

Complete Genome Sequence of *Burkholderia* sp. Strain RPE64, Bacterial Symbiont of the Bean Bug *Riptortus pedestris*

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We isolated *Burkholderia* symbiont strain RPE64 from the bean bug *Riptortus pedestris*. Analysis of the complete 6.96-Mb genome, which consists of three chromosomes and two plasmids, will facilitate further understanding of insect–microbe symbiosis and the development of pest-control technologies.

Received 22 May 2013 Accepted 24 May 2013 Published 5 July 2013

Citation Shibata TF, Maeda T, Nikoh N, Yamaguchi K, Oshima K, Hattori M, Nishiyama T, Hasebe M, Fukatsu T, Kikuchi Y, Shigenobu S. 2013. Complete genome sequence of *Burkholderia* sp. strain RPE64, bacterial symbiont of the bean bug *Riptortus pedestris*. *Genome Announc.* 1(4):e00441-13. doi:10.1128/genomeA.00441-13.

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The bean bug *Riptortus pedestris*, a leguminous-crop pest, is specifically associated with a betaproteobacterial *Burkholderia* symbiont in the posterior midgut (1). *R. pedestris* acquires the *Burkholderia* symbiont from the soil every generation (2), and the symbiont infection enhances growth and body size of the host insect (3). Here, we analyzed the whole-genome sequence of *Burkholderia* sp. strain RPE64, isolated from field-collected *R. pedestris* (4).

For whole-genome shotgun sequencing of the *Burkholderia* symbiont, we used paired-end sequencing with HiSeq 2000 (Illumina) and long sequencing with PacBio RS (Pacific Biosciences). Four Illumina libraries, with 180-, 300-, 500-, and 800-bp insertions, were constructed and sequenced 101 bp from both ends, yielding 32.6 Gb of raw data (accession no. DRA000987). After the removal of adaptor sequences and low-quality reads (quality value of <20), 25.6 Gb of high-quality sequences remained. An approximately-6-kb insert PacBio library was constructed and sequenced, yielding 189,571 reads with 2.1 to 2.7 kb mean maximum subread length, totaling 449 Mb of independent fragment reads (accession no. DRA000982). The Illumina and PacBio reads were assembled *de novo* using a hybrid assembly algorithm implemented in Allpaths-LG software (5), yielding 24 contigs. PacBio, which is tolerant to G+C bias, complemented the low coverage of the Illumina data in high-G+C regions. While scaffolds assembled from the Illumina reads contained 11,407 ambiguous bases (i.e., Ns), adding the PacBio reads resolved all but three Ns. Gaps between contigs were closed by Sanger sequencing of PCR-amplified fragments. Coding genes were predicted with Glimmer (6) and annotated by a BLAST search against UniProt.

The complete genome of *Burkholderia* sp. strain RPE64 is 6.96 Mb and comprises three circular chromosomes and two plasmids: chromosome 1 (3,013,410 bp, 2,907 protein-coding sequences [CDSs]), chromosome 2 (1,465,356 bp, 1,422 CDSs),

chromosome 3 (900,830 bp, 853 CDSs), plasmid 1 (1,275,199 bp, 1,222 CDSs), and plasmid 2 (309,692 bp, 328 CDSs). The G+C content is 60.1% to 63.5%.

The closest-sequenced-genome relative of strain RPE64 is the soil-isolated *Burkholderia* sp. strain YI23. The 16S rRNA sequences have high nucleic acid identity (98.6%) but different overall genomic structures; the total genome size of RPE64 is 1.93 Mb smaller than that of YI23. RPE64 lacks a plasmid equivalent to BYI23_F of YI23, where fenitrothion-degrading genes (*mhqA* and *mhqB*) are located. Consistent with the absence of these genes, RPE64 cannot degrade the insecticide.

While some genomes of human-pathogenic and plant-associated *Burkholderia* isolates have been sequenced (7), this is the first complete genome sequence of an insect-associated symbiotic *Burkholderia* strain. Together with the transcriptome data of the host midgut crypts (8), the complete sequence of this symbiotic *Burkholderia* strain will contribute to the molecular understanding of the insect–microbe symbiosis. We recently found that fenitrothion-degrading strains of symbiotic *Burkholderia* confer resistance of the host insect to fenitrothion and other organophosphorus insecticides (9). The RPE64 genome provides a good reference for comparatively analyzing the mechanisms of symbiont-mediated insecticide resistance.

Nucleotide sequence accession numbers. The complete genome sequence of *Burkholderia* sp. strain RPE64 (including three chromosomes and two plasmids) has been deposited in DDBJ/EMBL/GenBank under accession no. [AP013058](https://www.ncbi.nlm.nih.gov/nuccore/AP013058) to [AP013062](https://www.ncbi.nlm.nih.gov/nuccore/AP013062).

ACKNOWLEDGMENTS

We thank Hisayo Asao and Shoko Ohi for the Illumina and PacBio sequencing.

This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas, “Genetic Bases for the Evolution of Complex Adaptive

Traits” (Ministry of Education, Culture, Sports, Science, and Technology, Japan), by the NIBB Cooperative Research Program no. 11-727, and by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-Oriented Industry.

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