

Complete Genome Sequence of a Free-Living *Vibrio furnissii* sp. nov. Strain (NCTC 11218)[∇]

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The *Vibrionales* are widespread, free-living, Gram-negative proteobacteria (22) that have been linked to pathogenicity in animals and gastroenteric infection in humans. We report the annotated genome sequence of a free-living strain of *Vibrio furnissii* (NCTC 11218) harvested from an estuarine environment. It consists of two circular chromosomes (3.2 Mb and 1.6 Mb) and reveals novel genes likely to be involved in pathogenicity.

Interest in the *Vibrio* is stimulated mainly by the association of certain species with human and animal diseases (1, 4, 5, 6, 8, 20) and by quorum-derived bioluminescent symbiosis with marine animals (3, 24). The most intensively studied species, *V. cholerae*, can be lethally pathogenic (20). To date, 10 noncholeric *Vibrio* species have been implicated in human infection (11). These “emerging *Vibrio* species” include *V. furnissii*, a widespread, free-living, marine species that is associated with acute gastroenteritis (5, 9). More recently, *V. furnissii* has been reported to produce significant amounts of hydrocarbons that can be converted into renewable biofuels (18, 23).

The complete genome sequence of *V. furnissii* strain NCTC 11218 was determined using shotgun and pyrosequencing, at Agencourt Biosciences (Beckman Coulter Genomics). *V. furnissii* was sourced from the National Collection of Type Cultures (Health Protection Agency, United Kingdom) (13). Open reading frame (ORF) prediction was performed using GLIMMER software (10). The genome was scanned for tRNAs and tmRNAs by using tRNAscan-SE 1.23 and ARAGON, respectively (12, 14).

The assembled *V. furnissii* genome comprises two circular chromosomes. Chromosome I is 3,294,546 bp, and chromosome II is 1,621,862 bp. The purine content of chromosome I ($G+C_{\text{chr.I}}$) is 50.73%, and that of chromosome II ($G+C_{\text{chr.II}}$) is 50.54%, which is 4 to 10% higher than the other sequenced *Vibrio* species. Chromosome I contains 3,013 ORFs and 95 tRNA sequences, and chromosome II has 1,448 ORFs and 5 tRNA sequences, which is comparable to other *Vibrio*.

Despite reports of pathogenicity from *V. furnissii* (1, 21), the genome does not contain homologues to a number of the factors associated with virulence that have been identified in other *Vibrio* species, such as *toxRT*, *zot*, *ctxAB*, and genes encoding the type III secretion system. Conversely, other se-

quences, including *ompU*, *hlyA*, *toxS*, and *tcpA/tcpI*, are present (9, 15). However, the *V. furnissii* genome also contains novel sequences that may be associated with virulence. For example, VfuB00340 is an ORF encoding a 3,150-amino-acid polypeptide (329.18 kDa) with no homologues in the bacterial database. The protein is predicted to contain one signal peptide domain, 16 VCBS (hemolysin-type calcium-binding repeat) domains, and no transmembrane helix, which suggests that the gene product is a novel exoprotein that may have a role in cell adhesion and/or pathogenesis (2, 7). Therefore, despite scant genomic evidence that *V. furnissii* poses a significant pathogenic threat, the *V. furnissii* genome contains novel sequences that warrant further investigation.

In terms of bioluminescence and quorum sensing, the AI-2 production gene *luxS* is present in the *V. furnissii* genome, but the transcriptional regulator *luxR* (17) and the luciferase genes *luxAB* that mediate bioluminescence (16) are absent.

Concerning the recent reports of large-scale hydrocarbon production by *V. furnissii*, we confirm that the genome contains no obvious genes for the synthesis of alkanes (18, 19, 23).

Nucleotide sequence accession numbers. The *V. furnissii* NCTC 11218 sequences are available through the NCBI under accession numbers CP002377 (chromosome I) and CP002378 (chromosome II).

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REFERENCES

1. Austin, B. 2010. Vibrios as causal agents of zoonoses. *Vet. Microbiol.* **140**: 310–317.
2. Baumann, U., S. Wu, K. M. Flaherty, and D. B. McKay. 1993. Three-dimensional structure of the alkaline protease of *Pseudomonas aeruginosa*: a two-domain protein with a calcium binding parallel beta roll motif. *EMBO J.* **12**:3357–3364.
3. Belas, R., et al. 1982. Bacterial bioluminescence: isolation and expression of the luciferase genes from *Vibrio harveyi*. *Science* **218**:791–793.
4. Ben-Haim, Y., et al. 2003. *Vibrio corallilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *Int. J. Syst. Evol. Microbiol.* **53**:309–315.
5. Brenner, D., et al. 1983. *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. *J. Clin. Microbiol.* **18**:816–824.
6. Chakraborty, S., G. B. Nair, and S. Shinoda. 1997. Pathogenic vibrios in the natural aquatic environment. *Rev. Environ. Health* **12**:63–80.
7. Chung, Y. J., M. T. Steen, and J. N. Hansen. 1992. The subtilin gene of *Bacillus subtilis* ATCC 6633 is encoded in an operon that contains a homologue of the hemolysin B transport protein. *J. Bacteriol.* **174**:1417–1422.
8. Colwell, R. R. 2004. Infectious disease and environment: cholera as a paradigm for waterborne disease. *Int. Microbiol.* **7**:285–289.
9. Dalsgaard, A., et al. 1997. *Vibrio furnissii* isolated from humans in Peru: a possible human pathogen? *Epidemiol. Infect.* **119**:143–149.
10. Delcher, A., D. Harmon, S. Kasif, O. White, and S. Salzberg. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* **27**:4636–4641.

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11. **Igbnosa, E. O., and A. I. Okoh.** 2008. Emerging *Vibrio* species: an unending threat to public health in developing countries. *Res. Microbiol.* **159**:495–506.
12. **Laslett, D., and B. Canback.** 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res.* **32**:11–16.
13. **Lee, J. V., P. Shread, A. L. Furniss, and T. N. Bryant.** 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). *J. Appl. Bacteriol.* **50**:73–94.
14. **Lowe, T., and S. Eddy.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
15. **Magalhães, V., A. Castello Filho, M. Magalhães, and T. T. Gomes.** 1993. Laboratory evaluation on pathogenic potentialities of *Vibrio furnissii*. *Mem. Inst. Oswaldo Cruz* **88**:593–597.
16. **Meighen, E.** 1993. Bacterial bioluminescence: organization, regulation, and application of the lux genes. *FASEB J.* **7**:1016–1022.
17. **Ng, W., and B. Bassler.** 2009. Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.* **43**:197–222.
18. **Park, M.** 2005. New pathway for long-chain n-alkane synthesis via 1-alcohol in *Vibrio furnissii* M1. *J. Bacteriol.* **187**:1426–1429.
19. **Park, M., M. Tanabe, K. Hirata, and K. Miyamoto.** 2001. Isolation and characterization of a bacterium that produces hydrocarbons extracellularly which are equivalent to light oil. *Appl. Microbiol. Biotechnol.* **56**:448–452.
20. **Rivera, I., J. Chun, A. Huq, R. Sack, and R. Colwell.** 2001. Genotypes associated with virulence in environmental isolates of *Vibrio cholerae*. *Appl. Environ. Microbiol.* **67**:2421–2429.
21. **Tantillo, G. M., M. Fontanarosa, A. Di Pinto, and M. Musti.** 2004. Updated perspectives on emerging vibrios associated with human infections. *Lett. Appl. Microbiol.* **39**:117–126.
22. **Thompson, F., T. Iida, and J. Swings.** 2004. Biodiversity of vibrios. *Microbiol. Mol. Biol. Rev.* **68**:403–431.
23. **Wackett, L., J. Frias, J. Seffernick, D. Sukovich, and S. Cameron.** 2007. Genomic and biochemical studies demonstrating the absence of an alkane-producing phenotype in *Vibrio furnissii* M1. *Appl. Environ. Microbiol.* **73**:7192–7198.
24. **Yu, C., A. Lee, B. Bassler, and S. Roseman.** 1991. Chitin utilization by marine bacteria. A physiological function for bacterial adhesion to immobilized carbohydrates. *J. Biol. Chem.* **266**:24260–24267.