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Whole-Genome Sequence of *Priestia aryabhattai* Strain S2 Isolated from the Rhizosphere of Soybean *(Glycine max)*

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We present the complete genome sequence of *Priestia aryabhattai* strain S2 isolated from the soybean rhizosphere. The genome consists of a single circular chromosome of 5,070,860 bp with a G+C content of 38.3% and 2 plasmids, P1(148,124 bp, GC content 33.3%) and P2 (76,418 bp, GC content 36.5%).

Keywords: ***Priestia aryabhattai,*** **genome, soybean, rhizosphere**

The increasing reliance on chemical fertilizers for enhancing agricultural productivity has raised grave environmental concerns. The uncontrolled usage of these chemicals has led to the pollution of the ecosystem, the contamination of food, and significant risks to human health. As an alternative approach, exploring the potential of plant growth-promoting bacteria (PGPB) offers a sustainable solution to improve crop production and stress resilience while reducing our dependence on harmful chemicals such as pesticides [1]. Consequently, biofertilizers based on PGPB inoculants are becoming more prominent due to their eco-friendly nature and ability to enhance soil fertility and plant growth sustainably [2, 3].

Priestia aryabhattai, formerly known as *Bacillus aryabhattai* is, a rod-shaped Gram-positive bacterium [4]. It is known for its remarkable ability to promote plant growth through various mechanisms such as the synthesis of phytohormones, the assimilation of nutrients, and the enhancement of plant defense mechanisms, earning its classification as PGPB [5, 6].

In this study, *P. aryabhattai* strain S2 was isolated from the soybean rhizosphere collected in Daegu, South Korea (35°52'43.1"N 128°47'37.3"E). In short, 1 g of soil was serially diluted; subsequently, dilutions from 10^{-1} to 10^{-6} were spread on tryptic soy agar (TSA). The plates were incubated at 30℃ for 48 to 72 h. A bacterial colony of the strain was selected and sub-cultured twice on TSA for purification and isolation of a single colony.

Genomic DNA was extracted from bacterial cells grown overnight at 30℃ in tryptic soy broth using the Wizard® Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions. Further, DNA concentration and quality were measured using the Qubit fluorometer 2.0 (Thermo Fisher Scientific, USA) and the NanoDrop UV-Vis spectrophotometer (Thermo Fisher Scientific), respectively. Prior to the construction of the sequencing library, genomic DNA was not subjected to size selection. Furthermore, the sequencing library was constructed with the Oxford Nanopore Technology using the V14 kit chemistry (SQK-LSK114, Oxford Nanopore Technologies, UK) according to the

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manufacturer's instructions. This technique includes minimal fragmentation of the genomic input DNA in a sequence independent manner. The ligation kit was used to prepare the sequencing library using the NEBNext® Module (New England Biolabs, USA). Additionally, Genomic DNA was sequenced on an R10.4.1 flow cell using the MinION device. FASTQ files were generated by Guppy v4.4.1 software with high accuracy mode. In addition, low-quality reads (5% of worst fastq reads) were removed using Filtlong v0.2.1 with default parameters [7]. The sequencing was performed at the KNU NGS Core Facility (Korea).

The sequencing produced a total of 116,000 reads (791,903,685 bp) with an estimated genome coverage of 149 x and a relative N_{50} (rN_{50}) of 13,578 bp. A *de novo* genome assembly was conducted using Flye 2.9-b1768 with the default parameters expect for genome size (--genome-size 5.3m), number of iterations (--iterations 5), and assembly coverage (--asm-coverage 40) [8]. The genome assembly was evaluated using the Quality Assessment Tool for Genome Assemblies v5.0.2 software [9]. The assembly resulted in the generation of 3 circular contigs, with the largest one corresponding to *P. aryabhattai* chromosome of 5,070,860 bp, with a GC content of 38.3%. The second and the third contigs correspond to plasmids P1 and P2 with sizes of 148,124 bp and 76,418 bp, and GC content of 33.3% and 36.5%, respectively. The bacterial genome was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) build6494, and the Rapid Annotation using Subsystem Technology (RAST server) version 2.0, respectively [10, 11]. The annotation revealed that *P. aryabhattai* S2 chromosome encodes 5,324 coding genes, 42 ribosomal RNAs, 131 transfer RNAs, 6 non-coding RNAs and 42 pseudogenes (Table 1). Finally, bacterial genome and plasmids were visualized using Proskee online tool [12] (Fig. 1).

The average nucleotide identity was conducted between the genome of *Priestia aryabhattai* strain S2 and the NCBI deposited *Priestia aryabhattai* strain K13

Table 1. Genome features of *P. aryabhattai* S2 annotated by PGAP.

Genome features	Value
Number of contigs	3
Chromosome size (bp)	5,070,860 bp
Coding genes (CDSs)	5,324
Ribosomal RNAs (rRNAs)	42
Transfer RNAs (tRNAs)	131
Non-coding RNAs (ncRNAs)	6
Pseudogenes	42

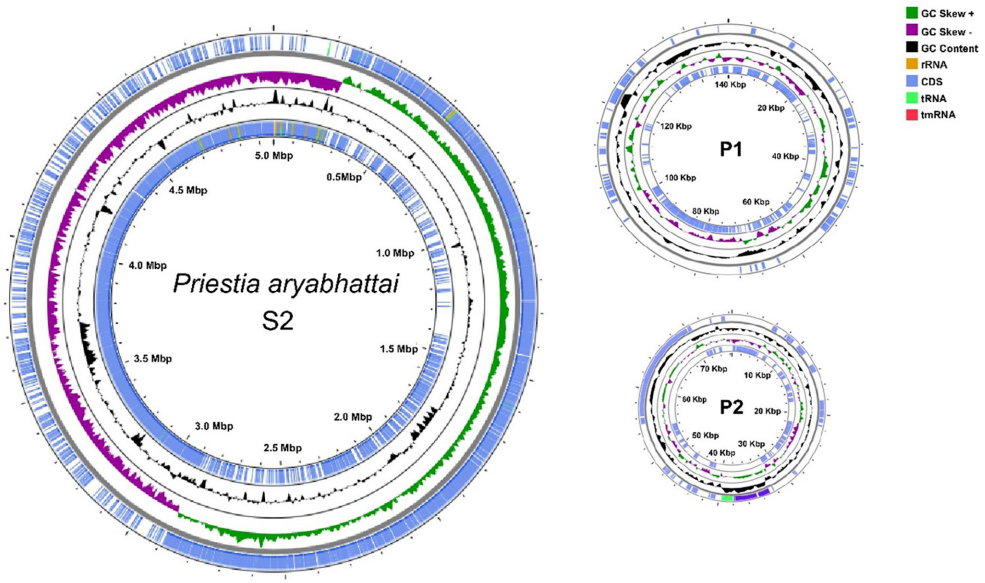

Fig. 1. Circular genome map of *Priestia aryabhattai* S2 with its plasmids P1 and P2. The outer blue circles present the annotation, location, and direction of predicted genes, the middle black circles show the GC% content and the inner circle indicates the GC skew, positive (green) and negative (purple).

Table 2. *Priestia aryabhattai* S2 PGP genes annotation.

Gene	Protein	Chromosome Location
gltD	Glutamate synthase [NADPH] small subunit	3287188-3288669
trpA	Tryptophan synthase subunit beta	1195140-1195955
trpB	Tryptophan synthase beta chain	1193939-1195153
trpC	Indole-3-glycerol phosphate synthase	1192558-1193325
trpD	Anthranilate phosphoribosyltransferase	1191543-1192568
trpE	Anthranilate synthase component I	1190036-1191550
ureC	Urease subunit alpha	2367895-2369604
ilvB	Acetolactate synthase large subunit	825575-827302
pstA	Phosphate ABC transporter permease	908697-909626
pstC	Phosphate ABC transporter permease subunit	907813..908700
nirB	Nitrite reductase large subunit	4172757-4175171
nirD	nitrite reductase small subunit	4172412-4172738
trxB	Thioredoxin-disulfide reductase	483345-484298
nhaC	Na+/H+ antiporter	483345-484298
hmpA	NO-inducible flavohemoprotein	4963721-4964902
msrA	Peptide-methionine (S)-S-oxide reductase	4972793-4973308

with the accession numbers CP024035.1, CP024036.1, CP024037.1.The comparison tool showed a 99.46% similarity between the 2 genomes.

Genomic functional annotation of *P. aryabhattai* S2 revealed the presence of potent genes associated with plant growth promotion (PGP) and stress resistance, as outlined in Table 2. Notable examples include *gltD*, involved in nitrogen assimilation; *trpABCDE* responsible for indole-3-acetic acid (IAA) synthesis; *ureC*, which catalyzes the hydrolysis of urea to produce ammonia; *ilvB*, responsible for the synthesis of acetoin, a plant growth-promoting signalling molecule; *pstAC*, facilitating the uptake and utilization of phosphate; and *nirBD* responsible for nitrite reduction and assimilation. Additionally, we assessed the ability of *P. aryabhattai* S2 to thrive under high salinity stress conditions, with concentrations of up to 10%, using TSA medium supplemented with NaCl. Furthermore, genomic analysis revealed the presence of genes with antioxidant properties that can mitigate oxidative stress in plants during both biotic and abiotic stress conditions, including *trxB, msrA, hmpA*, and *nhaC*. *trxB* and *msrA* are involved in suppressing oxidative stress responses, similarly *hmpA* is a NO-inducible flavohemoprotein contributing to the detoxification of free radicals under stress conditions, and *nhaC* plays a role in maintaining ion homeostasis by regulating extracellular ion concentrations. Owing to its unique PGP features, *P. aryabhattai* S2 has the potential to revolutionize sustainable agriculture practices and reduce reliance on chemical fertilizers and pesticides. For instance, it can be applied as a biofertilizer to enrich soil fertility and enhance nutrient uptake in plants by promoting phosphate solubilization and nitrogen. Furthermore, *P. aryabhattai* S2 can be employed to mitigate the impact of abiotic stress factors, such as salinity and drought, on plants through its genomic features related to oxidative stress control and alleviation.

The applications of *P. aryabhattai* S2 can be extended to other crops, including wheat, rice, and tomato plants, which are susceptible to many abiotic stressors such as salinity. Other studies showed the potential of distinctive strains of *P. aryabhattai* to enhance crop production by the alleviation of salinity stress impact on plants growth, yield, and quality [13, 14].

The genomic investigation of *P. aryabhattai* strain S2 has been pivotal to explore its unique genetic features. This valuable insight highlights the promising potential of this PGPB for future application as a sustainable biofertilizer to enhance plant growth, yield and stress tolerance.

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Nucleotide Sequence Accession Number(s)

The genome sequence of *P. aryabhattai* strain S2 and its plasmids P1 and P2 have been deposited in DDBJ/ENA/GenBank under the accession numbers CP129633, CP129634, and CP129635 respectively. The associated BioProject accession number is PRJNA991722, the BioSample accession number is SAMN36315346 and the SRA accession number is SRR25269555.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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