

Draft Genome Sequence of *Asaia* sp. Strain SF2.1, an Important Member of the Microbiome of *Anopheles* Mosquitoes

Jackie L. Shane,^a Nicholas J. Bongio,^a Guido Favia,^b David J. Lampe^a

Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania, USA^a; Scuola di Bioscienze e Biotecnologie, Università degli Studi di Camerino, Camerino, Italy^b

Asaia spp. are abundant members of the microbiota of *Anopheles* mosquitoes, the principle vectors of malaria. Here, we report the draft genome sequence of *Asaia* sp. strain SF2.1. This strain is under development as a platform to deliver antimalarial peptides and proteins to adult female *Anopheles* mosquitoes.

Received 12 December 2013 Accepted 13 December 2013 Published 9 January 2014

Citation Shane JL, Bongio NJ, Favia G, Lampe DJ. 2014. Draft genome sequence of *Asaia* sp. strain SF2.1, an important member of the microbiome of *Anopheles* mosquitoes. Genome Announc. 2(1):e01202-13. doi:10.1128/genomeA.01202-13.

Copyright © 2014 Shane et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to David J. Lampe, lampe@duq.edu.

saia sp. strain SF2.1 is a Gram-negative member of the Alphaproteobacteria, family Acetobacteraceae (1). Asaia spp. were first isolated from the nectar of tropical flowers and subsequently from insect midguts (1-4). The taxonomy of the genus Asaia is in flux and so we are hesitant to assign Asaia sp. SF2.1 to a specific species, although it seems to be most closely related to either Asaia bogorensis or Asaia platycodi (our unpublished data). Asaia sp. SF2.1 was isolated from a laboratory colony of Anopheles stephensi, where it is extremely abundant in the gut, salivary glands, ovaries, and testes of this insect (5). Asaia spp. have also been uncovered in Anopheles mosquitoes in the field, especially Anopheles gambiae, the most important vector of malaria in Africa (2, 6, 7). Efforts to genetically engineer Asaia sp. SF2.1 are under way in order to provide a platform to deliver antimalarial effector molecules to Anopheles mosquitoes in the field in an effort to block the transmission of malaria, a strategy called paratransgenesis (7-9). The sequence reported here is the first for the genus.

The sequencing and annotation of the genome of *Asaia* sp. SF2.1 was performed by ACGT, Inc. The standard protocol for the Nextera XT DNA sample preparation kit was used. The purified fragmented DNA was used as a template for a limited cycle PCR using Nextera primers and index adaptors. A second library was prepared using the Nextera mate-pair sample preparation kit.

In order to generate clusters of DNA, both libraries were sequenced in a paired-end 2×150 -bp protocol by MiSeq. The sequence reads passing the Illumina purity filter were demultiplexed. A total of 4,097,892 standard library reads were generated, giving an average coverage of $351 \times$ based on the 3.5-Mb genome. To generate additional mate-pair reads, a second MiSeq run was done using the mate-pair library. This run generated 999,241 mate-pair reads (86× coverage).

A 93× coverage subset of the small-insert library and the matepair library were assembled *de novo* using ABySS (10), Velvet (11), and SOAP*denovo*2 (12). The best Velvet, ABySS, and SOAP*denovo*2 contig sets were combined using CISA (13) to produce an assembly with 51 contigs. The largest contig is 506 kb, the N₅₀ length is 162 kb, and the total assembly length is 3.53 Mb. The G+C content is 59.5%.

Annotation of the genome was performed by the NCBI Prokaryotic Genome Annotation Pipeline version 2.0 (https://www .ncbi.nlm.nih.gov/genome/annotation_prok/). A total of 3,098 genes were predicted using this method, including 3,005 proteincoding genes, 44 pseudogenes, 3 rRNAs, and 45 tRNAs.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. AYXS00000000. The version described in this paper is version AYXS01000000.

ACKNOWLEDGMENTS

This work was supported by a Duquesne University Hunkele Dreaded Disease award to D.J.L.

We thank Jim Lund at ACGT, Inc., for expert technical assistance.

REFERENCES

- Yamada Y, Katsura K, Kawasaki H, Widyastuti Y, Saono S, Seki T, Uchimura T, Komagata K. 2000. Asaia bogorensis gen. nov., sp. nov., an unusual acetic acid bacterium in the alpha-proteobacteria. Int. J. Syst. Evol. Microbiol. 50(Pt 2):823–829. http://dx.doi.org/10.1099/00207713-50-2-823.
- Crotti E, Damiani C, Pajoro M, Gonella E, Rizzi A, Ricci I, Negri I, Scuppa P, Rossi P, Ballarini P, Raddadi N, Marzorati M, Sacchi L, Clementi E, Genchi M, Mandrioli M, Bandi C, Favia G, Alma A, Daffonchio D. 2009. *Asaia*, a versatile acetic acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically distant genera and orders. Environ. Microbiol. 11:3252–3264. http://dx.doi.org/10.1111/j.14 62-2920.2009.02048.x.
- Katsura K, Kawasaki H, Potacharoen W, Saono S, Seki T, Yamada Y, Uchimura T, Komagata K. 2001. Asaia siamensis sp. nov., an acetic acid bacterium in the alpha-proteobacteria. Int. J. Syst. Evol. Microbiol. 51: 559–563.
- 4. Yukphan P, Potacharoen W, Tanasupawat S, Tanticharoen M, Yamada Y. 2004. *Asaia krungthepensis* sp. nov., an acetic acid bacterium in the alpha-proteobacteria. Int. J. Syst. Evol. Microbiol. 54:313–316. http://dx .doi.org/10.1099/ijs.0.02734-0.
- 5. Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M, Rizzi A, Urso R, Brusetti L, Borin S, Mora D, Scuppa P, Pasqualini L, Clementi E, Genchi M, Corona S, Negri I, Grandi G, Alma A, Kramer

L, Esposito F, Bandi C, Sacchi L, Daffonchio D. 2007. Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. Proc. Natl. Acad. Sci. U. S. A. 104:9047–9051. http://dx .doi.org/10.1073/pnas.0610451104.

- Damiani C, Ricci I, Crotti E, Rossi P, Rizzi A, Scuppa P, Capone A, Ulissi U, Epis S, Genchi M, Sagnon N, Faye I, Kang A, Chouaia B, Whitehorn C, Moussa GW, Mandrioli M, Esposito F, Sacchi L, Bandi C, Daffonchio D, Favia G. 2010. Mosquito-bacteria symbiosis: the case of Anopheles gambiae and Asaia. Microb. Ecol. 60:644-654. http://dx.doi .org/10.1007/s00248-010-9704-8.
- 7. Favia G, Ricci I, Marzorati M, Negri I, Alma A, Sacchi L, Bandi C, Daffonchio D. 2008. Bacteria of the genus *Asaia*: a potential paratransgenic weapon against malaria. Adv. Exp. Med. Biol. 627:49–59. http://dx .doi.org/10.1007/978-0-387-78225-6_4.
- Abdul-Ghani R, Al-Mekhlafi AM, Alabsi MS. 2012. Microbial control of malaria: biological warfare against the parasite and its vector. Acta Trop. 121:71–84. http://dx.doi.org/10.1016/j.actatropica.2011.11.001.

- Wang S, Ghosh AK, Bongio N, Stebbings KA, Lampe DJ, Jacobs-Lorena M. 2012. Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. Proc. Natl. Acad. Sci. U. S. A. 109:12734–12739. http://dx.doi .org/10.1073/pnas.1204158109.
- Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res. 19:1117–1123. http://dx.doi.org/10.1101/gr.089532.108.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res. 18:821–829. http://dx.doi .org/10.1101/gr.074492.107.
- Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J. 2010. *De novo* assembly of human genomes with massively parallel short read sequencing. Genome Res. 20:265–272. http://dx.doi.org/10.1101/gr.097261.109.
- Lin SH, Liao YC. 2013. CISA: contig integrator for sequence assembly of bacterial genomes. PLoS One 8:e60843. http://dx.doi.org/10.1371/journal .pone.0060843.