






# Complete genome sequence of *Bacillus subtilis* PN176 with high fibrinolytic enzyme activity isolated from Cheonggukjang

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## 청국장에서 분리한 고효율성 혈전용해능을 지닌 *Bacillus subtilis* PN176 균주의 전체 게놈 서열

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*Bacillus subtilis* PN176 (KCCM80351) was isolated from Cheonggukjang as a starter strain of fermented foods such as Natto. The strain PN176 displayed a fibrinolytic activity on medium and fermented foods, and its whole-genome sequence was determined to confirm its genetic background. The whole-genome sequencing of the strain PN176 was performed using the PacBio Sequel (Pacific Biosciences) sequencing platform. The complete genome was 4,183,844 bp (chromosomal 4,119,229 bp and plasmid 64,615 bp) and the G + C content was 43.4% (chromosomal 43.5% and plasmid 37.8%). And *Bacillus subtilis* PN176 strain encoded 21 of protease genes that were correlated with fibrinolytic enzyme activity, as well as nattokinase.

**Keywords:** *Bacillus subtilis*, fibrinolytic enzyme activity, genome sequence, soybean fermented food, starter

*Bacillus subtilis* is a Gram-positive, rod-shaped and spore-forming bacterium and it's present in alkaline-fermented foods such as Cheonggukjang (Korea) and Natto (Japan) (Parvathi *et al.*, 2009; Jung *et al.*, 2016). Especially fibrinolytic enzymes from *Bacillus* species have been actively studied because of their potent fibrinolytic agents, nattokinase that is the most well-known example (Sumi *et al.*, 1987). In addition, *Bacillus*

strains can be used as a probiotic suitable for humans (Lee *et al.*, 2019). *Bacillus subtilis* PN176 was isolated from Cheonggukjang as a starter strain of fermented foods such as Natto. The strain PN176 had a fibrinolytic activity on medium and fermented foods, and its genome sequence was determined to confirm its genetic background.

Genomic DNA of strain PN176 was extracted from cultured cells grown to stationary phase in tryptic soy broth using a Maxwell<sup>®</sup> Prokaryote/ Eukaryote SEV DNA Purification Kit (Promega) according to the manufacturer's protocols. The genome library was prepared using PacBio SMRTbell Express Template Prep Kit 2.0 and SMRTbell templates were annealed using Sequel Binding and Internal Ctrl Kit 3.0. The whole genome sequencing of the strain PN176 was performed using the PacBio Sequel (Pacific Biosciences) sequencing platform by MacroGen Inc. Subsequent steps are based on the PacBio Sample Net-Shared Protocol, which is available at <http://pacificbiosciences.com/>. The *de novo* assembly step of short-read data was used by Hierarchical Genome Assembly (HGAP) version 4. After *de novo* assembly step, Illumina reads are applied for sequence correction to construct contigs more accurately. The correcting bases, fixing mis-assemblies and filling gaps were carried out using Pilon version 1.21. After whole genome was assembled, the location of protein-coding

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**Table 1.** Genome features of *Bacillus subtilis* PN176

Genome feature	Chromosome	Plasmid	Total
Genome size (bp)	4,119,229	64,615	4,183,844
G + C content (%)	43.5	37.8	43.4
Protein-coding genes (CDSs)	4,283	89	4,372
tRNAs genes	88	0	88
rRNA genes	30	0	30
GenBank accession No	CP103456.1	CP103457.1	-

sequences, tRNA genes, and rRNA genes were identified. The Prokaryotic Genome Annotation was used the NCBI Prokaryotic Genomes Automatic Annotation Pipeline ([http://www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](http://www.ncbi.nlm.nih.gov/genome/annotation_prok/)) and Prokaryotic Genome Annotation (Prokka) version 1.13 respectively (Seemann, 2014; Tatusova *et al.*, 2016). Open Reading Frames (ORFs) were also predicted by RAST (Rapid Annotation using Subsystem Technology) (<https://rast.nmpdr.org/rast.cgi>) (Aziz *et al.*, 2008).

The genome information of *B. subtilis* PN176 is summarized in Table 1. The whole genome sequence of *B. subtilis* PN176 was composed of 4,119,229 bp of chromosome, and its G + C content was 43.5%. The strain had one plasmid, and its length was 64,615 bp and G + C content was 37.8%.

*Bacillus subtilis* PN176 contains twenty-one of protease genes. Protease activity of *Bacilli* confers unique characteristic upon fermented foods such as cheonggukjang and Natto by breaking down proteins to peptides and various amino acids during fermentation. Among them, the strain PN176 also contains alkaline protease (subtilisin) gene, which encodes a nattokinase. Nattokinase has shown therapeutic pharmacological effects, including anti-thrombotic and antihypertensive properties (Fujita *et al.*, 2011). The gene fragment was 1,491 bp in length and contained the major fibrinolytic gene and encodes a protein of 382 amino acids consisting of signal sequence (30 amino acids), propeptide (77 amino acids) and mature enzyme (275 amino acids) (Jeong *et al.*, 2015). The complete genome sequence of the PN176 strain will be available as data on the strain-specific properties such as fibrinolytic enzymes and its usefulness.

#### Nucleotide sequence accession numbers

The accession number of genome sequence of *Bacillus subtilis* PN176 was CP103456.1 (chromosome) and CP103457.1

(plasmid) in the GenBank database of NCBI. The strain has been deposited at Korean Culture Center of Microorganisms (KCCM) under the accession no. KCCM80351.

## 적 요

*Bacillus subtilis* PN176은 한국 전통 발효식품인 청국장에서 분리된 균주이며, 혈전용해능이 우수한 특성으로 발효식품용 종균으로 선별되었다. 이 균주의 유전체는 4,119,229 bp 크기의 염색체와 64,615 bp 크기의 플라스미드로 구성되어 있으며, 전체 GC 함량은 43.4%이다. 그리고 이 균주는 혈전용해효소, 나포키나제와 관련성이 있는 21개의 프로테아제 유전자가 확인되었다.

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None.

## Conflict of Interest

The authors declare no conflict of interest.

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