

Draft Genome Sequence of Fusobacterium nucleatum subsp. fusiforme ATCC 51190^{T}

Soon-Nang Park,^a Si-Won Kong,^b Mo-Se Park,^b Jee-Won Lee,^b Eugene Cho,^a Yun Kyong Lim,^a Mi-Hwa Choi,^a Hwa-Sook Kim,^c Young-Hyo Chang,^d Jeong Hwan Shin,^e Hong-Seog Park,^{f,g} Sang-Haeng Choi,^f and Joong-Ki Kook^a

Korean Collection for Oral Microbiology and Department of Oral Biochemistry, School of Dentistry, Chosun University, Gwangju, Republic of Korea^a; SolGent Co., Ltd., Yuseong-Gu, Daejeon, Republic of Korea^b; Department of Dental Hygiene, Chunnam Techno University, Chunnam, Republic of Korea^c; Korean Collection for Type Cultures, Biological Resource Center, KRIBB, Daejeon, Republic of Korea^d; Department of Laboratory Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan, Republic of Korea^e; Genome Resource Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea^f; and University of Science and Technology (UST), Daejeon, Republic of Korea^g

Fusobacterium nucleatum, one of the major causative bacteria of periodontitis, is classified into five subspecies (*nucleatum*, *polymorphum*, *vincentii*, *animalis*, and *fusiforme*) on the basis of the several phenotypic characteristics and DNA homology. This is the first report of the draft genome sequence of *F. nucleatum* subsp. *fusiforme* ATCC 51190^T.

Fusobacterium nucleatum, the type species of the genus Fusobacterium, is a Gram-negative, nonmotile, obligately anaerobic rod bacterium which is frequently isolated from the oral cavity (5, 6). F. nucleatum is classified into five subspecies (nucleatum, polymorphum, vincentii, animalis, and fusiforme) on the basis of the polyacrylamide gel electrophoretic pattern of the whole-cell proteins and DNA homology (2) or electrophoretic patterns of glutamate dehydrogenase and 2-oxoglutarate reductase and DNA-DNA hybridization patterns (6). Except for F. nucleatum subsp. fusiforme, the genomes of the type strains of the other subspecies have been sequenced (9–12).

In this report, we present the draft genome sequence of *F. nucleatum* subsp. *fusiforme* ATCC 51190^{T} . Draft sequencing of the *F. nucleatum* subsp. *fusiforme* ATCC 51190^{T} genome was performed by SolGent, Co., Ltd. (Daejeon, South Korea), using 454 GS-FLX Titanium (Roche Diagnostics, Basel, Switzerland). Following an initial round of shotgun pyrosequencing, 402 contigs were assembled using the Newbler Assembler software gsAssembler V2.5.3 (454 Life Sciences, Branford, CT). An additional round of Sanger method sequencing was performed for gap closure. PCR products were subjected to cycle sequencing with ABI BigDye Terminator v3.1 and analyzed on a 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). The Phred-Phrap-Consed programs (3, 4, 7) were used for sequence assembly and editing of assembled sequences. We obtained 205 contigs, and the GC content was 27%.

Open reading frames (ORFs) were predicted and annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (13). The predicted protein sequences were annotated as gene ontology (GO) by the basic local alignment search tool (1). Then GO classes were grouped into a total of 124 GO-Slim terms using the web tool CateGOrizer (8).

A total of 1,344 (73.8%) of the ORFs were annotatable with known proteins. The genome contained 1,821 protein-coding genes, two 16S rRNA copies, one 23S rRNA copy, five 5S rRNA copies, and 45 tRNA genes. There were 1,821 possible ORFs in 198 contigs with a size range of 224 to 10,785 bp. There were not many ORFs with lengths of >2,000 bp (only 95); in fact, most were <1,500 bp (n = 1,597).

The draft genome sequence contains several key pathways for amino acids, carbohydrates, lipids, and organic acids. Biosynthetic pathways exist for at least four amino acids: aspartate, asparagine, glutamate, and glutamine. The amino acid biosynthesis activity is different from that of other F. nucleatum strains. For example, F. nucleatum subsp. nucleatum ATCC 25586^T can synthesize just 3 amino acids, glutamate, aspartate, and asparagine (9). GO analysis has revealed that 4% of the genes (n = 128) are transport-related genes for the uptake of peptides, sugars, metal ions, and cofactors. The draft genome sequence also contains genes for virulence factors such as porin, hemolysin, butyrate fermentation-related proteins, ion acquisition-related proteins, multidrug efflux proteins, 5-nitroimidazole antibiotic resistance proteins, beta-lactamase, macrolide efflux protein, toxin YoeB, virulence factor MviN, type II secretion proteins, and TonB and TolC proteins. The genome also contains genes for oxidative stress response factors such as glutathione peroxidase and NADH oxidase but not for superoxide dismutase.

Nucleotide sequence accession numbers. This Whole-Genome Shotgun project has been deposited at DDBJ/EMBL/ GenBank under accession no. AKX100000000. The version described in this paper is the first version, AKX101000000. The Bioproject designation for this project is PRJNA167963.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (2012-003324).

REFERENCES

- 1. Altschul S, et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402.
- Dzink JL, Sheenan MT, Socransky SS. 1990. Proposal of three subspecies of Fusobacterium nucleatum Knorr 1922: Fusobacterium nucleatum subsp. nucleatum subsp. nov., comb. nov.; Fusobacterium nucleatum subsp. poly-

Received 25 June 2012 Accepted 16 July 2012 Address correspondence to Joong-Ki Kook, jkkook@chosun.ac.kr. S.-N.P. and S.-W.K. contributed equally to this article. Copyright © 2012, American Society for Microbiology. All Rights Reserved.

copyright © 2012, American Society for Microbiology. All Rights Reserved doi:10.1128/JB.01138-12 *morphum* subsp. nov., nom. rev., comb. nov.; and *Fusobacterium nucleatum* subsp. *vincentii* subsp. nov., nom. rev., comb. nov. Int. J. Syst. Bacteriol. **40**:74–78.

- 3. Ewing B, Green P. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8(3):186–194.
- Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8(3): 175–185.
- Gharbia SE, Shah HN. 1990. Heterogeneity within Fusobacterium nucleatum, proposal of four subspecies. Lett. Appl. Microbiol. 10:105–108.
- 6. Gharbia SE, Shah HN. 1992. Fusobacterium nucleatum subsp. fusiforme subsp. nov. and Fusobacterium nucleatum subsp. animalis subsp. nov. as additional subspecies within Fusobacterium nucleatum. Int. J. Syst. Bacteriol. 42:296–298.
- 7. Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. Genome Res. 8:195–202.
- 8. Hu ZL, Bao J, Reecy JM. 2008. CateGOrizer: a web-based program to

batch analyze gene ontology classification categories. Online J. Bioinform. 9:108–112.

- 9. Kapatral V, et al. 2002. Genome sequence and analysis of the oral bacterium *Fusobacterium nucleatum* strain ATCC 25586. J. Bacteriol. **184**:2005–2018.
- Kapatral V, et al. 2003. Genome analysis of *F. nucleatum* sub spp *vincentii* and its comparison with the genome of *F. nucleatum* ATCC 25586. Genome Res. 13:1180–1189.
- Karpathy SE, et al. 2007. Genome sequence of *Fusobacterium nucleatum* subspecies *polymorphum*—a genetically tractable fusobacterium. PLoS One 2:e659. doi:10.1371/journal.pone.0000659.
- 12. Muzny D, et al. 2011. Fusobacterium nucleatum subsp. animalis ATCC 51191, whole genome shotgun sequencing project. NCBI, Bethesda, MD. http://www.ncbi.nlm.nih.gov/nuccore/339892075.
- 13. NCBI. 2012. NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP). NCBI, Bethesda, MD. http://www.ncbi.nlm.nih.gov/genomes /static/Pipeline.html.