

Genome Sequence of the Human Pathogen *Vibrio cholerae* Amazonia

Cristiane C. Thompson,¹ Michel A. Marin,¹ Graciela M. Dias,² Bas E. Dutilh,^{3,4} Robert A. Edwards,⁴ Tetsuya Iida,⁵ Fabiano L. Thompson,² and Ana Carolina P. Vicente^{1*}

Laboratory of Molecular Genetics of Microorganisms, Oswaldo Cruz Institute, Rio de Janeiro, Brazil¹; Department of Marine Biology, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil²; Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Geert Grootplein 28, 6525 GA Nijmegen, Netherlands³; Department of Biology and Department of Computer Science, San Diego State University, 5500 Campanile Drive, San Diego, California 92182⁴; and Laboratory of Genomic Research on Pathogenic Bacteria, International Research Center for Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan⁵

Received 22 June 2011/Accepted 4 August 2011

***Vibrio cholerae* O1 Amazonia is a pathogen that was isolated from cholera-like diarrhea cases in at least two countries, Brazil and Ghana. Based on multilocus sequence analysis, this lineage belongs to a distinct profile compared to strains from El Tor and classical biotypes. The genomic analysis revealed that it contains *Vibrio* pathogenicity island 2 and a set of genes related to pathogenesis and fitness, such as the type VI secretion system, present in choleraogenic *V. cholerae* strains.**

Vibrio cholerae O1 Amazonia was identified as the cause of cholera cases in villages in the Brazilian Amazon region in 1991, during the seventh cholera pandemic that reached South America and Brazil. Unlike the major human pathogen *V. cholerae* O1 El Tor biotype, these strains were characterized as not carrying the cholera toxin gene (*ctxAB*) or the *tcpA* gene, encoding the structural protein of the TCP pilus, the major virulence determinants of the epidemic strains, and they presented a distinct genomic profile (1). Multilocus sequence analysis (MLSA) revealed that the *V. cholerae* Amazonia strains belong to a profile distinct from the epidemic/pandemic strains, clustering with some isolates from cholera cases in Ghana in Africa (9). In order to reveal the genomic elements present in this lineage that could explain its pathogenesis and fitness, we sequenced the complete genome of a *V. cholerae* Amazonia strain.

The chromosomes of *V. cholerae* O1 Amazonia R-18332 were sequenced by using the Roche 454 pyrosequencing system according to the methods described Margulies et al. (4). Genomic DNA was extracted using a previously described method (8). The coverage was 20-fold, and raw reads were assembled using the Newbler software, generating 63 contigs. The genome was annotated and analyzed using the RAST annotation server (6). The estimated size of the *V. cholerae* O1 Amazonia R-18332 genome, comprising the two chromosomes, is 4,005,357 bp with an average G+C content of 47%. The number of coding sequences (CDS) is 3,622.

None of the genes of the two major virulence determinants, CTXPhi, the lysogenic filamentous bacteriophage that carries the cholera toxin genes, and *Vibrio* pathogenicity island 1 (VPI-1), which harbors the *tcpA* gene, were found in the *V. cholerae* O1 Amazonia R-18332 genome. However, a sequence was identified that showed 91% and 96% identity at the nucleotide

and amino acid levels, respectively, with the VPI-1 integrase gene from *V. cholerae* O1 pandemic lineages. *Vibrio* pathogenicity island 2 (VPI-2), carried by cholera-producing strains (7), was also present in this Amazonia strain. Most of the 57-kb region corresponding to VPI-2 in Amazonia presented 98% nucleotide identity with the pandemic lineages, but some genes, like *nanH* (neuraminidase) and *hsdM* (putative DNA methylase), presented distinct alleles. The genome sequence analysis also revealed 61 genes known to be related to virulence, disease, and defense. Some of these genes are related to invasion and intracellular resistance (e.g., metalloprotease, lipase precursor, cytolysin, hemolysin [*hlyA*], pore-forming toxin, methyl-accepting chemotaxis protein, hemolysin secretion protein [*hlyB*], lipase activator protein, and lipase-specific foldase). There is experimental evidence for both cytotoxic and enterotoxic activities of the hemolysin (2). The type VI secretion system (T6SS), recently identified in *V. cholerae* and associated with its survival in aquatic environments (3), was identified in *V. cholerae* O1 Amazonia R-18332. Another feature found in this strain was the *intI4* gene, the genomic signature of the chromosomal *V. cholerae* superintegron (5).

Nucleotide sequence accession numbers. *V. cholerae* O1 Amazonia R-18332 has been deposited in the BCCM/LMG Bacteria Collection at Ghent University and in the LGMM Vibrio Collection at Oswaldo Cruz Institute Collection (Rio de Janeiro, Brazil). The results of the whole-genome shotgun project have been deposited with DDBJ/EMBL/GenBank under accession number AFSV00000000. The version described in this paper is the first version, AFSV01000000.

This work was sponsored by CAPES, CNPq, and FAPERJ. R.A.E. acknowledges support of NSF grant DBI 0850356 from the Division of Biological Infrastructure. B.E.D. is supported by a Dutch Science Foundation (NWO) Veni grant (016.111.075).

REFERENCES

1. Coelho, A., J. R. Andrade, A. C. Vicente, and C. A. Salles. 1995. New variant of *Vibrio cholerae* O1 from clinical isolates in Amazonia. *J. Clin. Microbiol.* 33:114–118.

* Corresponding author. Mailing address: Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, P.O. Box 926, CEP 21045-900, Rio de Janeiro, Brazil. Phone: 55-21-38658176. Fax: 55-21-22604282. E-mail: anapaulo@ioc.fiocruz.br.

2. **Coelho, A., J. R. Andrade, A. C. Vicente, and V. J. Dirita.** 2000. Cytotoxic cell vacuolating activity from *Vibrio cholerae* hemolysin. *Infect. Immun.* **68**:1700–1705.
3. **Macintyre, D. L., S. T. Miyata, M. Kitaoka, and S. Pukatzki.** 2010. The *Vibrio cholerae* type VI secretion system displays antimicrobial properties. *Proc. Natl. Acad. Sci. U. S. A.* **107**:6–10.
4. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
5. **Mazel, D., B. Dychinco, V. A. Webb, and J. Davies.** 1998. A distinctive class of integron in the *Vibrio cholerae* genome. *Science* **280**:605–608.
6. **Meyer, F. D., et al.** 2008. The metagenomics RAST server: a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* **9**:386.
7. **Murphy, R. A., and E. F. Boyd.** 2008. Three pathogenicity islands of *Vibrio cholerae* can excise from the chromosome and form circular intermediates. *J. Bacteriol.* **190**:636–647.
8. **Pitcher, D. G., and O. R. Saunders.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.* **8**:151–156.
9. **Thompson, C. C., et al.** *Vibrio cholerae* O1 lineages driving cholera outbreaks during seventh cholera pandemic in Ghana. *Infect. Genet. Evol.* in press.