

Genome Sequence of *Vibrio cholerae* G4222, a South African Clinical Isolate

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Vibrio cholerae, a Gram-negative pathogen autochthonous to the aquatic environment, is the causative agent of cholera. Here, we report the complete genome sequence of *V. cholerae* G4222, a clinical isolate from South Africa.

Received 17 January 2013 Accepted 8 February 2013 Published 14 March 2013

Citation le Roux WJ, Chan WY, De Maayer P, Venter SN. 2013. Genome sequence of Vibrio cholerae G4222, a South African clinical isolate. Genome Announc. 1(2):e00040-13. doi:10.1128/genomeA.00040-13.

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ibrio cholerae is the causative agent of cholera, a severe diarrheal disease that remains a socioeconomic burden in many developing countries (1). As such, the species, also widely regarded as a model organism for studies pertaining to water-borne pathogens, has received much attention (2). Among other foci, emphasis has been placed on defining serotypes and biotypes in an attempt to understand this rapidly evolving pathogen (3). However, with the discoveries of intraspecies serogroup transfer and contradicting biotype-related phenotypes, the focus has shifted away from classic diagnostic markers to genome-based comparisons with an emphasis on mobile elements (3, 4). To this end, the genome sequence of V. cholerae G4222, a clinical O1 isolate obtained in South Africa during the 2000-2001 epidemic, was determined. This represents the first genome of a South African V. cholerae strain. In light of the dearth of African V. cholerae strain sequences and the recent discovery of new recombinant V. cholerae biotypes in southern Africa, this genome might provide valuable insights into the evolution of this pathogen (5, 6). Furthermore, the genome sequence of this strain may contribute to the understanding of the V. cholerae mobilome.

The genome was sequenced using a Roche 454 GS-FLX sequencer at Inqaba Biotec, South Africa. A total of 201,286 reads with an average read length of 236 bp were obtained, giving a total of 47,503,496 nucleotides and genome coverage of $11.6 \times$. The reads were assembled into 280 contigs using Newbler assembler v2.6 (454 Life Sciences). These contigs were then scaffolded by alignment against the complete genome sequences of V. cholerae MJ-1236 (7) and V. cholerae O1 biovar El Tor strain N16961 (8) with the NCBI Genomic (NG) Aligner tool of the NCBI Genome Workbench v2.5.5. A further 38 gaps were closed by PCR amplification and Sanger sequencing. This resulted in the assembly of the V. cholerae G4222 genome sequence into a total of 21 contigs. Protein-coding sequence (CDS) prediction and functional annotation of the predicted proteins were done using the Rapid Annotations using Subsystems Technology (RAST) Web server (9) before manual curation was performed.

The *V. cholerae* G4222 contigs could be scaffolded into two distinct chromosomes, as is typical of *V. cholerae* strains (10).

Chromosome I consists of 14 contigs amounting to a total length of 3,139,654 bp, with an average G+C content of 47.72% and 2,809 annotated CDS. Chromosome II consists of seven contigs with a total length of 1,061,058 bp and a G+C content of 46.88%, with 1,051 CDS annotated. The chromosome sizes and G+C compositions correlate well with those of other *V. cholerae* strains (6, 8). An ~150-kb integrative conjugative element (ICE), belonging to the SXT family, is located on chromosome I of *V. cholerae* G4222 and carries the genes involved in multiple-drug resistance. Given that an African origin for SXT-related ICEs in *V. cholerae* strains has been proposed, the genome of *V. cholerae* G4222 provides further opportunity to investigate the evolution of SXT elements (11). The strain might also provide insights into the biology of South African epidemic *V. cholerae* strains.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/ GenBank under the accession no. ANNB00000000. The version described in this paper is the first version, ANNB01000000.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation (NRF). We thank the National Institute for Communicable Diseases (NICD)

for providing the study strain.

REFERENCES

- World Health Organization (WHO). 2012. Cholera, 2011. Wkly. Epidemiol. Rec. 87(31/32):289–304. http://www.who.int/wer/2012/wer873132/en/.
- Yildiz F. 2007. Vibrio cholerae: model organism to study bacterial pathogenesis - interview. J. Vis. Exp. 4:e207.
- Cho YJ, Yi H, Lee JH, Kim DW, Chun J. 2010. Genomic evolution of Vibrio cholerae. Curr. Opin. Microbiol. 13:646–651.
- 4. Safa A, Nair GB, Kong RY. 2010. Evolution of new variants of *Vibrio cholerae* O1. Trends Microbiol. 18:46–54.
- Faruque SM, Tam VC, Chowdhury N, Diraphat P, Dziejman M, Heidelberg JF, Clemens JD, Mekalanos JJ, Nair GB. 2007. Genomic analysis of the Mozambique strain of *Vibrio cholerae* O1 reveals the origin of el Tor strains carrying classical CTX prophage. Proc. Natl. Acad. Sci. U. S. A. 104:5151–5156.
- Grim CJ, Hasan NA, Taviani E, Haley B, Chun J, Brettin TS, Bruce DC, Detter JC, Han CS, Chertkov O, Challacombe J, Huq A, Nair GB, Colwell RR. 2010. Genome sequence of hybrid *Vibrio cholerae* O1 MJ-

1236, B-33, and CIRS101 and comparative genomics with V. cholerae. J. Bacteriol. 192:3524–3533.

- Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, Haley BJ, Taviani E, Jeon YS, Kim DW, Lee JH, Brettin TS, Bruce DC, Challacombe JF, Detter JC, Han CS, Munk AC, Chertkov O, Meincke L, Saunders E, Walters RA, Huq A, Nair GB, Colwell RR. 2009. Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic *Vibrio cholerae*. Proc. Natl. Acad. Sci. U. S. A. 106:15442–15447.
- Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L, Gill SR, Nelson KE, Read TD, Tettelin H, Richardson D, Ermolaeva MD, Vamathevan J, Bass S, Qin H, Dragoi I, Sellers P, McDonald L, Utterback T, Fleishmann RD, Nierman WC, White O, Salzberg SL, Smith HO, Colwell RR, Mekalanos JJ, Venter JC, Fraser CM. 2000. DNA sequence of both

chromosomes of the cholera pathogen Vibrio cholerae. Nature 406: 477-483.

- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75.
- Trucksis M, Michalski J, Deng YK, Kaper JB. 1998. The Vibrio cholerae genome contains two unique circular chromosomes. Proc. Natl. Acad. Sci. U. S. A. 95:14464–14469.
- Burrus V, Marrero J, Waldor MK. 2006. The current ICE age: biology and evolution of SXT-related integrating conjugative elements. Plasmid 55:173–183.