Long-Read-Based Genome Sequences of Pandemic and Environmental *Vibrio cholerae* Strains

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**ABSTRACT** The bacterium *Vibrio cholerae* exhibits two distinct lifestyles, one as an aquatic bacterium and the other as the etiological agent of the pandemic human disease cholera. Here, we report closed genome sequences of two seventh pandemic *V. cholerae* O1 El Tor strains, A1552 and N16961, and the environmental strain Sa5Y.

Cholera is one of the oldest diseases known and is still a major burden for people in developing countries (1). The disease is caused by *Vibrio cholerae*, which also thrives in natural environments (2). Toxigenic strains are characterized by the presence of major virulence factors (3), while marine habitats are often dominated by nontoxigenic strains. Studying those strains helps us to understand pathogen emergence (4–8).

We sequenced three *V. cholerae* strains (A1552, N16961, and Sa5Y) using whole-genome PacBio sequencing. *V. cholerae* O1 El Tor (Inaba) strain A1552 (originally named 92A1552 [9]) was isolated by the California health authorities from a traveler returning from South America (10, 11), which links it to the Peruvian outbreak in the 1990s (12–14). First used for research in the Schoolnik laboratory at Stanford University, A1552 was rendered rifampicin resistant (9) and now represents the wild type in most laboratories, including ours. *V. cholerae* O1 El Tor strain N16961 was the first sequenced strain of this species (15). However, as a recent study suggested an inversion in the initial assembly (16), we resequenced N16961. *V. cholerae* Sa5Y is a 2004 environmental isolate from California (17).

Genomic DNA was isolated from bacteria cultured in lysogeny broth using a Qiagen genomic DNA buffer set combined with Qiagen 100/G Genomic-tips. Sequencing was performed by the Genomic Technology Facility of the University of Lausanne. DNA samples were sheared in Covaris g-TUBEs to obtain fragments with a mean length of 20 kb. The sheared DNA was used to prepare each library with the PacBio SMRTbell template prep kit 1 (Pacific Biosciences) according to the manufacturer’s recommendations. The resulting library was size selected on a BluePippin system (Sage Science, Inc.) for molecules larger than 15 kb, which excluded smaller plasmids. Each library was sequenced on one single-molecule real-time (SMRT) cell with P6/C4 chemistry and MagBeads on a PacBio RS II system at a movie length of 360 min. Genome assembly was performed using the protocol RS_HGAP_Assembly3 in SMRT Pipe 2.3.0, and circularization of the genomes was achieved using the Minimus assembler of the AMOS software package 3.1.0 using default parameters (18). The assembled genomes were annotated using Prokka 1.12 (19) (Table 1).

The stock of the A1552 strain described here was previously passed on to Kemter et al., who deposited it in the German Collection of Microorganisms and Cell Cultures (DSM 106276) concomitantly with the release of its genome sequence (20). To improve upon the automated annotation of this study, we checked the annotated gene names of all coding sequences (CDS) and manually added 1,269 commonly used gene names

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under “gene”/“gene_synonym” for CDS without/with an automatically assigned gene name. Allué-Guardia et al. also recently released an A1552 genome sequence. However, the absence of the mutation in \textit{rpoB} conferring rifampicin resistance (RpoB[S531F]) and the presence of a streptomycin resistance-causing mutation in \textit{rpsL} (RpsL[K88R]) (21) suggest that this isolate represents a lineage distinct from that of the more commonly used rifampicin-resistant strain A1552 described here.

**Data availability.** The genome sequences have been deposited in NCBI GenBank under the accession numbers CP028894 and CP028895 (A1552), CP028827 and CP028828 (N16961), and CP028892 and CP028893 (Sa5Y). The raw reads are available under SRA numbers SRX4011578, SRX4011577, and SRX4011579.

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N.M., N.C.D.D., and M.B. designed the research, N.M. and N.C.D.D. performed the experiments, M.B. assigned the gene/gene_synonym names, and N.M., N.C.D.D., and M.B. wrote the manuscript.

**REFERENCES**

1. World Health Organization. 2018. Cholera: the forgotten pandemic. World Health Organization, Geneva, Switzerland. https://www.who.int/cholera/the-forgotten-pandemic/en/.

2. Lipp EK, Huq A, Colwell RR. 2002. Effects of global climate on infectious disease: the cholera model. Clin Microbiol Rev 15:757–770. https://doi.org/10.1128/CMR.15.4.757-770.2002.

3. Nelson EJ, Harris JB, Morris JGJ, Calderwood SB, Camilli A. 2009. Cholera transmission: the host, pathogen and bacteriophage dynamic. Nat Rev Microbiol 7:689–702. https://doi.org/10.1038/nrmicro2204.

4. Faruque SM, Asadulghani Saha MN, Alim AR, Albert MJ, Islam KM, Mekalanos JJ. 1998. Analysis of clinical and environmental strains of nontoxigenic \textit{Vibrio cholerae} for susceptibility to CTXPhi: molecular basis for origin of new strains with epidemic potential. Infect Immun 66:5819–5825.

5. Faruque SM, Mekalanos JJ. 2003. Pathogenicity islands and phages in \textit{Vibrio cholerae} evolution. Trends Microbiol 11:505–510. https://doi.org/10.1016/j.tim.2003.09.003.

6. Blokesch M, Schoolnik GK. 2007. Serogroup conversion of \textit{Vibrio cholerae} in aquatic reservoirs. PLoS Pathog 3:e81. https://doi.org/10.1371/journal.ppat.0030081.

7. Shapiro BJ, Levade I, Kovacikova G, Taylor RK, Almagro-Moreno S. 2016. Origins of pandemic \textit{Vibrio cholerae} from environmental gene
8. Le Roux F, Blokesch M. 2018. Eco-evolutionary dynamics linked to horizontal gene transfer in vibrios. Annu Rev Microbiol 72:89–110. https://doi.org/10.1146/annurev-micro-090817-062148.

9. Yildiz FH, Schoolnik GK. 1998. Role of rpoS in stress survival and virulence of Vibrio cholerae. J Bacteriol 180:773–784.

10. Blokesch M. 2012. A quorum sensing-mediated switch contributes to natural transformation of Vibrio cholerae. Mob Genet Elements 2:224–227. https://doi.org/10.4161/mge.22284.

11. Eberhart-Phillips J, Besser RE, Tormey MP, Koo D, Feikin D, Araneta MR, Wells J, Kilman L, Rutherford GW, Baron R, Mascola L. 1996. An outbreak of cholera from food served on an international aircraft. Epidemiol Infect 116:9–13. https://doi.org/10.1017/S0950268800058891.

12. Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, Taviani E, Jeon Y-S, Kim DW, Lee J-H, Brettin TS, Bruce DC, Challacombe JF, Detter JC, Han CS, Muk AC, Cherakov O, Saunier S, 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 USA 106:15442–15447. https://doi.org/10.1073/pnas.0907787106.

13. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH, Kariuki S, Croucher NJ, Choi SY, Harris SR, Lebens M, Niigaki SK, Kim EJ, Ramamurthy T, Chun J, Wood JL, Clements JH, Taviani E, Nair GB, Colwell RR. 2011. Evidence for several waves of global transmission in the seventh cholera pandemic. Proc Natl Acad Sci U S A 108:15442–15447. https://doi.org/10.1073/pnas.0907787106.

14. Domman D, Quilici ML, Dorman MJ, Njamkepo E, Mutreja A, Mather AE, Delgado G, Morales-Espinosa R, Grimon PAD, Lizarraga-Partida ML, Boucher C, Anensen DM, Kariuki S, Taviani E, Nair GB, Colwell RR. 2017. Integrated view of Vibrio cholerae in the Americas. Science 358:789–793. https://doi.org/10.1126/science.aao2136.

15. 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, Melakalos JJ, Venter JC, Fraser CM. 2000. DNA sequence of both chromosomes of the cholera pathogen Vibrio cholerae. Nature 406:477–483. https://doi.org/10.1038/35020000.

16. Val ME, Marbouty M, de Lemos Martins F, Kennedy SP, Kemble H, Bland MJ, Zosso C, Skovgaard O, Mazel D. 2016. A checkpoint control orchestrates the replication of the two chromosomes of Vibrio cholerae. Sci Adv 2:e1501914. https://doi.org/10.1126/sciadv.1501914.

17. Keymer DP, Miller MC, Schoolnik GK, Bohm AB. 2007. Genomic and phenotypic diversity of coastal Vibrio cholerae strains is linked to environmental factors. Appl Environ Microbiol 73:3705–3714. https://doi.org/10.1128/AEM.02736-06.

18. Sommer DD, Delcher AL, Salzberg SL, Pod M. 2007. Minimus: a fast, lightweight genome assembler. BMC Bioinformatics 8:64. https://doi.org/10.1186/1471-2105-8-64.

19. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

20. Kemter FS, Messerschmidt SJ, Schalpp N, Sobetzko P, Lang E, Bunk B, Spröer C, Tschler JK, Yildiz FH, Overmann J, Waldminghaus T. 2018. Synchronous termination of replication of the two chromosomes is an evolutionary selected feature in Vibrionaceae. PLoS Genet 14:e1007251. https://doi.org/10.1371/journal.pgen.1007251.

21. Allué-Guardia A, Echazarreta M, Koenig SSK, Klose KE, Eppinger M. 2018. Closed genome sequence of Vibrio cholerae O1 El Tor Inaba Strain A1552. Genome Announc 6:e00098-18. https://doi.org/10.1128/genomeA.00098-18.