Whole-Genome Sequences of 26 Vibrio cholerae Isolates

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The human pathogen Vibrio cholerae employs several adaptive mechanisms for environmental persistence, including natural transformation and type VI secretion, creating a reservoir for the spread of disease. Here, we report whole-genome sequences of 26 diverse V. cholerae isolates, significantly increasing the sequence diversity of publicly available V. cholerae genomes.

### Table 1 List of V. cholerae strains sequenced in this study

| Strain | Location | Source | Yr of isolation | Type VI killing activity | NCBI accession no. |
|--------|----------|--------|-----------------|--------------------------|-------------------|
| 1496-86| United States (LA) | Moore swab | 1986 | – | MIPC00000000 |
| 2523-87| United States (LA) | Moore swab | 1974 | + | MIPE00000000 |
| VC48 | United States (FL) | Oyster | 1981 | + | MIOT00000000 |
| 2633-78| Brazil | Sewage | 1978 | + | MIPH00000000 |
| 857 | Bangladesh | Water | 1996 | + | MIKH00000000 |
| 3272-78| United States (MD) | Water | 1977 | + | MIOZ00000000 |
| TP | United States (CA) | Water | 2000 | + | MIPO00000000 |
| 2559-78| United States (LA) | Crab | 1978 | + | MIOV00000000 |
| HE46 | Haití (center) | Gray water | 2011 | + | MIPM00000000 |
| 2479-86| United States (LA) | Moore swab | 1986 | + | MIPB00000000 |
| 2497-86| United States (LA) | Moore swab | 1987 | + | MIPD00000000 |
| 2512-86| United States (LA) | Moore swab | 1986 | + | MIOT00000000 |
| 2631-78| United States (LA) | Moore swab | 1978 | + | MIOZ00000000 |
| VC22 | United States (FL) | Oyster | 1981 | + | MIKH00000000 |
| VC33 | United States (AL) | Oyster | 2009 | + | MIOW00000000 |
| VC36 | United States (AL) | Oyster | 2009 | + | MIOW00000000 |
| 3568-07| Mexico | Queso fresco | 2007 | + | MIPL00000000 |
| 1074-78| Brazil | Sewage | 1978 | + | MIPG00000000 |
| 3223-74| Guam | Storm drain | 1974 | + | MIZG00000000 |
| 3225-74| Guam | Storm drain | 1974 | + | MIPF00000000 |
| 2740-80| United States (Gulf Coast) | Water | 1980 | + | MIKH00000000 |
| 692-79| United States (LA) | Water | 1979 | + | MIPA00000000 |
| SIO | United States (CA) | Water | 2000 | + | MIPJ00000000 |
| C6706 | Peru | Patient | 1991 | – | MIPM00000000 |
| MZO-2 | Bangladesh | Patient | 2001 | – | MIKH00000000 |
| V52 | Sudan | Patient | 1968 | + | MIPO00000000 |

*Strains were isolated from an environmental source, except strains C6706, MZO-2, and V52.

*Presence (+) or absence (–) of constitutive type VI killing activity.
to pierce the membranes of adjacent cells and deliver toxic effectors that can lead to lysis of target (prey) cells. In a recent survey, Bernardy et al. (10) noted key differences within a diverse set of isolates for several phenotypes, including chitinase production, contact-dependent killing indicative of T6SS activity, and natural transformation, which can promote horizontal gene transfer. Both clinical and environmental isolates were rarely naturally transformable. In contrast, the majority of environmental, but not clinical, isolates constitutively killed Escherichia coli prey. Because different regulatory schemes control the phenotypes tested (11, 12), we sought to better understand the genetics that underlie these diverse V. cholerae phenotypes by characterizing whole-genome sequences of 23 environmental and three clinical isolates from Bernardy et al.

All strains were grown overnight in LB medium (Difco) at 37°C, with shaking. Genomic DNA was isolated using a ZR fungal/bacterial DNA mini prep kit (Zymo Research), and paired-end fragment libraries were constructed using a Nextera XT DNA library preparation kit (Illumina) with a fragment length of 300 bp. Libraries were sequenced by the High Throughput Sequencing Core at Georgia Institute of Technology on an Illumina HiSeq 2500 Rapid platform, producing approximately 280 million 100-bp reads in total. Reads were trimmed using Trimmomatic (13) to remove adapters and bases with a read quality score of <20. Genomes were assembled using SPAdes version 3.5 (14) and annotated using the Rapid Annotation and Subsystem Technology (RAST) web tool provided by the National Microbial Pathogen Data Resource (15–18). T6SS genes were annotated using T6SS Predictor (A. T. Chande et al., unpublished data).

T6SS loci were annotated in all genomes in an effort to characterize the genetic basis of T6SS-mediated killing among diverse environmental V. cholerae isolates. All genomes were found to encode the previously characterized large cluster and two auxiliary clusters, which together comprise the canonical T6SS loci. In addition, two previously unreported T6SS loci were discovered in six of the isolates. Numerous examples of novel effector-immunity protein pairs, which function together to catalyze T6SS-mediated killing, were characterized among the set of environmental isolate genomes. Taken together, our genome analysis illuminates the diverse repertoire of genetic mechanisms that underlie T6SS-mediated killing in V. cholerae.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

Strains were generously provided by D. Bartlett, Scripps Institution of Oceanography; C. Tarr and C. Bopp, Centers for Disease Control and Prevention; R. Colwell, University of Maryland; A. DePaolo, Food and Drug Administration; and J. Zhu, University of Pennsylvania. The Georgia Tech Sequencing Core assisted with genome sequencing.

FUNDING INFORMATION

This work, including the efforts of Aroon T. Chande, Lavanya Rishikshwar, and I. K. Jordan, was funded by the IHRC-Georgia Tech Applied Bioinformatics Laboratory. This work, including the efforts of Leonardo Marino-Ramirez, was funded by HHS | National Institutes of Health (NIH). This work, including the efforts of Brian K. Hammer and Samit S. Watve, was funded by the National Science Foundation (NSF) (MCB-1149925).

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