

Draft genome sequence of two *Fusobacterium varium* strains isolated from patients in Kazakhstan with colorectal cancer

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ABSTRACT *Fusobacterium varium* is a Gram-negative, invasive, obligate anaerobe in the gastrointestinal tract microbiota, associated with various clinical conditions, including colorectal cancer (CRC). Here, we announce the draft genome sequence of two *F. varium* strains Fv36kaz and Fv63kaz from patients with CRC in Kazakhstan.

KEYWORDS *Fusobacterium varium*, genome sequence, colorectal cancer

Fusobacterium varium, an opportunistic pathogen, is mostly reported in ulcerative colitis and should be considered a potential cause of colorectal cancer (CRC) (1–4).

Biopsy samples were collected from the CRC tissue of patients from the National Research Oncology Center. The homogenized solution from the biopsy, which was stored in 20% sucrose, was cultivated on Columbia agar (5% defibrinated sheep blood, 1 µg/mL menadione, 5 µg/mL hemin, 3 µg/mL josamycin, 4 µg/mL vancomycin, and 1 µg/mL neomycin) and incubated for 72 h under anaerobic conditions in an AnaeroGen system (Thermo) at 37°C. The isolated single colonies were identified as *F. varium* by V1–V4 region fragment of *16S rRNA* gene sequencing with more than 99% similarity for strains and the Bruker Biotyper microbial identification system. About 20 µL reactions mix contained template DNA, 0.2 U Taq Polymerase, 2 mM each dNTP, 10× PCR buffer, 25 mM MgCl₂, and 10 pmol primers (8 f 5′-AGAGTTTGATCCTGGCTCAG-3′, 806R 5′-GGAC TACCAGGGTATCTAAT-3′). The PCR annealing temperature was set to 55°C for 30 cycles. Sequencing was performed using a 3730 Genetic Analyzer (Thermo). The comparative method was constructed using the NCBI BLAST nucleotide, comparing the sequencing of both strains to the reference *Fv* (ATCC 27725). For DNA isolation, the colonies were grown at 37°C in Columbia agar (5% defibrinated sheep blood, 1 µg/mL menadione, 5 µg/mL hemin, 3 µg/mL josamycin, 4 µg/mL vancomycin, and 1 µg/mL neomycin) for 72 h under anaerobic condition. DNA was extracted using a QIAamp DNA mini kit (Qiagen). An Illumina MiSeq platform was used to carry out whole-genome sequencing, and a MiSeq reagent v3 600 cycles cartridge was used. DNA libraries were prepared using an Illumina DNA Prep kit. The total number of reads for Fv36kaz was 3,082,394, and the total number of reads for Fv63kaz was 2,402,302. Quality control and trimming of forward and reverse reads for both strains Fv36kaz and Fv63kaz were performed using Trim Galore version 0.6.5dev (5). The genome was assembled using Unicycler v0.4.8 (6). Contigs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.6 (7). Default parameters were used for all software. The genome of *F. varium* strain Fv36kaz with 72-fold coverage had a length of 3,388,962 bp, 145 contigs, an *N*₅₀ value was 74,423 bp, and a G + C content of 29.01%. The genome of *F. varium* strain Fv63kaz with 185-fold coverage had a length of 3,393,562 bp, 107 contigs, an *N*₅₀ value was 91,866 bp, and a G + C content of 29.11%. Additionally, annotation by the Bacterial and Viral Bioinformatics Resource Center Annotation Service of the Fv36kaz had 3,262 coding DNA sequences (CDSs), and Fv63kaz had 3,221 CDSs (8).

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Gulmira Kulmambetova, Conceptualization, Data curation, Formal analysis, Project administration, Supervision, Writing – original draft, Writing – review and editing.

DATA AVAILABILITY

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession [JBAJGW000000000](https://doi.org/10.1136/gut.52.1.79) (Fv36kaz) and [JBAKJD000000000](https://doi.org/10.1136/gut.52.1.79) (Fv63kaz). The raw data from BioProject numbers [PRJNA1078108](https://doi.org/10.1136/gut.52.1.79) (Fv36kaz) and [PRJNA1079513](https://doi.org/10.1136/gut.52.1.79) (Fv63kaz) were submitted to the NCBI Sequence Read Archive under experiment accession numbers [SRR28022417](https://doi.org/10.1136/gut.52.1.79) (Fv36kaz) and [SRR28059462](https://doi.org/10.1136/gut.52.1.79) (Fv63kaz). The *16S rRNA* sequences from two *F. varium* were deposited in GenBank as separate submissions under accession numbers [PP789727-PP789728](https://doi.org/10.1136/gut.52.1.79) (Fv36kaz) and [PP800236-PP800237](https://doi.org/10.1136/gut.52.1.79) (Fv63kaz).

REFERENCES

- Ohkusa T, Okayasu I, Ogihara T, Morita K, Ogawa M, Sato N. 2003. Induction of experimental ulcerative colitis by *Fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. *Gut* 52:79–83. <https://doi.org/10.1136/gut.52.1.79>
- Sekizuka T, Ogasawara Y, Ohkusa T, Kuroda M. 2017. Characterization of *Fusobacterium varium* Fv113-g1 isolated from a patient with ulcerative colitis based on complete genome sequence and transcriptome analysis. *PLoS ONE* 12:e0189319. <https://doi.org/10.1371/journal.pone.0189319>
- Lee SJ, Baek YJ, Kim JN, Lee KH, Lee EH, Yeom JS, Choi JY, Ku NS, Ahn JY, Kim JH, Jeong SJ. 2022. Increasing *Fusobacterium* infections with *Fusobacterium varium*, an emerging pathogen. *PLoS ONE* 17:e0266610. <https://doi.org/10.1371/journal.pone.0266610>
- Kulmambetova G, Utupov T, Kurentay B, Aukanova D, Tarlykov P, Shevtsov A, Daniyarov A, Khamzina S, Mamlin M, Kozhakhmetov A, Shalekenov S. 2023. Draft genome sequence of a *Fusobacterium nucleatum* strain isolated from a patient in Kazakhstan with colorectal cancer. *Microbiol Resour Announc* 12:e0036723. <https://doi.org/10.1128/MRA.00367-23>
- Krueger F. 2012. Trim galore: a wrapper Tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ Files, with some extra functionality for MspI-digested RRBS-type (reduced representation bisulfite-seq) libraries. Available from: http://www.bioinformatics.babraham.ac.uk/projects/trim_galore
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>
- Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, Dempsey DM, Dickerman A, Dietrich EM, Kenyon RW, et al. 2023. Introducing the bacterial and viral bioinformatics resource center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic Acids Res* 51:D678–D689. <https://doi.org/10.1093/nar/gkac1003>