




Complete Genome Sequencing of *Elizabethkingia* sp. Strain 2-6

Kuan-Ming Liu,^a Hui-Lan Chang,^{a,b} Ming-Hong Hsu,^a Yi-Zhen Lin,^a Yu-Lin Lee,^{a,b}  Ying-Tsong Chen^{a,c}

^aInstitute of Genomics and Bioinformatics, National Chung Hsing University, Taichung, Taiwan

^bDepartment of Infection, Changhua Christian Hospital, Changhua, Taiwan

^cInstitute of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Taiwan

ABSTRACT *Elizabethkingia* sp. strain 2-6 was collected from a water faucet in the intensive care unit of a medical center in Taiwan. The complete genome sequence and annotation are reported. Analysis of the genetic relatedness to the known *Elizabethkingia* genomes indicated that strain 2-6 may be a new genomospecies of *Elizabethkingia*.

Elizabethkingia is a genus of highly resistant, Gram-negative bacilli that is ubiquitous in the environment. The genus contains six species, namely, *E. meningoseptica*, *E. miricola*, *E. anophelis*, *E. bruuniana*, *E. ursingii*, and *E. occulta* (1–3). They are phenotypically very similar, sometimes leading to misidentifications in clinical laboratories (4). Nosocomial infections caused by *Elizabethkingia* spp. are an emerging problem in Taiwan. The majority of the infections in Taiwan have been meningitis outbreaks in premature newborns or infants. Nosocomial infections caused by *Elizabethkingia* spp. in adult patients are rare, and most cases have been reported in Taiwan (5). The cases of infections caused by *Elizabethkingia* spp. in Changhua Christian Hospital (CCH), a medical center in central Taiwan, began to soar since 2015, from 1 to 3 cases a month in 2000 to 12 cases a month in 2017. Almost all of the cases were from the respiratory care unit (RCU) and intensive care unit (ICU) of the hospital. In a surveillance project carried out in CCH from 2016 to 2018, *Elizabethkingia* isolates from clinical samples and the environment of the RCU/ICU were collected for analysis.

Elizabethkingia sp. strain 2-6 was isolated from a swab sample from one of the water faucets in the ICU. Bacteria from the swab were first cultured in thioglycolate broth for 48 h at 35°C and then transferred to a blood agar plate/eosin methylene blue (BAP/EMB) agar plate for another 24 h at 35°C. The strain was identified either as *Elizabethkingia* species or *E. meningoseptica* using the API20NE system (bioMérieux), Vitek mass spectrometer (MS) (bioMérieux), and Bruker MALDI Biotyper (BD). In an attempt to determine the genetic relatedness of the *Elizabethkingia* isolates from the ICU, pulsed-field gel electrophoresis (PFGE) analysis was performed on the bacterial genomic DNA samples digested by the restriction endonuclease XhoI. Interestingly, strain 2-6 was found to have a PFGE pattern different from the rest of those of the *Elizabethkingia* isolates we collected in the hospital.

Sequencing of the genome was carried out using the Illumina iSeq 100 and Nanopore MinION platforms. For short-read sequencing, the genomic DNA of *Elizabethkingia* sp. strain 2-6 was prepared using the DNeasy UltraClean microbial kit (Qiagen). Sequencing was carried out using an iSeq 100 sequencer. The Nextera DNA Flex kit (Illumina) and barcode kit were used for shotgun library generation, following standard protocols. Sequencing of the library generated 150-bp paired-end reads, followed by adapter trimming using the standard pipeline provided by the vendor. This generated a total of 1,343,458 reads with a total of 198.5 Mbp (~45× coverage). For Nanopore sequencing, the genomic DNA was purified from the cultured bacteria using a DNeasy blood and tissue kit (Qiagen). The sequencing library was prepared using a

Citation Liu K-M, Chang H-L, Hsu M-H, Lin Y-Z, Lee Y-L, Chen Y-T. 2019. Complete genome sequencing of *Elizabethkingia* sp. strain 2-6. Microbiol Resour Announc 8:e00805-19. <https://doi.org/10.1128/MRA.00805-19>.

Editor Irene L. G. Newton, Indiana University, Bloomington

Copyright © 2019 Liu et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Ying-Tsong Chen, onion@nchu.edu.tw.

Received 3 July 2019

Accepted 19 September 2019

Published 31 October 2019

rapid 1D sequencing kit with barcoding, following standard protocols. Sequencing was performed using a Nanopore MinION device. Reads were called using Guppy version 2.3.7 (Nanopore). Only the reads that passed the initial quality check (QC) were used. This generated 31,175 reads for a total of 320 Mbp ($\sim 72.8\times$ coverage). The maximum, N_{50} , and average read lengths are 115 kb, 17.4 kb, and 11 kb, respectively.

Hybrid assembly of the Illumina short paired-end reads and Nanopore long reads was performed using Unicycler version 0.4.8 (6), which resulted in a single circular chromosome of 4,394,713 bp, with an average G+C content of 35.86%. The correctness of the assembly result was rechecked by using another assembler, Canu 1.8 (7), with the aid of CLC Genomics Workbench 11 (Qiagen). The genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (8). The genome of *Elizabethkingia* sp. 2-6 is predicted to contain 4,038 genes, of which 3,910 are protein-coding genes. There are 73 RNA genes, including 15 rRNA (5 each of 5S, 16S, and 23S rRNA), 55 tRNA, and 3 noncoding RNA genes.

Interestingly, average nucleotide identity (ANI) analysis (9) resulted in ANI scores of <95% between strain 2-6 and the 6 known species of the *Elizabethkingia* genus (ANI score, 79.86% for *E. meningoseptica* G4120 [GenBank accession number [NZ_CP016378](#)], 92.78% for *E. miricola* BM10 [GenBank accession number [NZ_CP011059](#)], 91.67% for *E. anopheles* R26 [GenBank accession number [NZ_CP023401](#)], 92.63% for *E. bruuniana* G0146 [GenBank accession number [NZ_CP014337](#)], 90.96% for *E. ursingii* G4123 [GenBank accession number [NZ_CP016377](#)], and 90.81% for *E. occulta* G4070 [GenBank assembly number [GCA_002023715](#)]). The results suggest that strain 2-6 may be another new addition to the *Elizabethkingia* genus.

Data availability. This complete genome sequence of *Elizabethkingia* sp. 2-6 has been deposited at GenBank under the accession number [NZ_CP039929](#) and BioProject number [PRJNA540378](#). The sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under the accession numbers [SRR9643623](#) and [SRR9643624](#).

ACKNOWLEDGMENTS

This work was supported by research grant MOST107-2320-B-005-006 from the Ministry of Science and Technology (MOST) Taiwan and research grant MG-108-PP-13 from the National Health Research Institutes, Taiwan.

REFERENCES

- Kim KK, Kim MK, Lim JH, Park HY, Lee ST. 2005. Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. *Int J Syst Evol Microbiol* 55:1287–1293. <https://doi.org/10.1099/ijs.0.63541-0>.
- 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. <https://doi.org/10.1099/ijs.0.026393-0>.
- Nicholson AC, Gulvik CA, Whitney AM, Humrighouse BW, Graziano J, Emery B, Bell M, Loparev V, Juieng P, Gartin J, Bizet C, Clermont D, Criscuolo A, Brisse S, McQuiston JR. 2018. Revisiting the taxonomy of the genus *Elizabethkingia* using whole-genome sequencing, optical mapping, and MALDI-TOF, along with proposal of three novel *Elizabethkingia* species: *Elizabethkingia bruuniana* sp. nov., *Elizabethkingia ursingii* sp. nov., and *Elizabethkingia occulta* sp. nov. *Antonie Van Leeuwenhoek* 111:55–72. <https://doi.org/10.1007/s10482-017-0926-3>.
- Eriksen HB, Gumpert H, Faurholt CH, Westh H. 2017. Determination of *Elizabethkingia* diversity by MALDI-TOF mass spectrometry and whole-genome sequencing. *Emerg Infect Dis* 23:320–323. <https://doi.org/10.3201/eid2302.161321>.
- Lin JN, Lai CH, Yang CH, Huang YH. 2018. Comparison of clinical manifestations, antimicrobial susceptibility patterns, and mutations of fluoroquinolone target genes between *Elizabethkingia meningoseptica* and *Elizabethkingia anophelis* isolated in Taiwan. *J Clin Med* 7:538. <https://doi.org/10.3390/jcm7120538>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.