genomeA
Journals.ASM.org

High-Quality Draft Genome Sequence of *Vagococcus lutrae* Strain LBD1, Isolated from the Largemouth Bass *Micropterus salmoides*

François Lebreton,[a,b] Michael D. Valentino,[a,b] Lucile B. Duncan,[a] Qiandong Zeng,[b] Abigail Manson Mcguire,[b] Ashlee M. Earl,[b] Michael S. Gilmore[a,b]

Departments of Ophthalmology, Microbiology, and Immunobiology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, USA[a]; The Broad Institute, Cambridge, Massachusetts, USA[b]

Vagococci are usually isolated from marine hosts and occasionally from endodontic infections. Using 16S rRNA gene comparison, the closest relatives are members of the genera *Enterococcus* and *Carnobacterium*. A draft sequence of *Vagococcus lutrae* was generated to clarify the relationship of *Vagococcus* to these and other related low-G+C Gram-positive bacteria.

Received 18 November 2013 **Accepted** 26 November 2013 **Published** 26 December 2013

Citation Lebreton F, Valentino MD, Duncan LB, Zeng Q, Manson Mcguire A, Earl AM, Gilmore MS. 2013. High-quality draft genome sequence of *Vagococcus lutrae* strain LBD1, isolated from the largemouth bass *Micropterus salmoides*. Genome Announc. 1(6):e01087-13. doi:10.1128/genomeA.01087-13.

Address correspondence to Michael S. Gilmore, michael_gilmore@meei.harvard.edu.

The bacterial genus *Vagococcus* was proposed in 1989 for Gram-positive, catalase-negative, motile, coccus-shaped bacteria that react with Lancefield group N antisera (1). Phylogenetic trees based on 16S rRNA gene sequences place *Vagococcus* adjacent to the genera *Enterococcus* and *Carnobacterium* (2). The genus *Vagococcus* currently consists of eight species (*V. fluvialis, V. salmoninarum, V. lutrae, V. fessus, V. carniphilus, V. elongatus*, *V. penaei*, and *V. acidifermentans*) (1–8).

Most representatives of *Vagococcus* have been isolated from aquatic environments, suggesting that members of this genus have traits optimized for existence and survival in marine habitats (1–8). *V. fluvialis* strains have been suggested as a promising candidate probiotic for aquaculture, a critical economic activity practiced worldwide (9). Interestingly, strains of this genus have also been isolated from patients receiving endodontic treatment for periradicular lesions (10).

In this study, we sampled the intestine of a largemouth bass (*Micropterus salmoides*) that was caught in the wild in Maine. Following outgrowth on bile esculin azide agar, we isolated a strain of *V. lutrae* named LBD1. This strain was subjected to whole-genome sequencing and constitutes the first report of a genome of the species *V. lutrae* and the genus *Vagococcus*.

Genomic DNA was isolated with a DNeasy kit (Qiagen, Valencia, CA) and was quantified by a Qubit fluorometric assay (Invitrogen, Carlsbad, CA). The paired-end library (2 × 250 bp) was prepared using a Nextera XT DNA sample preparation kit (Illumina, San Diego, CA). The quality and quantity of the library DNA fragments were measured on an Agilent Technologies 2100 Bioanalyzer (Santa Clara, CA). Sequencing was carried out on the Illumina MiSeq personal sequencer platform at the Massachusetts Eye and Ear Infirmary (MEEI) Ocular Genomics Institute (Boston, MA). CLC Genomics Workbench version 4.9 software (CLC bio, Cambridge, MA) was used for *de novo* assembly on an i7 Intel dual-core workstation. For *V. lutrae* LBD1, 7.48 million paired-end reads were collected. The average coverage of the 1.83-Mb LBD1 assembled genome (21 scaffolds; scaffold N_{50}, 81.28 kb) was 720×.

Protein-coding genes were predicted with Prodigal (11) and filtered to remove genes with >70% overlap with tRNAs or rRNAs, which were identified using tRNAscan-SE (12) and RNAmmer (13), respectively. The gene product names were assigned based on top BLAST hits against the SwissProt protein database and a protein family profile search against the TIGRfam equivalogs, followed by top BLAST hits to KEGG protein sequences. For *V. lutrae* LBD1, we identified 1,736 protein-coding genes (48% with "hypothetical protein" as the gene product name), 3 rRNA genes (one each for 5S, 16S, and 23S), and 49 tRNAs (19 amino acids with tRNA-Asn missing, probably in the contig gap regions). Additional analyses performed included Pfam (14), TIGRfam (15), KEGG (16), COG (17), GO (18), and TMHMM (19) analyses.

The availability of this genome sequence begins to illuminate the roles of vagococci as members of the microbiome of fish and other marine animals and may aid future studies related to the aquaculture industry and/or human medicine.

Nucleotide sequence accession numbers. This whole-genome project has been deposited at DDBJ/EMBL/GenBank under accession no. AYSH00000000. The version of *V. lutrae* LBD1 described in this paper is version AYSH01000000.

ACKNOWLEDGMENTS

This project was funded in part by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract no. HHSN272200900018C. Portions of this work were also supported by NIH/NIAID grants no. AI083214 (Harvard-wide Program on Antibiotic Resistance) and no. AI072360.

REFERENCES

1. **Collins MD, Ash C, Farrow JA, Wallbanks S, Williams AM.** 1989. 16S ribosomal ribonucleic acid sequence analyses of lactococci and related

taxa. Description of *Vagococcus fluvialis* gen. nov., sp. nov. J. Appl. Bacteriol. **67:**453–460.
2. **Wallbanks S, Martinez-Murcia AJ, Fryer JL, Phillips BA, Collins MD.** 1990. 16S rRNA sequence determination for members of the genus *Carnobacterium* and related lactic acid bacteria and description of *Vagococcus salmoninarum* sp. nov. Int. J. Syst. Bacteriol. **40:**224–230.
3. **Lawson PA, Foster G, Falsen E, Ohlén M, Collins MD.** 1999. *Vagococcus lutrae* sp. nov., isolated from the common otter (*Lutra lutra*). Int. J. Syst. Bacteriol. **49**(Pt 3):1251–1254.
4. **Hoyles L, Lawson PA, Foster G, Falsen E, Ohlén M, Grainger JM, Collins MD.** 2000. *Vagococcus fessus* sp. nov., isolated from a seal and a harbour porpoise. Int. J. Syst. Evol. Microbiol. **50**(Pt 3):1151–1154.
5. **Shewmaker PL, Steigerwalt AG, Morey RE, Carvalho Mda G, Elliott JA, Joyce K, Barrett TJ, Teixeira LM, Facklam RR.** 2004. *Vagococcus carniphilus* sp. nov., isolated from ground beef. Int. J. Syst. Evol. Microbiol. **54:**1505–1510.
6. **Lawson PA, Falsen E, Cotta MA, Whitehead TR.** 2007. *Vagococcus elongatus* sp. nov., isolated from a swine-manure storage pit. Int. J. Syst. Evol. Microbiol. **57:**751–754.
7. **Jaffrès E, Prévost H, Rossero A, Joffraud JJ, Dousset X.** 2010. *Vagococcus penaei* sp. nov., isolated from spoilage microbiota of cooked shrimp (*Penaeus vannamei*). Int. J. Syst. Evol. Microbiol. **60:**2159–2164.
8. **Wang L, Cui YS, Kwon CS, Lee ST, Lee JS, Im WT.** 2011. *Vagococcus acidifermentans* sp. nov., isolated from an acidogenic fermentation bioreactor. Int. J. Syst. Evol. Microbiol. **61:**1123–1126.
9. **Sorroza L, Padilla D, Acosta F, Román L, Grasso V, Vega J, Real F.** 2012. Characterization of the probiotic strain *Vagococcus fluvialis* in the protection of European sea bass (*Dicentrarchus labrax*) against vibriosis by *Vibrio anguillarum*. Vet. Microbiol. **155:**369–373.
10. **Al-Ahmad A, Pelz K, Schirrmeister JF, Hellwig E, Pukall R.** 2008. Characterization of the first oral *Vagococcus* isolate from a root-filled tooth with periradicular lesions. Curr. Microbiol. **57:**235–238.
11. **Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics **11:**119. doi:10.1186/1471-2105-11-119.
12. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25:**955–964.
13. **Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35:**3100–3108.
14. **Finn RD, Tate J, Mistry J, Coggill PC, Sammut SJ, Hotz HR, Ceric G, Forslund K, Eddy SR, Sonnhammer EL, Bateman A.** 2008. The Pfam protein families database. Nucleic Acids Res. **36:**D281–D288. doi:10.1093/nar/gkm960.
15. **Haft DH, Selengut JD, White O.** 2003. The TIGRfams database of protein families. Nucleic Acids Res. **31:**371–373.
16. **Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M.** 1999. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. **27:**29–34.
17. **Tatusov RL, Koonin EV, Lipman DJ.** 1997. A genomic perspective on protein families. Science **278:**631–637.
18. **Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M.** 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics **21:**3674–3676.
19. **Krogh A, Larsson B, von Heijne G, Sonnhammer EL.** 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. **305:**567–580.