GENOME SEQUENCES

Complete Genome Sequence of Akkermansia muciniphila JCM 30893, Isolated from Feces of a Healthy Japanese Male

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ABSTRACT Akkermansia muciniphila is an anaerobic and mucin-degrading bacterium in the human gut. Here, we report the complete genome sequence of Akkermansia muciniphila JCM 30893 harboring the plasmid pJ30893.

Akkermansia muciniphila is an anaerobic and Gram-negative bacterium and was first
isolated from human feces [\(1\)](#page-1-0). A. muciniphila degrades and utilizes mucin in the human intestine and was also reported to have associations with several diseases, including obesity and diabetes [\(2](#page-1-1)[–](#page-1-2)[4\)](#page-1-3). Here, we report the genome of a strain, A. muciniphila JCM 30893, isolated from feces of a 45-year-old healthy Japanese male.

A total of 0.5 g of a fecal sample was suspended in 4.5 ml of prereduced phosphatebuffered saline (PBS). Each dilution of a fresh fecal sample was plated onto Columbia blood agar supplemented with 5% (vol/vol) horse blood. After 2 to 4 days of incubation at 37°C under a H_2 -CO₂-N₂ (1:1:8 [vol/vol/vol]) gas mixture, strain CBH12S (= JCM 30893) was isolated. Genomic DNA extraction, sequencing with the Illumina MiSeq and PacBio Sequel platforms, quality checking of reads, de novo hybrid assembly of both reads, and quality checking of the genome were performed as previously described [\(5\)](#page-1-4), and default parameters were used for all software unless otherwise specified. We obtained a total of 278,916,914 bases from 467,638 filter-passed Illumina paired reads with an average length of 298.2 bp, and a total of 676,968,912 bases from 43,760 filter-passed PacBio reads with an average length of 15,470 bp. The assembly generated two circular single contigs, corresponding to the A. muciniphila JCM 30893 chromosome and the plasmid pJ30893. The ratio of the average read depth of the two contigs estimated the copy number of pJ30893 to be \sim 3 per chromosome, which was estimated by mapping the reads to contigs using minimap2 (v. 2.13-r850) [\(6\)](#page-1-5).

The A. muciniphila JCM 30893 chromosome was 2,845,645 bp long, with a $G+C$ content of 55.6%, and encoded 2,332 protein-coding genes and 54 tRNA, 3 5S rRNA, 3 16S rRNA, and 3 23S rRNA genes. pJ30893 was 32,814 bp long, with a $G+C$ content of 54.6%, and encoded 43 protein-coding genes. The quality was checked using CheckM (v. 1.0.11) [\(7\)](#page-1-6), estimating the genome completeness and contamination of the A. muciniphila JCM 30893 chromosome to be 98.0% and 0.68% without strain heterogeneity, respectively. The average and the highest nucleotide identity of the A. muciniphila JCM 30893 chromosome was 98.9% with the published A. muciniphila DSM 22959T chromosome using ANI Calculater [\(8\)](#page-1-7). In contrast, pJ30893 had no significant similarity with any genome in the publicly available databases. A similarity search of the 43 genes in pJ30893 against the Clusters of Orthologous Groups of proteins (COGs) database (v 2014 update) [\(9\)](#page-1-8) using blastp (v. $2.6.0+)$ hit eight COGs with an E value of \leq 0.00001, of which four (COG0582, COG1783, COG3600, and COG4388) were assigned as category X (mobilome). A similarity search against the Prokaryotic Virus Orthologous **Citation** Ogata Y, Sakamoto M, Ohkuma M, Hattori M, Suda W. 2020. Complete genome sequence of Akkermansia muciniphila JCM 30893, isolated from feces of a healthy Japanese male. Microbiol Resour Announc 9:e01543-19. [https://doi.org/10.1128/MRA](https://doi.org/10.1128/MRA.01543-19) [.01543-19.](https://doi.org/10.1128/MRA.01543-19)

Editor David Rasko, University of Maryland School of Medicine

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Received 18 December 2019 **Accepted** 23 January 2020 **Published** 13 February 2020

Groups (pVOG) database (v. 2016 update) [\(10\)](#page-1-9) using blastp also hit nine VOGs with an E value of \leq 0.00001, including VOG1298 with 65.1% identity and 95.5% coverage. These data suggested that pJ30893 was a plasmid.

Data availability. The complete genome sequence of A. muciniphila JCM 30893 was deposited in DDBJ/ENA/GenBank under the accession no. [AP021898](https://www.ncbi.nlm.nih.gov/nuccore/AP021898) and [AP021899,](https://www.ncbi.nlm.nih.gov/nuccore/AP021899) which are linked to the BioProject accession no. [PRJDB8988,](https://www.ncbi.nlm.nih.gov/bioproject/590660) the BioSample accession no. [SAMD00192834,](https://www.ncbi.nlm.nih.gov/biosample/SAMD00192834) and the DDBJ Sequence Read Archive (SRA) accession no. [DRX188527](https://www.ncbi.nlm.nih.gov/sra/DRX188527%5baccn%5d) and [DRX188528.](https://www.ncbi.nlm.nih.gov/sra/DRX188528%5baccn%5d)

ACKNOWLEDGMENTS

We thank K. Kaida, C. Shindo, and M. Tanokura (RIKEN) for technical support of Illumina and PacBio sequencing and Y. Kiguchi (Waseda University) for bioinformatics analysis. We also thank W. Bunryo for cultivation of A. muciniphila JCM 30893.

This work was supported by PRIME, the Japan Agency for Medical Research and Development (AMED) under grant no. JP19gm6010007 (to M.S.), the RIKEN Competitive Program for Creative Science and Technology (to M.O.), and RIKEN Integrated Symbiology (to M.H.).

REFERENCES

- 1. Derrien M, Vaughan EE, Plugge CM, de Vos WM. 2004. Akkermansia municiphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 54:1469 –1476. [https://doi.org/10](https://doi.org/10.1099/ijs.0.02873-0) [.1099/ijs.0.02873-0.](https://doi.org/10.1099/ijs.0.02873-0)
- 2. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, De Vos WM, Cani PD. 2013. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 110:9066-9071. [https://doi.org/10.1073/pnas.1219451110.](https://doi.org/10.1073/pnas.1219451110)
- 3. Caesar R, Tremaroli V, Kovatcheva-Datchary P, Cani PD, Bäckhed F. 2015. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. Cell Metab 22:658 – 668. [https://doi](https://doi.org/10.1016/j.cmet.2015.07.026) [.org/10.1016/j.cmet.2015.07.026.](https://doi.org/10.1016/j.cmet.2015.07.026)
- 4. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, Kayser BD, Levenez F, Chilloux J, Hoyles L, MICRO-Obes Consortium, Dumas ME, Rizkalla SW, Doré J, Cani PD, Clément K. 2016. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut 65:426 – 436. [https://doi.org/10.1136/gutjnl-2014-308778.](https://doi.org/10.1136/gutjnl-2014-308778)
- 5. Ogata Y, Suda W, Ikeyama N, Hattori M, Ohkuma M, Sakamoto M. 2019. Complete genome sequence of Phascolarctobacterium faecium JCM

30894, a succinate-utilizing bacterium isolated from human feces. Microbiol Resour Announc 8:1–2. [https://doi.org/10.1128/MRA.01487-18.](https://doi.org/10.1128/MRA.01487-18)

- 6. Li H. 2018. Sequence analysis minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094 –3100. [https://doi.org/10.1093/](https://doi.org/10.1093/bioinformatics/bty191) [bioinformatics/bty191.](https://doi.org/10.1093/bioinformatics/bty191)
- 7. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. [https://doi.org/10.1101/gr.186072.114.](https://doi.org/10.1101/gr.186072.114)
- 8. Yoon S-H, Ha S, Min Lim J, Kwon S, 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/](https://doi.org/10.1007/s10482-017-0844-4) [s10482-017-0844-4.](https://doi.org/10.1007/s10482-017-0844-4)
- 9. Galperin MY, Makarova KS, Wolf YI, Koonin EV. 2015. Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res 43:D261–D269. [https://doi.org/10.1093/nar/](https://doi.org/10.1093/nar/gku1223) [gku1223.](https://doi.org/10.1093/nar/gku1223)
- 10. Grazziotin AL, Koonin EV, Kristensen DM. 2017. Prokaryotic Virus Orthologous Groups (pVOGs): a resource for comparative genomics and protein family annotation. Nucleic Acids Res 45:D491–D498. [https://doi](https://doi.org/10.1093/nar/gkw975) [.org/10.1093/nar/gkw975.](https://doi.org/10.1093/nar/gkw975)