



# Complete Genome Sequence of *Veillonella nakazawae* JCM 33966<sup>T</sup> (=CCUG 74597<sup>T</sup>), Isolated from the Oral Cavity of Japanese Children

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**ABSTRACT** We report the complete genome sequence of *Veillonella nakazawae* JCM 33966<sup>T</sup> (=CCUG 74597<sup>T</sup>). This bacterium is a member of the oral *Veillonella* and has the potential to be anticariogenic as an oral probiotic seed.

The genus *Veillonella* consists of small, strictly anaerobic, Gram-negative cocci that can be isolated from the oral cavity and intestinal tract of humans and animals. The bacteria gain energy from the utilization of short-chain organic acids, especially lactate, instead of using carbohydrates or amino acids and subsequently produce acetate and propionate (1–3). Recent reports have shown that the gut bacterium *Veillonella atypica* can improve the running efficiency of the host by converting the lactate produced during exercise into propionate, thus enhancing athletic performance (4). Therefore, members of the genus *Veillonella* are attractive for use as natural probiotic seeds and need further research.

*Veillonella nakazawae* was isolated from the oral cavity of Japanese children and was established as a novel *Veillonella* species in 2020 (5). At present, 8 species from the genus *Veillonella* have been isolated from the human oral cavity, and they are called oral *Veillonella* (5–11). These 8 species have shown lactate consumption ability (5–11). As lactate causes dental caries, it has been suggested that oral *Veillonella* may be anti-cariogenic (12). However, the anticariogenic mechanisms or functions of these species have not been clarified. Therefore, to promote the study of the mechanisms underlying the functioning of oral *Veillonella*, we determined the complete genome sequence of *V. nakazawae* JCM 33966<sup>T</sup> (=CCUG 74597<sup>T</sup>), a *V. nakazawae* type strain (5).

Towards this end, *V. nakazawae* JCM 33966<sup>T</sup> (=CCUG 74597<sup>T</sup>) was grown on Bacto brain heart infusion (BHI) medium (Difco Laboratories, BD) supplemented with 5% (vol/vol) defibrinated sheep blood for 2 days at 37°C under anaerobic conditions (N<sub>2</sub>, 80%; H<sub>2</sub>, 10%; CO<sub>2</sub>, 10%). Genomic DNA was extracted from this strain using the phenol-chloroform extraction and ethanol precipitation procedures (13). The quality of the genomic DNA obtained was checked using the Qubit 4 fluorometer (Thermo Fisher Scientific, MA, USA). The same genomic DNA was used for both long- and short-read sequencing. All DNA sequencing was performed and *de novo* assembly was generated by the Taniguchi Dental Clinic/Oral Microbiome Center (Kagawa, Japan) as per the manufacturer's instructions.

For long-read sequencing, a DNA library was prepared using a native barcoding expansion kit (EXP-NBD104; barcodes 1 to 12; Oxford Nanopore, Oxford, UK) and ligation sequencing kit (SQK-LSK108; Oxford Nanopore) without DNA sharing according to the manufacturer's instructions and was subsequently sequenced using the GridION X5 genome sequencing system with an R9.4.1 flow cell (FLO-MIN106). The long-read sequences were base called using Guppy v.3.6.0 (Oxford Nanopore), and the estimated *N*<sub>50</sub> value was 10.9 kbp. After quality trimming (average Phred quality value of >10.0, short reads

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of >1,000 bp, and adaptor sequences) using NanoFilt v.2.7.1 software (14), a total of 40,817 reads were generated. Concurrently, for short-read sequencing, libraries were prepared for Illumina sequencing using a Nextera DNA Flex library prep kit (Illumina, San Diego, CA, USA), followed by sequencing using the MiSeq platform (Illumina). The raw sequencing data were processed using FASTQ v.0.20.1 (15) for trimming the adaptors, low-quality data (average Phred quality value of >30.0), and low sequencing data (short reads of >10 bp), yielding 1,825,183 short reads with an average length of 149.2 bp.

The remaining high-quality reads were then assembled *de novo* using the Unicycler v.0.4.8 software (16) and were visualized using Bandage v.0.8.1 software (17) to confirm a closed circular sequence. All software was operated using default settings and parameters unless otherwise specified. The final chromosome sequence was 2,097,818 bp (G+C content, 38.7%), and the final coverage of the genome was 259.8 $\times$ .

The final chromosome was annotated using the DDBJ Fast Annotation and Submission Tool (DFAST) v.1.2.7.0 (<https://dfast.nig.ac.jp/>) with default parameters (18). The chromosome contained 1,925 coding DNA sequences (CDSs), 12 rRNAs, 48 tRNAs, and 1 CRISPR.

With the addition of the annotated genome sequence of *V. nakazawae* described here, the genome sequences of 8 species of oral *Veillonella* are now available to the public. In addition, the results of a genome comparison of *V. nakazawae* JCM 33966<sup>T</sup> (=CCUG 74597<sup>T</sup>) against reported type strains of the genus *Veillonella* were reported in our recent study (5). The data obtained will help clarify the anticariogenic mechanisms or functions of *Veillonella* in future studies.

**Data availability.** The raw sequence data were deposited in the DDBJ Sequence Read Archive (SRA), and the complete genome sequence was deposited in DDBJ/GenBank/EMBL under the accession numbers [DRA009432](https://accession.ddbj.ac.jp/DR/DR_A0009432.html) and [AP022321](https://accession.ddbj.ac.jp/AP/AP022321.html), respectively. The versions in this paper are the first versions reported. These accession numbers were originally published by Mashima et al. (5).

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## REFERENCES

- Foubert EL, Jr, Douglas HC. 1948. Studies on the anaerobic micrococci. II. The fermentation of lactate by *Micrococcus lactilyticus*. *J Bacteriol* 56:35–36. <https://doi.org/10.1128/JB.56.1.35-36.1948>.
- Rogosa M. 1984. Anaerobic Gram-negative cocci, p 680–685. In Krieg NR, Holt JG (ed), *Bergey's manual of systematic bacteriology*, vol 1. Williams & Wilkins, Baltimore, MD.
- Ng SKC, Hamilton IR. 1971. Lactate metabolism by *Veillonella parvula*. *J Bacteriol* 105:999–1005. <https://doi.org/10.1128/JB.105.3.999-1005.1971>.
- Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham L-D, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT, Yang Z, Hattab MW, Avila-Pacheco J, Clish CB, Lessard S, Church GM, Kostic AD. 2019. Metagenomics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat Med* 25:1104–1109. <https://doi.org/10.1038/s41591-019-0485-4>.
- Mashima I, Theodorea CF, Djais AA, Kunihiro T, Kawamura Y, Otomo M, Saitoh M, Tamai R, Kiyoura Y. 2021. *Veillonella nakazawae* sp. nov., an anaerobic Gram-negative coccus isolated from the oral cavity of Japanese children. *Int J Syst Evol Microbiol* 71. <https://doi.org/10.1099/ijsem.0.004583>.
- Mays TD, Holdeman LV, Moore WEC, Rogosa M, Johnson JL. 1982. Taxonomy of the genus *Veillonella* Prévot. *Int J Syst Bacteriol* 32:28–36. <https://doi.org/10.1099/00207713-32-1-28>.
- Rogosa M. 1965. The genus *Veillonella*. IV. Serological groupings, and genus and species emendations. *J Bacteriol* 90:704–709. <https://doi.org/10.1128/JB.90.3.704-709.1965>.
- Byun R, Carlier J-P, Jacques NA, Marchandin H, Hunter N. 2007. *Veillonella denticariosi* sp. nov., isolated from human carious dentin. *Int J Syst Evol Microbiol* 57:2844–2848. <https://doi.org/10.1099/ijms.0.65096-0>.
- Arif N, Do T, Byun R, Sheehy E, Clark D, Gilbert SC, Beighton D. 2008. *Veillonella rogosae* sp. nov., an anaerobic, Gram-negative coccus isolated from dental plaque. *Int J Syst Evol Microbiol* 58:581–584. <https://doi.org/10.1099/ijms.0.65093-0>.
- Mashima I, Kamaguchi A, Miyakawa H, Nakazawa F. 2013. *Veillonella tobetsuensis* sp. nov., an anaerobic, Gram-negative coccus isolated from human tongue biofilms. *Int J Syst Evol Microbiol* 63:1443–1449. <https://doi.org/10.1099/ijms.0.042515-0>.
- Mashima I, Liao Y-C, Miyakawa H, Theodorea CF, Thawboon B, Thawboon S, Scannapieco FA, Nakazawa F. 2018. *Veillonella infantium* sp. nov., an anaerobic Gram-stain-negative coccus isolated from tongue biofilm of a Thai child. *Int J Syst Evol Microbiol* 68:1101–1106. <https://doi.org/10.1099/ijsem.0.002632>.
- Delwiche EA, Pestka JJ, Tortorello ML. 1985. The veillonellae: Gram-negative cocci with a unique physiology. *Annu Rev Microbiol* 39:175–193. <https://doi.org/10.1146/annurev.mi.39.100185.001135>.
- Sambrook J, Russell DW. 2006. Purification of nucleic acids by extraction with phenol:chloroform. *Cold Spring Harb Protoc* 2006:pdb.prot4455. <https://doi.org/10.1101/pdb.prot4455>.
- De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics* 34:2666–2669. <https://doi.org/10.1093/bioinformatics/bty149>.

15. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ pre-processor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
16. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long read sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
17. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of *de novo* genome assemblies. *Bioinformatics* 31:3350–3352. <https://doi.org/10.1093/bioinformatics/btv383>.
18. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. *Bioinformatics* 34:1037–1039. <https://doi.org/10.1093/bioinformatics/btx713>.