

Draft Genome Sequences of *Elizabethkingia anophelis* Strains R26^T and Ag1 from the Midgut of the Malaria Mosquito *Anopheles gambiae*

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Elizabethkingia anophelis is a species in the family *Flavobacteriaceae*. It is a dominant resident in the mosquito gut and also a human pathogen. We present the draft genome sequences of two strains of *E. anophelis*, R26^T and Ag1, which were isolated from the midgut of the malaria mosquito *Anopheles gambiae*.

Received 31 October 2013 Accepted 1 November 2013 Published 5 December 2013

Citation Kukutla P, Lindberg BG, Pei D, Rayl M, Yu W, Steritz M, Faye I, Xu J. 2013. Draft genome sequences of *Elizabethkingia anophelis* strains R26^T and Ag1 from the midgut of the malaria mosquito *Anopheles gambiae*. Genome Announc. 1(6):e01030-13. doi:10.1128/genomeA.01030-13.

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he mosquito gut accommodates a diverse microbiota (1–4). Elizabethkingia sp. has been identified as a dominant resident in the gut of Anopheles gambiae (1, 5, 6) and Anopheles stephensi (3). Recently, Kämpfer and collaborators described the *Elizabeth*kingia anophelis type strain R26, isolated from the midgut of the mosquito Anopheles gambiae, as a novel taxon in the genus Elizabethkingia (7). Another strain, designated Ag1, was isolated from the midgut of the mosquito Anopheles gambiae G3 strain in the Xu laboratory at New Mexico State University, and the strain was identified as Elizabethkingia anophelis based on the bacterial 16S rRNA gene sequence (99.8% homology). The genomes were sequenced using Illumina HiSeq 2000 paired-end technology at BGI, Hong Kong. The R26^T genomic reads (652 Mbp) were *de* novo assembled using DNASTAR NGen v 10.0, which generated 66 contigs, totaling 4.03 Mbp with an average GC content of 35.4%. The Ag1 genomic reads (620 Mbp) were de novo assembled in CLC Genomics Workbench v.4.9, which yielded 51 contigs, totaling 4.05 Mbp. The draft genomes were annotated using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (http: //www.ncbi.nlm.nih.gov/genome/annotation_prok/), which predicted 3,687 protein coding sequences (CDS) and 44 RNA genes in R26^T and 3,648 CDS and 38 RNA genes in Ag1. Strikingly, 112 protein features were identified in the category "Resistance to antibiotics and toxic compounds." This included drug efflux/transport (36 features); resistance to β -lactam antibiotics, fluoroquinolones, and heavy metals (28, 4 and 25 features, respectively); and 19 additional features involved in resistance to a diverse set of antibiotics. The large genetic capacity against various antibiotics is consistent with the observation that E. anophelis has natural antibiotic resistance to several antibiotics (7). Recently, E. anophelis was reported as a human pathogen in Central Africa (8) and an outbreak was also seen in an intensive care unit in Singapore (9). In both clinical cases multidrug resistance was reported. Further analysis of the genomic background would improve our understanding of antibiotic resistance mechanisms and their significance in shaping a microbial community in natural environments

and the host-associated metagenomic ecosystem (10, 11). Like some *Bacteroides* (12), *E. anophelis* possesses polysaccharide utilization loci (PUL), which suggests the genetic capability to utilize various plant polysaccharides. This implies an intriguing ecological connection with the nectar and plant sap feeding behavior of mosquitoes in nature. The genome of *E. anophelis* plus other bacterial genomes that Xu and collaborators isolated from the mosquito guts, *Pseudomonas* sp. (13) and *Enterobacter* sp. (14), will serve as references for subsequent characterization of the mosquito gut microbiome and its impact on *Anopheles gambiae* life traits. Additionally, the pathogenic and multiresistant nature of the bacteria prompts investigations of the vector potential of mosquitoes for *E. anophelis* transmission to humans.

Nucleotide sequence accession numbers. The draft genome sequences of strains R26^T and Ag1 are available in DDBJ/EMBL/ GenBank under the GenBank accession numbers ANIW00000000 and AHHG00000000, respectively.

ACKNOWLEDGMENTS

This work was funded by a grant from the National Institutes of Health (1SC2GM092789-01A1) to J.X. M.S. was an undergraduate research scholar supported by the NMSU Howard Hughes Medical Institute (HHMI) research scholars program. I.F. was supported by a grant from the Research Infrastructure-Integrating Activity project supported by the European Community (INFRAVEC) FP7 Infrastructures (grant agreement number: 228421).

REFERENCES

- 1. Wang Y, Gilbreath TM III, Kukutla P, Yan G, Xu J. 2011. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. PLoS One 6:e24767. doi:10.1371/journal.pone.0024767.
- 2. Gusmao DS, Santos AV, Marini DC, Bacci M, Jr, Berbert-Molina MA, Lemos FJ. 2010. Culture-dependent and culture-independent characterization of microorganisms associated with *Aedes aegypti (Diptera: Culicidae)* (L.) and dynamics of bacterial colonization in the midgut. Acta Trop. 115:275–281.
- 3. Rani A, Sharma A, Rajagopal R, Adak T, Bhatnagar RK. 2009. Bacterial diversity analysis of larvae and adult midgut microflora using culture-

dependent and culture-independent methods in lab-reared and fieldcollected *Anopheles stephensi---*an Asian malarial vector. BMC Microbiol. **9:96**.

- 4. Boissière A, Tchioffo MT, Bachar D, Abate L, Marie A, Nsango SE, Shahbazkia HR, Awono-Ambene PH, Levashina EA, Christen R, Morlais I. 2012. Midgut microbiota of the malaria mosquito vector *Anopheles* gambiae and interactions with *Plasmodium falciparum* infection. PLoS Pathog. 8:e1002742. doi:10.1371/journal.ppat.1002742.
- Lindh JM, Borg-Karlson AK, Faye I. 2008. Transstadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (*Diptera: Culicidae*) and oviposition response to bacteria-containing water. Acta Trop. 107:242–250.
- Lindh JM, Terenius O, Faye I. 2005. 16S rRNA gene-based identification of midgut bacteria from field-caught *Anopheles gambiae sensu lato* and *A. Funestus* mosquitoes reveals new species related to known insect symbionts. Appl. Environ. Microbiol. 71:7217–7223.
- Kämpfer P, Matthews H, Glaeser SP, Martin K, Lodders N, Faye I. 2011. Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae. Int. J. Syst. Evol. Microbiol. 61:2670–2675.
- 8. Frank T, Gody JC, Nguyen LB, Berthet N, Le Fleche-Mateos A, Bata P,

Rafaï C, Kazanji M, Breurec S. 2013. First case of *Elizabethkingia anophelis* meningitis in the Central African Republic. Lancet **381**:1876.

- 9. Teo J, Tan SY, Tay M, Ding Y, Kjelleberg S, Givskov M, Lin RT, Yang L. 2013. First case of *E anophelis* outbreak in an intensive-care unit. Lancet **382**:855–856.
- 10. Schmieder R, Edwards R. 2012. Insights into antibiotic resistance through metagenomic approaches. Future Microbiol. 7:73–89.
- 11. Sommer MO, Church GM, Dantas G. 2010. The human microbiome harbors a diverse reservoir of antibiotic resistance genes. Virulence 1:299–303.
- 12. Martens EC, Lowe EC, Chiang H, Pudlo NA, Wu M, McNulty NP, Abbott DW, Henrissat B, Gilbert HJ, Bolam DN, Gordon JI. 2011. Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. PLoS Biol. 9:e1001221. doi:10.1371/journal.pbio. 1001221.
- 13. Alvarez C, Kukutla P, Jiang J, Yu W, Xu J. 2012. Draft genome sequences of *Pseudomonas* sp. Ag1 isolated from the midgut of malaria mosquito *Anopheles gambiae*. J. Bacteriol. **194**:5449.
- 14. Jiang J, Alvarez C, Kukutla P, Yu W, Xu J. 2012. Draft genome sequences of *Enterobacter* sp. isolate Ag1 from the midgut of the malaria mosquito *Anopheles gambiae*. J. Bacteriol. **194**:5481.