been deposited in association with BioProject PRJNA610624. The plasmid sequence has been deposited in GenBank under accession number MT645221.

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REFERENCES

1. Marchal E, Hult EF, Huang J, Stay B, Tobe SS. 2013. *Diploptera punctata* as a model for studying the endocrinology of arthropod reproduction and development. Gen Comp Endocrinol 188:85–93. https://doi.org/10.1016/j.ygcen.2013.04.018.
2. Roth LM, Willis ER. 1955. Intra-uterine nutrition of the "beetle-roach" *Diploptera dytiscoides* (Serv.) during embryogenesis, with notes on its biology in the laboratory (Blattaria: Diplopteridae). Psyche (Camb Mass) 62:55–68. https://doi.org/10.1155/1955/12542.
3. Stay B, Coop AC. 1974. "Milk" secretion for embryogenesis in a viviparous cockroach. Tissue Cell 6:669–693. https://doi.org/10.1016/0040-8166(74)90009-3.
4. Williford A, Stay B, Bhattacharya D. 2004. Evolution of a novel function: nutritive milk in the viviparous cockroach, *Diploptera punctata*. Evol Dev 6:67–77. https://doi.org/10.1111/j.1525-142x.2004.04012.x.
5. Jennings EC, Korthauer MW, Hendershot JM, Bailey ST, Weirauch MT, Ribeiro JM, Benoit JB. 2020. Molecular mechanisms underlying milk production and viviparity in the cockroach, *Diploptera punctata*. Insect Biochem Mol Biol 120:103333. https://doi.org/10.1016/j.ibmb.2020.103333.
6. Ingram MJ, Stay B, Cain GD. 1977. Composition of milk from the viviparous cockroach, *Diploptera punctata*. Insect Biochem 7:257–267. https://doi.org/10.1016/0020-1790(77)90023-3.
7. Jennings EC, Korthauer MW, Hamilton TL, Benoit JB. 2019. Matrotrophic viviparity constrains microbiome acquisition during gestation in a live-bearing cockroach, *Diploptera punctata*. Ecol Evol 9:10601–10614. https://doi.org/10.1002/ece3.5580.
8. Sabree ZL, Kambhampati S, Moran NA. 2009. Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. Proc Natl Acad Sci U S A 106:19521–19526. https://doi.org/10.1073/pnas.0907504106.
9. López-Sánchez MJ, Neef A, Peretó J, Patiño-Navarrete R, Pignatelli M, Latorre A, Moya A. 2009. Evolutionary convergence and nitrogen metabolism in *Blattabacterium* strain Bge, primary endosymbiont of the cockroach *Blattella germanica*. PLoS Genet 5:e1000721. https://doi.org/10.1371/journal.pgen.1000721.
10. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
11. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Woyke T, Mclean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/cmb.2013.0084.
12. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia J-M, Chia J-M, Colasanti R, Conrad N, Davis JJ, Davison BH, DeJongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Jain R, Joachimiak MP, Keegan KP, Kondo S, Kumar V, Land ML, Meyer F, Mills M, Novichkov PS, Oh T, Olsen GJ, Olson R, Parrello B, Pasternak S, Pearson E, Poon SS, Price GA, Ramakrishnan S, Ranjan P, Ronald PC, Schatz MC, Seaver SMD, Shukla M, Sutormin RA, Syed MH, Thomason J, Tintle NL, Wang D, Xia F, Yoo H, Yoo S, Yu D. 2018. KBase: the United States Department of Energy Systems Biology Knowledgebase. Nat Biotechnol 36:566–569. https://doi.org/10.1038/nbt.4163.
13. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.
14. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
15. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P. 2016. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res 44:D286–D293. https://doi.org/10.1093/nar/gkv1248.
16. Kanehisa M, Sato Y, Furumichi M, Morishima K, Tanabe M. 2019. New approach for understanding genome variations in KEGG. Nucleic Acids Res 47:D590–D595. https://doi.org/10.1093/nar/gky962.