Genome sequence of *Prevotella intermedia* strain originally isolated from cervicofacial actinomycosis

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경부안면형 방선균증에서 분리된 Prevotella intermedia의 유전체 염기서열 해독

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Anaerobic Gram-negative bacterium *Prevotella intermedia* is a part of normal flora of the oral cavity and associated with various types of oral and systemic diseases. We present here a draft genome sequence of *P. intermedia* ATCC 15032, originally isolated from cervicofacial actinomycosis. The genome is 2,848,426 bp in length and has a GC content of 43.45%. The genome includes 2,358 protein-coding genes, 5 rRNAs, and 43 tRNA. The sequence information will provide important clues in understanding the genome diversity within the bacterial species, and genetic basis for phenotypic differences among *P. intermedia* strains.

Keywords: Prevotella intermedia, actinomycosis isolate, genome

Anaerobic Gram-negative bacterium *Prevotella intermedia*, a part of normal flora of the oral cavity, acts as an opportunistic pathogen. Besides being involved in oral diseases, *P. intermedia*

*For correspondence. (J.Y. Lee) E-mail: ljinyong@khu.ac.kr; Tel.: +82-2-961-9544; Fax: +82-2-962-0598 / (J.H. Lee) E-mail: jaehlee@khu.ac.kr; Tel.: +82-2-961-9290; Fax: +82-2-962-0598 often colonizes the respiratory tract and is associated with cystic fibrosis and chronic bronchitis (Ruan et al., 2015). Like many other pathogenic bacteria characterized by extensive intraspecies variation, the phenotypic difference related to pathogenicity exists among P. intermedia strains (Takahashi et al., 2006; Moon et al., 2013). The phenotypic intraspecies difference could be non-genetic, such as the interaction with surrounding microorganisms or the regulation of gene expression under different niches (Ruan et al., 2015). However, there has been accumulating evidence to show that bacteria gain and lose genes and other genomic sequences to adapt to specific ecological niches, and that certain bacterial clones are often specialized in the disease they cause and the hosts they colonize (Lan and Reeves, 2000). Therefore, analyzing the genome composition of diverse strains from different niches and examining the intraspecies genomic variation are crucial not only to gain insight into the genomic structure of the species but also to predict how much gene mutation occurs in a strain to adapt to different environment (Ruan et al., 2015).

P. intermedia strains that have been analyzed so far for their genomic sequences are mainly from periodontal pockets and saliva. Here we first report a draft genome sequence of *P*.

Table 1. Genome features of Prevotella intermedia ATCC 15032
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Attribute	Value
Genome size (bp)	2,848,426
GC content (%)	43.45
No. of scaffolds	58
Total genes	2,540
Protein-coding genes	2,358
tRNAs	43
Complete rRNAs (5S, 16S, 23S)	2, 1, 1
Partial rRNAs (23S)	1
ncRNAs	4
Pseudogenes	130
N50	99,144
Overall estimated genome coverage	879x

intermedia ATCC 15032 originally isolated from cervicofacial actinomycosis. The sequence information of this strain of cervicofacial actinomyces will help understand the general characteristics of *P. intermedia*, genome diversity within the bacterial species, and genetic basis for phenotypic differences among *P. intermedia* strains.

P. intermedia ATCC 15032 was grown at 37°C on brucella agar supplemented with 5% sheep blood, 5 µg/ml of hemin and 1 µg/ml of vitamin K₁ under anaerobic conditions (85% N₂, 10% H₂, and 5% CO₂). The bacterial genomic DNA was extracted, and the sequencing library was constructed. Briefly, purified DNA was randomly fragmented to 400–500 bp, and the resulting overhangs were converted to blunt ends. After a cleaning step using the AMPure XP Beads, the 3' ends of the fragmented genomic DNA were adenylated, then ligated to the index adapters. DNA fragments containing adapter sequences (500–600 bp) were extracted from a 2% agarose gel, then

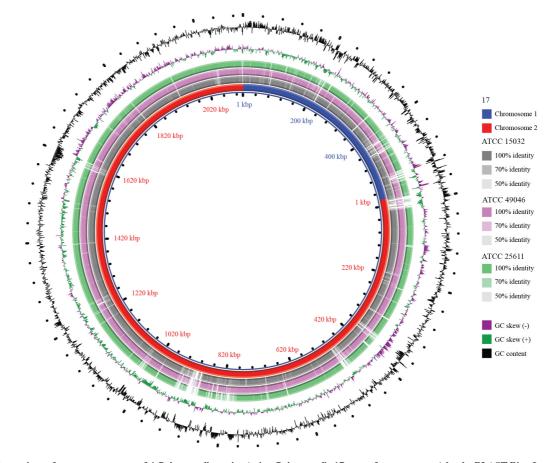


Fig. 1. Comparison of genome sequences of 4 *P. intermedia* strains (using *P. intermedia* 17 as a reference genome) by the BLAST Ring Image Generator (BRIG) (Alikhan *et al.*, 2011). From outer to inner ring: ATCC 25611, ATCC 49046, ATCC 15032, and 17. The color intensity in each ring represents the BLAST match identity.

enhanced via PCR using adapter specific primers. The constructed library was used for the paired-end sequencing on the Illumina Hi-Seq 2500 platform. In total, 12,774,525 paired-end read pairs (2×101 bp) were obtained. After filtering out low quality raw reads, A5 assembler (version A5-miseq 20140604) (Tritt et al., 2012) with default parameters was used for the construction of the genome. The genome assembly resulted in 58 scaffolds (> 500-bp length). Gene annotation of the draft genome was performed by the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016). The genome of P. intermedia ATCC 15032 is 2,848,426 bp long and has a GC content of 43.45% (Table 1). A total of 2,358 protein-coding genes, 5 rRNAs, and 43 tRNAs were annotated (Table 1). Cas1, 2, and 9 genes were located in the same scaffold, and 2 clustered regularly interspaced short palindromic repeats (CRISPRs) arrays were identified. Global pairwise comparison of the genome sequences between ATCC 15032 and other strains (ATCC 25611^T, ATCC 49046, and 17) showed that 92.44, 91.76, and 94.15% of the genomic regions were similar to each other, respectively (Fig. 1). Meanwhile, several ATCC 15032 strain-specific genes were identified, including genes related to DNA rearrangements (such as XerC and XerD) and antibiotic resistance (such as beta-lactamase and multidrug export protein MepA).

Nucleotide sequence accession number

This Whole Genome Shotgun sequencing project for *P. intermedia* ATCC 15032 is available at GenBank under the accession QXEM00000000. The BioProject ID in GenBank is PRJNA488694.

적 요

혐기성 그람 음성 세균인 Prevotella intermedia는 사람의 구 강 내 정상세균총의 하나이고 다양한 구강 및 전신 질환과 관련 이 있다. 본 논문에서는 경부안면형 방선균증으로부터 분리된 P. intermedia ATCC 15032 균주의 유전체 염기서열을 분석하 여 보고한다. 이 균주의 유전체는 2,848,426 bp의 크기로 GC 함 량은 43.45%이다. 이 유전체 서열 정보는 P. intermedia 종 내 에서의 균주 간 유전체 다양성 및 표현형 차이의 유전적 기초 를 이해하는데 중요한 정보를 제공할 것이다.

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