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RESEARCH ARTICLE OPEN ACCESS

Panchagavya-Derived *Brevibacillus brevis* S1-3: Insights from the Draft Genome on its Antimicrobial and Plant Growth-Promoting Ability

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Abstract

***Panchagavya* has traditionally been used in Indian Ayurvedic practices because of its pro-agricultural and medicinal properties. This study presents the draft genome of a new *Brevibacillus brevis* S1-3 strain isolated from the fermented product *Panchagavya*. Through whole-genome sequencing, we determined that the genome of *B. brevis* S1-3 was 6,348,716 base pairs with a GC content of 54.3%. Genome assembly revealed the presence of 6107 protein-coding genes, 186 tRNA genes, and 13 rRNA genes. Genome annotation and analysis identified the genes involved in metabolism and other cellular processes. We also predicted the presence of several gene clusters associated with plant growth promotion, including indole acetic acid (IAA), gibberellic acid, ammonia, and nitrogen. Our study also revealed the genes responsible for the production of secondary metabolites that displayed a significant correlation with antimicrobial activity. Our results provide new insights into the genomic basis of the plant growth-promoting abilities of *B. brevis* and pave the way for further research in this field.**

Keywords: *Brevibacillus brevis*, Draft Genome Sequencing, *Panchagavya*, Plant Growth Promotion, Secondary Metabolite, Ayurvedic Practices

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INTRODUCTION

Brevibacillus brevis is a gram-positive, motile, rod-shaped, aerobic spore-forming bacterium known to be present in various environmental conditions, including soil and the animal guts.[1,2] This bacterium has been shown to possess antimicrobial activity against soil-borne pathogens such as *Phytophthora nicotianae* and *Ralstonia solanacearum,*[3,4] making it a potential control agent against plant pathogens. Additionally, *B. brevis* produces a variety of secondary metabolites, such as tyrocidine, grastin, and adenine, which are responsible for its antimicrobial activity.[5-7] *Brevibacillus brevis* has also been studied to identify its role and interaction with plants and has been found to confer disease resistance against fungal agents in plants like tomatoes,[8] grapes,[9] pigeon pea,[10] tea,[11] etc. Furthermore, *B. brevis* has been identified as a plant-growth-promoting rhizobacterium (PGPR),[12-14] which can act as a biofertilizer, increasing crop yield and soil fertility, while reducing the need for chemical fertilizers.[15]

Several studies have reported draft genome sequences of several strains of *B. brevis*, including NBRC 100599,[16] *B. brevis* X23,[14] and *B. brevis* strain FJAT-0809-GLX.[13] These genome sequences typically range in size from 6Mb and contain more than 5600 protein-coding genes. However, these previously published genomes are yet to undergo functional annotation to identify the genes responsible for the biosynthesis of secondary metabolites or plant growth regulators. In the field of plant-microbe interactions, biocontrol is a dynamic strategy that uses beneficial microbes to control plant pathogens. The biocontrol arsenal includes systemic resistance, antimicrobial compounds, competitive exclusion, and nutrient enhancement.[17] The success of biocontrol depends on factors such as compatibility, adaptability, persistence, and specificity, which collectively determine its effectiveness. Integrating these methods with other pest management approaches is essential for sustainable agriculture.[18] However, achieving a delicate balance between inducing resistance without harmful effects and addressing practical application challenges remains a complex task.

Recent advancements highlight the crucial roles of plant-associated microorganisms in maintaining plant health and ecological balance. Utilizing beneficial microbes is a promising approach for disease mitigation and improved crop yields.[19] Genomic and proteomic analyses of microbial genomes provide insights into the molecular intricacies of these interactions, which are critical for refining control measures. While previous research focused on the rhizosphere, the phyllosphere, which includes aboveground plant parts, is less explored.[20]

B. brevis is recognized as a noteworthy inhabitant of the rhizosphere, showcasing remarkable biocontrol capabilities through its interactions with plants. In this study, we isolated a new strain of *Brevibacillus brevis* S1-3 from Panchagavya, a fermented product traditionally used in Indian Ayurvedic practices that is composed of five cow products, including clarified butter, curd, milk, urine, and fermenting dung.[21,22] Through genome sequencing and functional annotation, we characterized the genome of *B. brevis* S1-3, providing new insights into the genomic basis of the biosynthesis of secondary metabolites and plant growth regulators in this strain.

MATERIALS AND METHODS

Isolation and Molecular Identification

The *Panchagavya* used in this study were obtained from a commercial market in Chennai, India. After serial dilutions, the bacterial species present in *Panchagavya* were grown in Luria-Bertani medium at 37°C. Distinct colonies were then selected and cultured separately before storage at -20°C. Genomic DNA was extracted from the selected bacteria using the QIAamp DNA Microbiome Kit (Qiagen India Pvt. Ltd., India), according to the manufacturer's instructions. The quality and quantity of the extracted bacterial genomic DNA were analyzed using agarose gel electrophoresis and Nanodrop (Tecan-Infinite 200 PRO, Switzerland). PCR was performed using the 16s rDNA universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3').[23,24] The

amplified PCR product was purified (using a Qiagen PCR product purification kit) and sequenced using the Sanger DNA sequencing method (Applied Biosystems Genetic Analyzer, Saint Aubin, France). The resulting 16s rDNA sequences were compared to those in the NCBI database using the Basic Local Alignment Search Tool (BLAST). The bacterial species were identified based on sequence similarity, and a phylogenetic tree was constructed using the MEGAX software. Evolutionary distances were inferred using the neighbour-joining method.[25-27]

Genome Sequencing and Annotation

Paired-end sequencing libraries were prepared using a Nextera XT DNA Library Preparation Kit (Illumina). The final library was analyzed using a Bioanalyzer 2100 (Agilent Technologies, USA) with a high-sensitivity DNA kit according to the manufacturer's instructions. The paired-end Illumina library was sequenced using 2 x 150 bp chemistry on a NextSeq-500 sequencer. Quality control of the raw reads was performed using FastQC v.0.11.5,[28-30] and the low-quality reads were filtered. The Cutadapt tool was used to remove adapter regions from sequencing reads. High-quality reads obtained from Illumina NextSeq-500 were assembled into scaffolds using SPAdes (version 3.7.1) with default parameters.[31-33] The quality of the assembled genome was analyzed using QUAST.

Genome assembly was annotated using Prokka v.2.1.1 and Rapid Annotation using Subsystems Technology (RAST) server v.2.0. Secondary metabolite gene clusters were identified using the antiSMASH version 5. The various biological features of the annotated genome were analyzed using RAST. Antimicrobial resistance genes and other protein functions were identified using PATRIC genome analysis server.[34-36]

RESULTS AND DISCUSSION

Isolation of culture and phylogenetic analysis

We isolated various bacterial strains and evaluated their antimicrobial activity. Antibacterial activity was examined using broth microdilution assays against *Streptococcus aureus (*NCBI_CP00253)*, E. coli (*NCBI_U00096)*, and Vibrio cholerae (*NCBI_CP043554). One of the bacterial

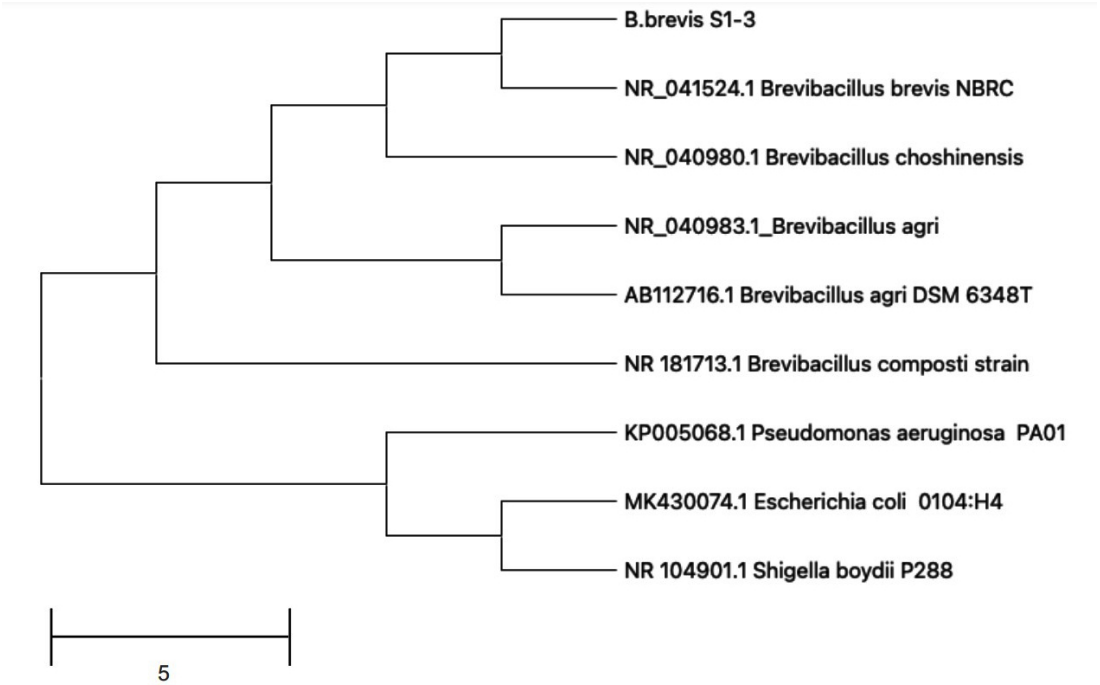

Figure 1. Phylogenetic analysis of 16S rDNA sequence of *Brevibacillus brevis* S1-3 strain using neighbor-joining method. *Pseudomonas aeruginosa* was used as an outgroup

isolates that exhibited antimicrobial activity was selected for this study.

We isolated several bacterial strains from *Panchagavya* and assessed their antimicrobial potential. The antibacterial activity of these strains was evaluated using broth microdilution assays against three target pathogens: *Streptococcus aureus, E. coli*, and *Vibrio cholerae* (data not shown). One strain demonstrated notable antimicrobial activity among the bacterial isolates tested, prompting its selection for further investigation. The selected bacterial isolate was identified by 16s rDNA sequencing; and showed high similarity to *Brevibacillus brevis* (NR_041524). The 16s rDNA gene sequence of *Brevibacillus brevis* S1-3 was used to construct a phylogenetic tree (Figure 1), which revealed that the isolate was closely related to *B. brevis* NBRC *and B. choshinensis* with 99.2% and 98.38% sequence similarity, respectively. Other closely related species included *B. agri* and *B. agri* DSM 6348T, with 97.5% and 97.3% sequence similarity, respectively. The bacterial isolate identified in this study was named *Brevibacillus brevis* S1-3. The efficiency of *Brevibacillus brevis* as a plant growth-promoting rhizobacterium (PGPR) has been determined through studies evaluating its application in fostering plant growth.[2] Through the examination of several plant growth-promoting (PGP) features, such as ammonia synthesis, and the generation of phytohormones, such as indole-3-acetic acid (IAA), *Brevibacillus brevis*' efficiency in promoting plant growth, evaluations of seed germination, and several plant growth metrics

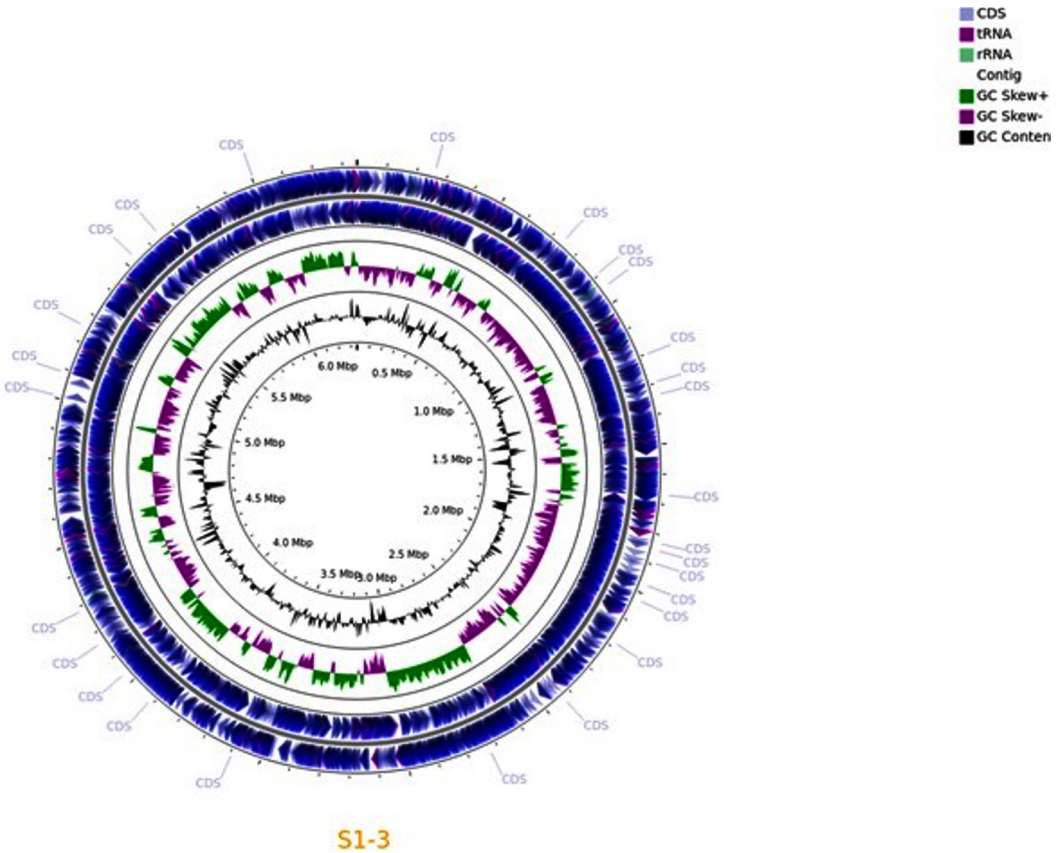

Figure 2. The chromosome organization of *Brevibacillus brevis* S1-3, a plant growth-promoting bacteria isolated from *Panchagavya.* Circularized DNA plotter diagram of the chromosome of *B. brevis,* oriented from the origin; the outer light blue circle designates the genome base positions, and the outer blue circles depict predicted 5800 CDSs on both forward and reverse strands. The purple and green combination circle states important chromosomal core structures with DNA elements like tRNA, GC skew^{+}, GC skew^{-} and rRNA contig. The inner black circle denotes GC content.

Table 1. General genome features of *Brevibacilus brevis* S1-3 strain plant growth promoting bacteria isolated from *Panchagavya*

Features	S1-3 chromosome
Genome size	6,348,716
G + C (%)	55.2
Predicted CDS	5800
rRNAs	13
tRNAs	186

G+C (%): guanine and cytosine content; CDS: protein-coding genes; rRNAs: ribosomal RNA; tRNAs: transfer RNA

have also been made.[37] *Bacillus brevis* has been found to provide a multi-pronged defense against fungal and microbial pathogens by means of extracellular secretion of gramicidin S, gramicidin A, and a biosurfactant, thereby functioning as a biological control agent and aiding plant growth, apart from the production of PGPs.[38]

Whole Genome Sequencing of *B. brevis* S1-3

Genome sequencing of *B. brevis* S1-3 was performed using the Illumina NextSeq-500 platform. A total of 1,602,833 paired-end reads of 101bp were generated, with an average GC content of 54.3% (Table 1). These reads were assembled using SPAdes software, resulting in a draft genome of 5,845,263 bp in size, comprising 187 contigs (N50 - 88,031 bp) and 20 scaffolds (N50 - 678,417 bp). The genome contained 6,107 protein-coding sequences (CDS), 186 tRNA genes, and 13 rRNA operons (16S-23S-5S rRNA) (Figure 2). Genome annotation was performed using the Prokka and RAST servers, which revealed that out of the total of 2,616 proteins, 2,492 were annotated as 'hypothetical' while the remaining proteins had non-hypothetical functions. The annotation included 5,259 proteins with functional assignments, including 1,592 proteins with Enzyme Commission numbers, 1,355 with Gene Ontology (GO) assignments, and 1,201 proteins mapped to KEGG pathways. The quality of the genome assembly was evaluated using QUAST and showed that the genome assembly of *B. brevis* S1-3 was of high quality.

Genome annotation

Genome annotation of *B. brevis* S1-3 assigned many genes to cellular processes related to metabolism, such as membrane transport, dormancy and sporulation, cellular signalling and regulation, cell wall synthesis, and capsule formation. Additionally, many genes were correlated with biosynthesis of a diverse group of macromolecules, such as amino acids, carbohydrates, cofactors, vitamins, prosthetic groups, and pigments (Table 2). A similar study conducted on *Brevibacillus brevis* LABIM17 proved its antimicrobial property against plant pathogens by *brevis* through the production of octapeptin and, auranticin.[39]

Table 2. Annotation of genes involved in metabolism and other cellular processes of *Brevibacillus brevis* S1-3 plant growth-promoting bacteria isolated from *Panchagavya*

Genes function	Compounds	No. of genes
Genes related to metabolism	Fatty acids, lipids and isoprenoids	215
	Amino acids and derivatives	625
	Sulphur	57
	Carbohydrates	560
	Cofactors, vitamins, prosthetic groups and pigments	381
	Aromatic compounds	45
	DNA	140
	Phosphorous	85
	Iron	29
	Secondary metabolism	8
	Nitrogen	16
	Nucleosides and nucleotides	163
	Potassium	13
	RNA	207
Genes related to cellular processes	Cell division and cellular cycle	56
	Dormancy and sporulation	141
	Cellular wall and capsule formation	145
	Photosynthesis	0
	Miscellaneous	67
	Motility and chemotaxis	118
	Regulation and cell signalling	115
	Phages, prophages, transposable elements and plasmids	14
	Respiration	109
	Response to stress	124
	Membrane transport	226
	Virulence, disease and defence	128

Table 3. Plant growth promotor (PGP) gene cluster identified in *B. brevis* S1-3 strain

Plant growth promotor	Genes
IAA (Indole Acetic Acid)	*Iaam, Iac, IaaH, IaaL, trpE(G), ipdC*
Ammonia and Nitrogen	*amoA, amoCAB, nifD, nifK, nifH*
Siderophore	*Sid, agbB, entB, entC, entA*
Cytokines	*Tzs, TLRs, PDGFA, PDGFB, PDGFC, PDGFD*
GA3	*P450-3, P450-4, NPB20, ggs1, ggs2*

Table 4. Antimicrobial Resistance Genes from *Brevibacillus brevis* S1-3

AMR Mechanism	Genes
Antibiotic inactivation enzyme	*ANT(6)-I, FosB, PDC* family
Antibiotic target in susceptible species	*Alr, Ddl, dxr, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, MurA, rho, rpoB, rpoC, S10p, S12p*
Antibiotic target modifying enzyme	*Cfr*
Efflux pump conferring antibiotic resistance	*EmrAB-OMF, EmrAB-TolC, FexA family, MdtABC-OMF, MdtABC-TolC, MexAB-OprM, MexCD-OprJ, MexCD-OprJ system, MexEF-OprN, MexHI-OpmD, MexHI-OpmD* system, *MexJK-OprM/OpmH, MexVW-OprM, MexXY-OMP, YkkCD*
Gene conferring resistance via absence	*gidB*
Protein altering cell wall charge conferring antibiotic resistance	*GdpD, PgsA*
Protein modulating permeability to antibiotic	*OccD4/OpdT, OccD6/OprQ, OccK8/OprE, OprD* family
Regulator modulating expression of antibiotic resistance genes	*LiaF, LiaR, LiaS*

Identification of genes involved in plant growth promotion and secondary metabolite biosynthesis

B. brevis also exhibits PGP traits at high temperatures, making it a valuable inoculant for cotton crops. Previous studies have reported that *B. brevis* enhances plant growth by increasing the expression of plant growth promoters such as IAA, ammonia, siderophores, cytokinins, and GA3.[2,40,41] Analysis of *B. brevis* S1-3 revealed that the genome contains many genes involved in the biosynthesis of plant growth promoters (PGP) (Table 3). The presence of five structural genes, *trpE*, *trpD*, *trpC*, *trpB*, and *trpA* in *B. brevis* S1-3 predicted the indole acetic acid production through the tryptophan pathway.[42] The *amoA* and *amoCAB* code ammonia monooxygenase, which is essential for ammonia production. *nifD*, *nifK*, and *nifH* are responsible for metabolism involved in nitrogen fixation. *entA*, *entB*, and *entC* encode 2,3-dihydro-2,3-dihydroxybenzoate synthetase, which is essential for siderophore production.[43] Cytokinin production was predicted based on the presence of *Tzs* genes, which encode cytochrome P450 monooxygenase, the key enzyme for cytokinin production.[44] *ggs1* and *ggs2* initiate the GGDP pathway for primary metabolism of gibberellic acid.[45] The presence of these genes in *B. brevis* S1-3 suggests that this strain has potential applications in agriculture as a biofertilizer and for controlling plant pathogens.

The gene clusters involved in the biosynthesis of secondary metabolites in *B. brevis* S1-3 were identified using the antiSMASH 5.1.2 software (Table 4). This analysis revealed 97 genes associated with antibiotic resistance, 47 genes related to drug targets, 79 transporter genes, and 96 virulence factor genes. The genes were classified based on their antimicrobial resistance mechanisms, as determined by various antimicrobial resistance databases.[46-49] This study provides a comprehensive understanding of the genomic basis for the plant growth-promoting and secondary metabolite biosynthetic abilities of *B. brevis* S1-3 and, provides a foundation for future research in this area.

CONCLUSION

This study isolated and characterized a new strain of *Brevibacillus brevis*, designated as S1-3, from *Panchagavya*. The 16s rDNA sequencing and phylogenetic analysis revealed that the isolate was closely related to *B. choshinensis* and *B. agri 5*-2. Genome sequencing of *B. brevis* S1-3 revealed that the genome is of high quality and contains a wide range of genes involved in various cellular processes, including metabolism, cell wall synthesis, and capsule formation. In addition, the genome contains many genes involved in the biosynthesis of plant growth promoters and secondary metabolites. The presence of genes involved in the biosynthesis of indole acetic acid, ammonia, nitrogen fixation, siderophores, cytokinins, and gibberellic acid suggests that this strain has potential applications as a biofertilizer and in controlling plant pathogens. Furthermore, identifying the genes involved in antibiotic resistance, drug targets, transport, and virulence factors may provide insights into the potential biotechnological applications of this strain. The results of this study expand our understanding of the genetic and functional diversity of *B. brevis* and provide a foundation for future research.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

PR and MRam conceptualized the idea. SS, AA and VS isolated and sequenced the genome of *Brevibacillus brevis* S1-3. JJ and MRan performed Genome annotation. PR, VS and JJ wrote the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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