Microbiol. Biotechnol. Lett. (2024), 52(3), 331–334
http://dx.doi.org/10.48022/mbl.2408.08001
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Genome Reports

# Complete Genome Sequence of *Priestia megaterium* Strain 10 Isolated from Soybean Rhizosphere (*Glycine max*)

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Received: August 2, 2024 / Revised: September 2, 2024 / Accepted: September 6, 2024

**We present the complete genome sequence analysis of Priestia megaterium strain 10, isolated from the soybean rhizosphere. The genome consists of a single circular chromosome of 4,815,034 bp with a G+C content of 38.2% and 4 plasmids named P1 (198,305 bp), P2 (139,815 bp), P3 (79,328 bp), and P4 (61,901 bp).**

**Keywords:** **Complete genome, *Priestia megaterium*, rhizosphere, soybean**

Plants are a major living organism form, essential for ecosystem sustainability. Due to their beneficial roles, plants provide nutrient sources, regulate the hydroclimate, improve soil structure and function, and clean the biosphere of contaminants (gas emissions, biochemical fertilizers, etc.) [1, 2]. However, various environmental stress conditions, including abiotic (drought, salinity, floods, cold/heat) and biotic factors (pathogen infection), can have detrimental impacts on plant growth and agricultural productivity. To mitigate the impact of harsh environmental conditions and enhance crop yield, the application of plant growth-promoting bacteria (PGPB) has shown promising results [3].

*P. megaterium*, previously known as *Bacillus megaterium* is a Gram-positive, rod-shaped bacterium belonging to the *Bacillota*. Several studies have demonstrated its plant growth-promoting ability in normal and stress conditions. Through nutrient uptake, phytohormone synthesis, and enhancement of plant defense mechanisms, *P. megaterium* has been recognized as a PGPB [4].

In the present study, *P. megaterium* strain 10 was isolated from the rhizosphere of soybean soil collected from Daegu, South Korea (35°52'43.1"N 128°47'37.3"E). Briefly, 1 g of soil was serially diluted, then dilutions from $10^{-1}$ to $10^{-5}$ were spread on tryptic soy agar (TSA) and incubated at 30℃ for 24 to 48 h. Additionally, and for purification purposes, a bacterial colony was selected and sub-cultured twice on TSA agar and subsequently incubated for 24 to 48 h.

The genomic DNA extraction was performed from an overnight cultured bacterial cell at 30℃ in tryptic soy broth (TSB) using the Wizard® Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions. The concentration and quality of DNA were assessed using the Qubit fluorometer 2.0 (Thermo Fisher Scientific, USA) and the NanoDrop UV-Vis spectrophotometer (Thermo Fisher Scientific), respectively. The sequencing library was prepared without DNA size selection and was generated using the Oxford Nanopore technology (ONT). The V14 kit chemistry (SQK-LSK114, Oxford Nanopore Technologies, United Kingdom) and the ligation kit NEBNext® Companion Module (New

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England Biolabs, USA) were utilized for library preparation based on the manufacturer's protocol. The genomic DNA was sequenced on an R10.4.1 flow cell using the MinION device of the ONT. In addition, Guppy v4.4.1 software was used to produce FASTQ files of the sequenced DNA [5]. Low-quality reads (5% worst fastq reads) were removed using Filtlong v0.2.1 with default settings. The genomic DNA sequencing was performed at the KNU NGS Core Facility (Republic of Korea).

The sequencing data showed the generation of 144,000 reads equivalent to 862,137,845 bp with an approximate coverage of 164 x and a relative $N_{50}$ of 11,632 bp. Flye 2.9.1-b1780 was used for *de novo* genome assembly using the default parameters except for genome size which was set to 5 m (--genome-size 5m) [6]. Assembly results showed the presence of 5 circular contigs, with the biggest corresponding to *P. megaterium* chromosome of 4,815,034 bp with a G+C content of 38.2%. The rest of the four contigs correspond to plasmids P1, P2, P3, and P4 with sizes of 198,305 bp, 139,815 bp, 79,328 bp, and 61,901 bp, and G+C contents of 39.8%, 34.2%, 35.1% respectively. The bacterial genome was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP), and the Rapid Annotation using Subsystem Technology (RAST server) version 2.0 [7, 8].

The annotation indicated that the chromosome of *P. megaterium* 10 encodes 5317 coding genes, 38 ribosomal RNAs, 130 transfer RNAs, 8 non-coding RNAs, and 45 pseudogenes (Table 1). Bacterial chromosome and plasmids were visualized using Proskee tool [9] (Fig. 1).

The average nucleotide identity (ANI) was performed between the genome of *P. megaterium* strain 10 and the NCBI deposited sequence of *P. megaterium* strain WSH-

**Table 1. Genomic features of *P. megaterium* 10.**

| Feature | Number |
|---|---|
| Number of contigs | 5 |
| Chromosome size (bp) | 4,815,034 |
| Coding genes (CDSs) | 5317 |
| Ribosomal RNAs (rRNAs) | 38 |
| Transfer RNAs (tRNAs) | 130 |
| Non-coding RNAs (ncRNAs) | 8 |
| Pseudogenes | 45 |

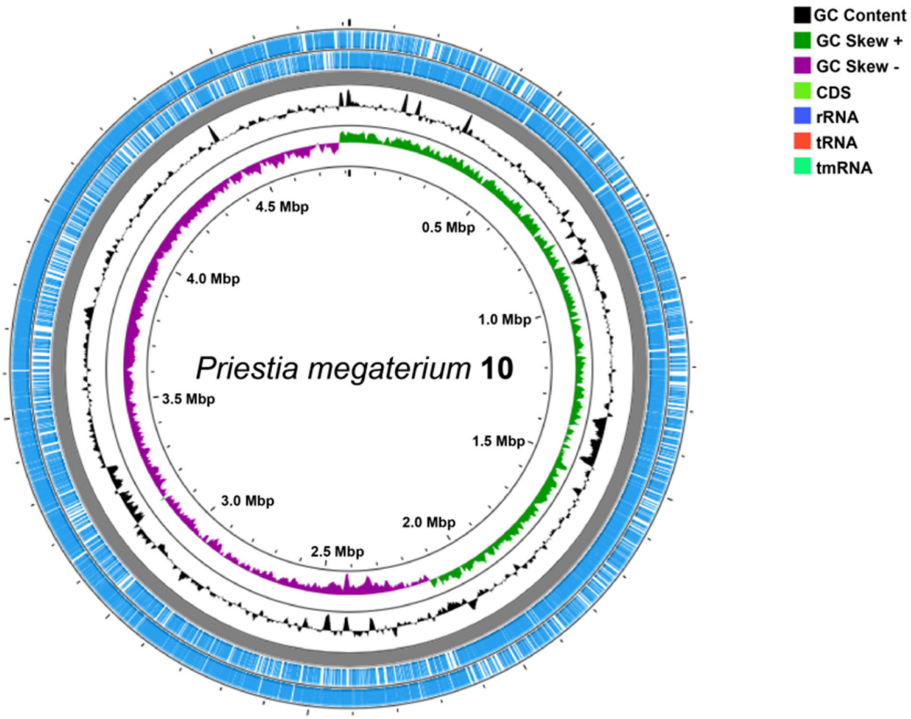


**Fig. 1. Circular genome map of *P. megaterium* 10.** The outer blue circles present the annotation, location, and direction of predicted genes, the middle black circles show the GC% content and the inner circle indicates the GC skew, positive (green) and negative (purple).

http://dx.doi.org/10.48022/mbl.2408.08001

**Table 2. PGP and stress resistance genes annotation of *P. megaterium* 10.**

| | Gene | Product | Chromosome location |
|---|---|---|---|
| PGP related genes | *NorD* | Nitric oxide reductase activation | 1842547-1844463 |
| | *NorQ* | Nitric oxide reductase activation | 1841645-1842535 |
| | *Amt* | Ammonium transporter | 2288510-2287227 |
| | *GltD* | Glutamate synthase small subunit | 1772515-1773996 |
| | *PhoH* | Phosphate starvation-inducible protein | 1103104-1104432 |
| | *PhoP* | Alkaline phosphatase synthesis transcriptional regulatory protein | 4271064-4270348 |
| | *PhoU* | Phosphate transport system regulatory protein | 4024271-4023612 |
| | *PhoR* | Phosphate regulon sensor protein PhoR | 4270355-4268583 |
| | *CysA* | Sulfate and thiosulfate import ATP-binding protein CysA | 1798136-1799202 |
| | *CysC* | Adenylyl-sulfate kinase | 4420749-4421348 |
| | *KdpA* | Potassium-transporting ATPase A chain | 2715008-2713341 |
| | *KdpB* | Potassium-transporting ATPase B chain | 2713319-2711244 |
| | *KdpC* | Potassium-transporting ATPase C chain | 2711230-2710664 |
| | *X-ABC3* | Uncharacterized iron compound ABC uptake transporter, ATP-binding protein | 2840979-2840221 |
| | *Fe-ABC1* | Iron compound ABC uptake transporter substrate-binding protein | 2840197-2839241 |
| | *TrpB* | Anthranilate phosphoribosyltransferase | 3848094-3844706 |
| | *TrpD* | Indole-3-glycerol phosphate synthase | 3847079-3846312 |
| Stress response related genes | *AhpC* | Alkyl hydroperoxide reductase subunit C-like protein | 1073806-1074354 |
| | *BetB* | Betaine aldehyde dehydrogenase | 4592262-4593746 |
| | *OpuAA* | Glycine betaine ABC transport system, ATP-binding protein OpuAA | 1272624-1273877 |
| | *OpuAB* | Glycine betaine ABC transport system, permease protein OpuAB | 1273880-1274743 |
| | *GbsB* | Alcohol dehydrogenase GbsB (type III) | 4593768-4594970 |
| | *SodB* | Superoxide dismutase [Fe] | 2390241-2389357 |
| | *SodC* | Superoxide dismutase [Cu-Zn] precursor | 1813126-1813716 |
| | *GlpF* | Glycerol uptake facilitator protein | 262037-262864 |
| | *GlpK* | Glycerol kinase | 262932-264422 |
| | *Chb* | Cyanoglobin; Hemoglobin-like protein HbN | 2080787-2081149 |
| | *ProA* | Gamma-glutamyl phosphate reductase | 1969310-1970572 |
| | *ProB* | Glutamate 5-kinase / RNA-binding C-terminal domain PUA | 1968089-1969195 |

002 with the accession number CP003017.1. An ANI of 99.2% was shared between the two genomes.

The functional annotation of *P. megaterium* genome revealed the presence of plant growth-promoting (PGP) and stress-resistance genes, as highlighted in Table 2. Notably, genes involved in nitrogen metabolism, such as *NorDQ, Amt,* and *GltD* were identified. Additionally, genes associated with phosphate, sulfate, potassium, and indole-3-acetic acid (IAA) metabolism, including *PhoHPUR, CysAC, KdpABC,* and *TrpBD,* were present [10, 11]. The annotation further showed the presence of iron acquisition systems, specifically siderophores production, which play a role in pathogen inhibition and nutrient assimilation [12]. These systems are encoded by genes *X-ABC3, Fe-ABC1, ABC2,* and *ABC3* [13]. Furthermore, genes associated with stress resistance, including *BetB, OpuAA, OpuAB, GbsB, SodBC,* and *GLPFK,* were detected [14]. Osmoprotectant molecules like betaines, amino acids, and sugars synthesized under stress conditions are crucial for reactive oxygen species (ROS) detoxification [15]. Similarly, other detoxification genes, such as *Chb*, and *ProAB,* involved in stress alleviation, were also identified.

*P. megaterium* 10 has been proven to harbor potential genes that enhance plant growth and stress resistance. Hence, the agricultural application of this strain may improve plant yield and stress resilience under both biotic and abiotic conditions. The investigation of the

functional properties of *P. megaterium* 10 has shown its potential role as a beneficial PGPB for maintaining plant fertility and productivity.

## Nucleotide Sequence Accession Number(s)

The genome of *P. megaterium* strain 10 and its plasmids P1, P2, P3, and P4 have been submitted to DDKJ/ENA/GenBank under the accession numbers CP162497.1, CP162498.1, CP162499.1, CP162500.1, and CP162501.1 respectively. BioProject and BioSample numbers are PRJNA1133222 and SAMN42379998 respectively.

## Acknowledgments

This research was supported by the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ015697)" Rural Development Administration, and the biological materials specialized graduate program through the Korea Environmental Industry & Technology Institute(KEITI), funded by the Ministry of Environment(MOE), and by a grant from the Korea Basic Science Institute (National Research Facilities and Equipment Center) funded by the Ministry of Education(2021R1A6C101A416), Republic of Korea.

## Conflict of Interest

The authors have no financial conflicts of interest to declare.

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