



Draft Genome Sequences of *Cetobacterium somerae* 2G Large and Two Novel *Cetobacterium* Isolates from Intestines of Channel Catfish (*Ictalurus punctatus*)

 Benjamin R. LaFrentz,^a Stacey A. LaFrentz,^b Benjamin H. Beck,^a Covadonga R. Arias^{b†}

^aAquatic Animal Health Research Unit, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Auburn, Alabama, USA

^bSchool of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama, USA

ABSTRACT We report the draft genome sequences of *Cetobacterium somerae* 2G Large and two *Cetobacterium* isolates, 2A and 8H, which may represent novel species. The isolates were recovered from the intestines of catfish, and the genomes will assist in research to understand their potential use as probiotics in aquaculture.

The genus *Cetobacterium* is comprised of two described species, *C. ceti* (1) and *C. somerae* (2). Our research, among that of others, has shown a high prevalence of *Cetobacterium* in the gastrointestinal tracts of fish (3–6). This finding has led us to explore the role of these bacteria in the physiology of the fish gastrointestinal tract and their potential use as probiotics in aquaculture. The draft genomes reported here will assist in this research.

Pond-raised channel catfish (*Ictalurus punctatus*) were collected at the E. W. Shell Fisheries Center in Auburn, AL, and euthanized with 300 mg liter⁻¹ neutral buffered Tricaine-S (Western Chemical) according to approved Auburn University IACUC protocol 2016–2946. The intestines were aseptically removed and transferred to an anaerobic chamber (Shel Lab) with 5% hydrogen, 5% carbon dioxide, and 90% nitrogen. The intestinal contents were removed, diluted 1:1 (vol/vol) with Gifu anaerobic broth (GAM; HiMedia Laboratories) containing 0.05% L-cysteine (GAM-L), and serially diluted, and then dilutions were plated on GAM agar and brucella blood agar with hemin and vitamin K (Hardy Diagnostics). The plates were incubated at 25°C for 48 h under anaerobic conditions. Colonies with typical characteristics of *Cetobacterium* spp. (gray, smooth, and circular) were subcultured to GAM agar. Single isolated colonies were expanded in GAM-L broth anaerobically at 25°C and cryopreserved at –80°C in 20% glycerol. Isolates were identified as *Cetobacterium* using partial 16S rRNA sequencing, and three were chosen for genome sequencing (2G Large, 2A, and 8H) based on phylogenetic analysis of the 16S rRNA gene sequences (data not shown).

Genomic DNA (gDNA) was extracted using a Qiagen DNeasy blood and tissue kit (Qiagen) and sent to MR DNA (Molecular Research LP, Shallowater, TX) for sequencing using a Pacific Biosciences (PacBio) Sequel sequencer. The gDNA was sheared using Covaris g-TUBEs (Covaris, Inc.), and a multiplex microbial library was prepared using a SMRTbell Express template prep kit 2.0 (PacBio). The three DNA samples were pooled in equimolar ratios prior to the final library purification, and the average fragment size was determined to be 9,160 bp. The SMRTbell library pool was then sequenced using a 10-h movie time on the PacBio Sequel, and default parameters were used for all software unless otherwise noted. *De novo* assembly of each genome was accomplished using the SMRT Analysis Hierarchical Genome Assembly Process (HGAP) and Falcon pipelines and then polished with Arrow (SMRT Link version 7.0). The single-pass reads were mapped against the seed reads, which averaged 15.6 kb in length across the three genomes. The average percentage of bases successfully realigned to each draft assembly was 95.5%,

Citation LaFrentz BR, LaFrentz SA, Beck BH, Arias CR. 2020. Draft genome sequences of *Cetobacterium somerae* 2G Large and two novel *Cetobacterium* isolates from intestines of channel catfish (*Ictalurus punctatus*). *Microbiol Resour Announc* 9:e01006-20. <https://doi.org/10.1128/MRA.01006-20>.

Editor Frank J. Stewart, Georgia Institute of Technology

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply.

Address correspondence to Benjamin R. LaFrentz, benjamin.lafrentz@usda.gov.

†Deceased.

Received 27 August 2020

Accepted 7 October 2020

Published 29 October 2020

TABLE 1 Assembly and annotation metrics

Parameter	Data for:		
	<i>Cetobacterium somerae</i> 2G Large	<i>Cetobacterium</i> sp. strain 2A	<i>Cetobacterium</i> sp. strain 8H
Genome size (bp)	2,984,168	2,593,282	2,569,949
No. of contigs	9	14	6
N_{50} contig length (bp)	1,628,627	1,299,302	1,746,580
Avg genome coverage (×)	1,810	2,416	1,820
G+C content (%)	29.09	29.85	29.88
Total no. of genes	2,814	2,647	2,499
No. of rRNA genes	44	34	34
No. of tRNA genes	82	71	77
GenBank accession no.	JACHTH000000000	JACFAJ000000000	JACHTG000000000
SRA accession no.	SRR12455216	SRR12455218	SRR12455217

with a mean concordance of 88.8%. The assembled genome sequences were submitted to NCBI for annotation using the Prokaryotic Genome Annotation Pipeline (PGAP; version 4.12). The assembly and annotation metrics are shown in Table 1.

Average nucleotide identities (ANI) between isolates 2G Large, 2A, and 8H and the genomes of *C. somerae* ATCC BAA-474^T (NCBI accession no. AXZF000000000) and *C. ceti* ATCC 700028^T (NCBI accession no. FUWX000000000) were calculated using the OrthoANLu algorithm at EZBioCloud (7). Genome distances revealed that isolate 2G Large had high genomic similarity to *C. somerae* (98.57%). The ANI values obtained for comparisons of all other genomes were less than 80%, well below the 95 to 96% threshold used to distinguish between members of closely related species (8, 9), suggesting that isolates 2A and 8H represent two novel species in the genus *Cetobacterium*.

Data availability. The draft genome sequences and raw read sequences for the three strains have been deposited in GenBank and SRA under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

Funding for this research was provided through a Non-Assistance Cooperative Agreement between Auburn University and the United States Department of Agriculture–Agricultural Research Service (project no. 6010-32000-027-04-S; Examining the interrelationships between virulent *Aeromonas hydrophila* and the microflora of farmed catfish). The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

REFERENCES

- Foster G, Ross HM, Naylor RD, Collins MD, Ramos CP, Fernández-Garayzábal F, Reid RJ. 1995. *Cetobacterium ceti* gen. nov., sp. nov., a new Gram-negative obligate anaerobe from sea mammals. *Lett Appl Microbiol* 21:202–206. <https://doi.org/10.1111/j.1472-765x.1995.tb01041.x>.
- Finegold SM, Vaisanen M-L, Molitoris DR, Tomzynski TJ, Song Y, Liu C, Collins MD, Lawson PA. 2003. *Cetobacterium somerae* sp. nov. from human feces and emended description of the genus *Cetobacterium*. *Syst Appl Microbiol* 26:177–181. <https://doi.org/10.1078/072320203322346010>.
- Tsuchiya C, Sakata T, Sugita H. 2008. Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett Appl Microbiol* 46:43–48. <https://doi.org/10.1111/j.1472-765x.2007.02258.x>.
- Larsen AM, Mohammed HH, Arias CR. 2014. Characterization of the gut microbiota of three commercially valuable warmwater fish species. *J Appl Microbiol* 116:1396–1404. <https://doi.org/10.1111/jam.12475>.
- Ray C, Bujan N, Tarnecki A, Davis AD, Browdy C, Arias CR. 2017. Analysis of the gut microbiome of Nile tilapia *Oreochromis niloticus* L. fed diets supplemented with Previda and saponin. *J Fish Sci* 11:036–045.
- Burgos FA, Ray CL, Arias CR. 2018. Bacterial diversity and community structure of the intestinal microbiome of channel catfish (*Ictalurus punctatus*) during ontogenesis. *Syst Appl Microbiol* 41:494–505. <https://doi.org/10.1016/j.syapm.2018.04.006>.
- Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 110:1281–1286. <https://doi.org/10.1007/s10482-017-0844-4>.
- Goris J, Konstantinidis KT, Klappenbach A, Coenye T, Vandamme P, Tiedje JM. 2007. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijs.0.64483-0>.
- Chun J, Rainey FA. 2014. Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *Int J Syst Evol Microbiol* 64:316–324. <https://doi.org/10.1099/ijs.0.054171-0>.