UNRAVELING THE PLANT GROWTH-PROMOTING POTENTIAL OF *BACILLUS SAFENSIS* P1.5S THROUGH GENOME ANALYSIS

Loredana-Elena Mantea[a], Amada El-Sabeh[a], Marius Mihasan[a] and Marius Stefan[a,*]

[a] Department of Biology, Faculty of Biology, Alexandru Ioan Cuza University of Iasi, Bd. Carol I, No. 11, 700506 Iasi, Romania;
*Correspondence: stefanm@uaic.ro

Abstract

Plant growth-promoting bacteria have emerged as promising eco-friendly alternatives to traditional agricultural practices. These beneficial microbes promote plant growth through various mechanisms including nitrogen fixation, production of indole-3-acetic acid (IAA), salicylic acid (SA), volatile organic compounds (VOCs), and antimicrobial secondary metabolites. In this study, we performed a genome-based characterization of bacterial strain P1.5S using bioinformatic tools to identify genes associated with plant growth promotion. The draft genome of strain P1.5S is 3,667,318 bp in size, assembled into 13 contigs. Taxonomic analysis confirmed the identity of the strain as *Bacillus safensis* (dDDH: 80.6%; ANI: 97.84%). Our *in silico* investigation revealed gene clusters related to nitrogen fixation (10 genes), as well as genes involved in the production of IAA (12 genes), SA (7 genes) and VOCs biosynthesis. Additionally, the genome encodes biosynthetic gene clusters for secondary metabolites with antimicrobial properties such as lipopeptides, peptides and polyketides. The presence of genes related to siderophore and hydrolytic enzymes production highlights the strain's potential for biocontrol. Moreover, genes associated with root colonization further support the plant-beneficial potential of this strain. *Bacillus safensis* P1.5S is a promising candidate for agricultural practices, but further greenhouse and field studies are necessary to validate its potential.

Key words: draft genome, *Bacillus safensis* P1.5S, plant growth promoting traits, nitrogen fixation, phytohormone production, biocontrol.

Introduction

One of the greatest challenges of the 21st century is meeting the food demands of a growing global population, while preserving the environment and maintaining biodiversity. Historically, food production has relied heavily on the use of chemical fertilizers, which significantly increased crop productivity. However, considerable environmental drawbacks related to this practice were evidenced, including water contamination, soil degradation and loss of biodiversity (Pahalvi et al. 2021). Given the negative impact of excessive chemical fertilizer use, there is an increasing need for innovative solutions and more sustainable agricultural practices to help farmers manage their fields more efficiently (Saleem et al. 2023).

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Plant growth-promoting bacteria (PGPB) have emerged as a promising solution to enhance crop yields and sustainability, providing a more eco-friendly alternative to conventional agricultural methods (Katsenios et al. 2022). These bacteria colonize the rhizosphere of plants and promote growth through both direct and indirect mechanisms, including biological nitrogen fixation (BNF), solubilization of phosphorus compounds, production of indole-3-acetic acid (IAA), salicylic acid (SA), siderophores and volatile organic compounds (VOCs), activity of 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), and microbial antagonism (Kumar et al. 2022). In addition, it has been shown that PGPB improves crop tolerance to abiotic stress, as well as enhance their growth and quality characteristics. Bacteria such as *Rhizobium*, *Azospirillum*, *Bacillus*, and *Pseudomonas* have been utilized as effective plant growth promoters (Katsenios et al. 2022). Several benefits of PGPB agricultural use were outlined, including environmental sustainability, improved soil health, nutrient availability and fixation, enhanced plant growth and productivity, and reduction of soil and water contamination (Souza et al. 2015).

In this study, we isolated a *Bacillus safensis* strain (named P1.5S), from a phosphorus-deficient soil. *In vitro* tests revealed that the P1.5S strain possesses several traits involved in plant growth promotion, including phosphorus solubilization, production of IAA, siderophores, ACC deaminase and antimicrobial lipopeptides. It is also a stress-tolerant bacterium, capable of solubilizing phosphorus under pH, salt, and temperature stress (Mantea et al. 2025). Additionally, *Bacillus safensis* P1.5S forms a robust biofilm, which plays a crucial role in root colonization. Here, we report the draft genome sequence of *B. safensis* P1.5S, providing important insights into the genes involved in the plant growth-promoting capabilities. Our results highlight the potential of P1.5S strain to be used as a biofertilizer in agricultural applications. Nonetheless, additional research is required to evaluate the strain's performance under field conditions.

Materials and methods

Isolation of *Bacillus safensis P1.5S* and genomic DNA sequencing

The strain used in this study was isolated from phosphorus-deficient soil cultivated with maize located in the north-eastern part of Iasi County, Romania. The soil sample was serially diluted with distilled sterile water, and 50 µL from the suspension were plated onto Pikovskaya (PVK) medium containing insoluble $Ca_3(PO_4)_2$ (TCP) as the sole phosphorus source. After incubation at 28 °C for 7 days, a small, bright yellow colony was selected based on the formation of a clear zone around it (indicating TCP solubilization) and inoculated into 10 mL Luria-Bertani (LB) broth. Following a 14-hours incubation at 28 °C, 9 mL of the culture was centrifuged, and the pellet was used for genomic DNA extraction using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The whole genome *de novo* sequencing was performed using the Illumina NovaSeq 6000 (Macrogen Europe BV, Amsterdam, Netherlands), and library preparation was conducted with the TruSeq DNA PCR-Free Kit, generating paired-end 151 nt reads.

Genome assembly, annotation and taxonomic identification

First, the obtained raw data was assessed for read quality using FastQC v0.11.9 (Andrews 2010) and filtered with Fastp v0.23.2 (Chen et al. 2018) to remove the adapters and low-quality sequences. *De novo* genome assembly of the resulting filtered reads was performed using Unicycler v0.4.9 (Wick et al. 2017). The quality and contiguity statistics of the assemblies were evaluated using QUAST v5.2.0 (Gurevich et al. 2013), while completeness and contamination were assessed using CheckM v1.2.2 (Parks et al. 2015). The draft genome was uploaded to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP v6.5) for automatic annotation (Tatusova et al. 2016). In addition, the draft genome was submitted to TYGS (Type

(Strain) Genome Server) for taxonomic identification (Meier-Kolthoff and Göker 2019) and to antiSMASH 7.0 for the prediction of secondary metabolite biosynthesis gene clusters (Blin et al. 2023). The TBLASTN algorithm (Altschul et al. 1990) was used to search for genes related to plant growth promotion, chemotaxis, motility and biofilm formation. Genes with an identity higher than 30% and a query coverage value above 70% were considered positive hits. A database of UniProt protein sequences of interest was created and subsequently searched against the draft genome (The UniProt Consortium, 2025). For all tools and servers, default parameters were used unless otherwise stated.

Results and discussion

Genomic characterization of *Bacillus safensis* P1.5S

The sequencing runs allowed the acquisition of 13.6 million reads totaling 2.0 Gbp. After filtering > 91% of the reads had Q scores > 30 and an average Phred score/read of 36. A total of 13.47 million reads were used for the genome assembly. The draft genome assembly has a length of 3,667,318 bp and consists of 13 contigs, with an N50 of 975,127 bp and a C+G content of 41.5% (Table 1). The CheckM analysis indicated a completeness of 99.22% with minimal contamination (0.29%). According to the functional annotation performed by NCBI PGAP, a number of 3,756 genes, of which 3,667 genes are protein-coding sequences, were identified (Table 1).

Table 1. *Bacillus safensis* P1.5S genome features

Attribute	Value
Total reads (before filtering)	13,617,686
Total reads (after filtering)	13,475,572
Genome coverage (×)	530
Genome size (bp)	3,667,318
Genes (total)	3756
CDSs (total)	3705
CDSs (with protein)	3667
Number of contigs	13
Contig N50 (bp)	975,127
Contig L50	2
GC content (%)	41.5
Genes (RNA)	51
tRNA	44
rRNA	1, 1 (16S, 23S)
ncRNA	5
Pseudogenes	38
Accession number	JARZFW000000000

CDS – coding sequence; GC – guanine-cytosine content; ncRNA – non-coding RNA; rRNA – ribosomal RNA; tRNA – transfer RNA.

Phylogenomic analysis of *Bacillus safensis* P1.5S

Based on TYGS analysis, a phylogenetic tree divided into two clades with a common ancestor was constructed - Figure 1. The first clade is divided into two branches. One of the branches contains P1.5S strain along with *Bacillus safensis* subsp. *osmophilus* CECT 9344T, *B. safensis* FO-36b, *B. australimaris* NH7I_1 and two strains of *B. pumilus* (ATCC 7061 and NCTC 10337); the other branch consists of *B. zhangzhouensis* MCCC 1A08372. The P1.5S strain is clustered along with *B. safensis* FO-36b and *B. safensis* subsp. *osmophilus* CECT

9344T in a unique branch. TYGS analysis also revealed a digital DNA-DNA hybridization (dDDH) value of 80.6% when compared to *Bacillus safensis* subsp. *osmophilus* CECT 9344T and 70.7% when compared to *Bacillus safensis* FO-36b (Table 2). The dDDH values are above the common threshold accepted for species-level delineation (70%) (Meier-Kolthoff and Göker 2019), suggesting a high similarity between P1.5S strain and *B. safensis subsp. osmophilus* CECT 9344T/*Bacillus safensis* FO-36b. Based on these results we concluded that P1.5S strain belongs to *Bacillus safensis* species. Also, the average nucleotide identity (ANI) of the P1.5S strain genome was compared with that of *Bacillus safensis* subsp. *osmophilus* CECT 9344T and *Bacillus safensis* FO-36b, yielding values of 97.84% and 96.59%, respectively. These high ANI values confirm the close genetic similarity between the P1.5S strain and the two *Bacillus safensis* strains. In support of our conclusion, the G+C content difference between the P1.5S strain and *Bacillus safensis* subsp. *osmophilus* CECT 9344T (0.6%) and *Bacillus safensis* FO-36b (0.11%) falls within the 1% variation range typically considered for strains of the same species (Palma et al. 2024).

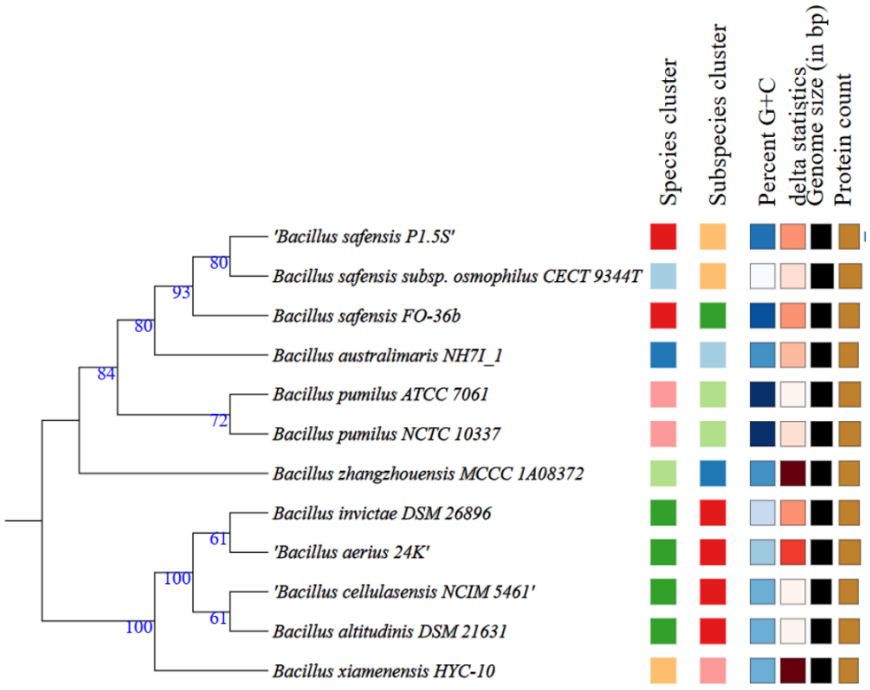

Figure 1. Phylogenetic tree based on the whole genome of P1.5S strain. Tree inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. The numbers are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 81.2 %. The tree was rooted at the midpoint.

Table 2. Pairwise dDDH comparisons between P1.5S strain genome and the type-strain genomes selected by TYGS. The dDDH values, their confidence intervals for the d4 formula and the G+C content difference are shown.

Subject strain	dDDH (d4 in %)	C.I. (d4 in %)	G+C content difference (in %)
Bacillus safensis subsp. *osmophilus* CECT 9344T	80.6	(77.7 - 83.2)	0.6
Bacillus safensis FO-36b	70.7	(67.7 - 73.6)	0.11
Bacillus australimaris NH7I 1	52.3	(49.7 - 55.0)	0.15
Bacillus pumilus NCTC 10337	45	(42.4 - 47.6)	0.22

Bacillus pumilus ATCC 7061	44.9	(42.4 - 47.5)	0.17
Bacillus zhangzhouensis MCCC 1A08372	42.1	(39.6 - 44.7)	0.11
Bacillus xiamenensis HYC-10	37.5	(35.1 - 40.0)	0.2
Bacillus invictae DSM 26896	36.6	(34.2 - 39.2)	0.39
Bacillus aerius 24K	36.6	(34.1 - 39.1)	0.28
Bacillus altitudinis DSM 21631	36.6	(34.1 - 39.1)	0.23
Bacillus cellulasensis NCIM 5461	36.5	(34.0 - 39.0)	0.17

dDDH = digital DNA-DNA hybridization calculated using d4 formula (Meier-Kolthoff and Göker 2019)
C.I. = confidence intervals

Prediction of plant growth promotion traits

Several genes involved in plant growth promotion were detected in *B. safensis* P1.5S genome (Table 3). P1.5S strain harbors genes important for agricultural applications, influencing plant growth through both direct and indirect mechanisms. Thus, 10 genes related to nitrogen fixation such as *nifU*, as well as several nitrogen metabolism-related genes including those responsible for nitrogen transport (*gltP*, *amtB*/*nrgA*, and *nrgB*/*glnK*), nitrogen assimilation (*glnA*, *glnH* and *glnR*), nitrate reduction (*nasE*, *nasD*) and glutamate metabolism (*gltX*) were identified (Hazarika et al. 2023). These findings suggest a putative direct role of the P1.5S strain in plant growth promotion by increasing nitrogen availability, thereby enhancing photosynthesis, improving yields, and contributing to stress tolerance (Vats et al. 2021). Additionally, the P1.5S strain may improve soil nitrogen availability, potentially reducing the reliance on chemical fertilizers (Timofeeva et al. 2023).

Table 3. Genes involved in plant growth promotion putatively encoded by the *Bacillus safensis* P1.5S genome

NCBI Gene ID*	UniProt Gene name	UniProt Protein ID*	Identity (%)	Query coverage (%)	E-value	Total score
Nitrogen fixation and metabolism						
3026303	***nifU***	Q631Z1	83.45	97	2e-77	246
938421	***gltP***	P39817	71.43	100	0.0	1011
			40.53	97	2e-93	305
			39.02	97	8e-90	294
936862	***gltX***	P22250	82.82	100	0.0	845
940070	***glnR***	P37582	81.48	99	1e-70	226
940020	***glnA***	P12425	94.82	100	0.0	880
938139	***glnH***	O34563	68.27	99	4e-113	432
			31.46	96	7e-29	112
936937	***nrgB***	Q07428	84.07	97	2e-61	199
936933	***nrgA***	Q07429	73.38	100	0.0	592
938331	***nasE***	P42436	64.36	95	2e-41	141
938329	***nasD***	P42435	68.83	100	0.0	1702
IAA production						
939011	***trpA***	P07601	71.16	100	9e-129	399
939010	***trpB***	P07600	80.40	100	0.0	674
939004	***trpC***	P03964	69.92	94	1e-104	329

939007	***trpD***	P03947	57.35	100	2e-118	373
939008	***trpE***	P03963	68.50	99	0.0	842
			38.24	92	7e-86	287
939006	***trpF***	P20167	51.38	99	2e-70	229
939361	***trpS***	P21656	85.11	100	0.0	584
939963	***dhaS***	O34660	79.19	100	0.0	1375
			42.29	96	2e-125	567
			37.04	92	3e-88	293
			34.55	96	5e-73	486
937252	***ywdH***	P39616	65.43	100	0.0	800
			30.80	93	8e-53	471
			31.67	88	1e-50	182
937464	***ysnE***	P94562	61.59	100	4e-60	197
936875	***ywkB***	P45869	72.19	95	7e-137	425
938351	***amhX***	P54983	65.16	98	2e-167	516
Salicylic acid production						
939014	***aroA***	P20691	76.06	100	0.0	650
939001	***aroB***	P31102	59.72	99	6e-156	482
938963	***aroC***	P35146	31.84	96	6e-41	147
939000	***aroF***	P31104	82.05	100	0.0	643
937820	***aroE***	P54374	67.14	100	3e-129	401
			33.56	96	6e-47	165
939005	***aroH***	P19080	69.29	100	1e-57	189
937190	***menF***	P23973	55.77	99	2e-174	540
Siderophores production and iron transport						
936592	***besA***	O32102	49.10	97	6e-86	335
936579	***dhbA***	P39071	55.94	100	6e-95	490
			33.98	97	2e-38	546
			30.23	97	5e-21	89.4
937162	***dhbC***	P45744	58.40	100	6e-153	475
936582	***dhbE***	P40871	70.94	96	0.0	763
936582	***dhbB***	P45743	54.81	100	8e-114	358
936569	***dhbF***	P45745	59.63	100	0.0	2932
			31.48	89	0.0	2343
			31.75	90	0.0	2402
			32.81	94	0.0	3400
936706	***sufB***	O32162	95.05	100	0.0	1096
938871	***sufD***	O32165	81.01	100	0.0	933
Volatile organic compounds						
936852	***alsS***	Q04789	66.97	98	0.0	771
936852	***alsR***	Q04778	58.74	95	2e-117	534
939490	***bdhA***	O34788	79.30	100	3e-180	768
936152	***acoA***	O31404	73.99	96	3e-168	516
			49.21	98	5e-98	590
939697	***acoB***	O34591	80.18	99	0.0	572
			47.76	98	7e-99	911
Hydrolytic enzymes						
939313	***aprE***	P04189	62.04	100	1e-141	442
			50.00	98	1e-113	653

			37.89	97	3e-61	333
938861	***lipA***	O32129	97.32	100	0.0	656

* Gene IDs and protein IDs are derived from the genome of the reference strain *Bacillus subtilis* 168.

Percent identity (%) is the percentage of nucleotides that are identical between the genome of the bacterial strain and the sequence of interest.

Query cover (%) is the percentage of the query sequence (strain genome) overlapping the reference sequence.

E value is a parameter that describes the number of hits that can be "expected" to be found by chance when searching a database of a given size. The lower the E-value, the better the match.

Genome analysis revealed the presence of several genes that contribute to production of two key plant hormones – IAA (12 genes) and SA (7 genes). Some genes are directly involved in the biosynthesis of tryptophan, a precursor to IAA (*trpA* to *trpF* genes) (Natori et al. 1990), while others are related to IAA biosynthesis or the regulation of IAA levels in plants (*dhaS*, *ywdH*, *ysnE*, *ywkB*, and *amhX* genes) (Shao et al. 2015). *aroA*, *aroB*, *aroC*, *aroE* and *aroH* genes play a direct role in the production of SA and *aroF* contributes to the production of phenylalanine, a precursor for SA (Polen et al. 2005). By producing IAA and SA, *Bacillus safensis* P1.5S may promote plant growth by enhancing cell elongation, division, and differentiation, stimulating root formation, improving germination and seedling growth, and modulating responses to environmental factors (Hayat et al. 2010).

Several genes associated with siderophore production and the assembly of iron-sulfur clusters such as *dhbA*, *dhbB*, *dhbC*, *dhbE*, *dhbF, besA* (involved in bacillibactin synthesis) or *sufB*, *sufD* (associated with the iron-sulfur cluster assembly pathway) were detected in *B. safensis* P1.5S genome (Blahut et al. 2020). By producing siderophores capable of binding, extracting and transporting iron near the plant roots, soil bacteria play an important role in iron acquisition, stimulating plant development and health (Leal et al. 2021).

The release of VOCs can directly influence plant growth by enhancing nutrient availability. Our data sustains the presence of *alsS*, *alsR*, *bdhA*, *acoA*, and *acoB* genes involved in the biosynthesis of acetoin and 2,3-butanediol, two potent volatile organic compounds that play significant roles in plant growth promotion (Altimira et al. 2022). Moreover, the presence of the *aprE* and *lipA* genes, encoding an alkaline protease (Jan et al. 2000) and a lipase respectively (Akatsuka et al. 1994), suggests that *Bacillus safensis* P1.5S may contribute to nutrient release and availability, root development, and plant stress tolerance. In addition, several genes involved in phosphorous solubilization, encoding the biosynthesis of organic acids, such as gluconic, formic, malic, citric, lactic, acetic, and succinic acids, or the synthesis of acid and alkaline phosphatases were also predicted in P1.5S genome (Mantea et al. 2025). These results highlight the potential of *Bacillus safensis* P1.5S to promote plant growth through direct mechanisms, including enhanced uptake of essential nutrients such as nitrogen, phosphorus, and iron, regulation of root development, cell elongation and division, and improved stress tolerance.

Regarding the indirect mechanisms, antiSMASH analysis predicted 12 biosynthetic gene clusters involved in the synthesis of secondary metabolites (Table 4). Eight of these clusters showed similarity to known compounds, including lipopeptides (fengycin, surfactin, and lichenysin production), peptides (bottromycin A2), polyketides (bacilysin), and siderophores (bacillibactin and schizokinen). These compounds exhibit a wide range of biological activities: antifungal and antibacterial (fengycin, bottromycin A2, bacilysin bacillibactin) (Coutte et al. 2010; Özcengiz and Öğülür 2015; Franz et al. 2021; Chakraborty et al. 2022), antibiofilm (lichenysin) (Yeak et al. 2022) and biosurfactant properties (surfactin) (Coutte et al. 2010). In addition, identification of genes involved in the production of

siderophores, hydrolytic enzymes or VOCs (Table 3) highlights the potential role of *B. safensis* P1.5S in the biocontrol and pathogen defense, induction of systemic resistance, and improvement of plant health and stress tolerance (Chen et al. 2007). Many of the secondary metabolites identified in the P1.5S genome have also been reported in other *Bacillus safensis* strains (Li et al. 2021; Mateus et al. 2021).

Table 4. Biosynthetic gene clusters predicted in the *Bacillus safensis* P1.5S genome using antiSMASH

Contig	Region	Type	From (nt)	To (nt)	Most similar known cluster	Similarity (%)
JARZFW010000001	Region 1.1	betalactone	616,475	644,887	fengycin	53
	Region 1.2	terpene	714,926	736,800	bottromycin A2	6
	Region 1.3	Type III PKS	777,306	818,406	-	-
	Region 1.4	betalactone	1,270,349	1,302,80	-	-
JARZFW010000002	Region 2.1	other	632,972	674,393	bacilysin	85
	Region 2.2	NRP-metallophore/ NRPS	897,950	949,678	bacillibactin/ bacillibactin E/bacillibactin F	80
JARZFW010000003	Region 3.1	NI-siderophore/ terpene	111,749	149,400	schizokinen	60
	Region 3.2	RRE-containing	292,542	313,447	-	-
JARZFW010000005	Region 5.1	RiPP-like	27,719	38,045	-	-
	Region 5.2	NRPS	179,437	208,163	lichenysin	50
JARZFW010000006	Region 6.1	NRPS	1	44,033	surfactin	39
JARZFW0100000010	Region 10.1	NRPS	1	10,479	lichenysin	14

PKS – Poliketide synthetase;
NRPS – Non-ribosomal peptide synthetase;
NI-siderophore – NRPS-independent;
NRP-metallophore – Non-ribosomal peptide metallophores;
RRE-containing – Regulatory RNA Element;
RiPP-like - Other unspecified ribosomally synthesised and post-translationally modified peptide product.

Genes involved in root colonization predicted in *Bacillus safensis* P1.5S genome

According to BLAST analysis, the draft genome of *B. safensis* P1.5S contains several genes associated with plant root colonization capabilities (Table 5). First, the genome encodes at least 20 genes responsible for chemotaxis and motility, such as *mcpA*, *mcpB*, and *mcpC* involved in eliciting a response to changes in the concentration of environmental attractants and repellents (Müller et al. 1997; Liu et al. 2023). Like many *Bacillus* species, P1.5S strain may

use a chemosensory pathway involving a histidine kinase (*cheA*) and a response regulator (*cheY*) to transmit signals from receptors to the flagellar motors, and *motA* and *motB* to encode membrane proteins that form the bacterial flagellar motor, essential for flagellar rotation and motility (Vats et al. 2021).

Four genes involved in the *quorum sensing* (QS) signaling system - critical for effective root colonization - were identified in the draft genome. These include *luxS* which is responsible for the synthesis of autoinducer-2 (AI-2), a universal signaling molecule used in interspecies communication (Lombardía et al. 2006); *comP* and *comA* which encode a protein kinase and a response regulator protein, forming the two-component ComP-ComA signal transduction system essential for QS (Wolf et al. 2015); and *comQ* which is involved in the post-translational modification and export of the ComX quorum-sensing pheromone. This pheromone regulates key population-dependent processes that are important for the strain's ability to colonize plant roots and promote plant growth (Kalamara et al. 2018).

Moreover, 47 genes related to exopolysaccharide (EPS) production, biofilm formation and regulation were identified in *B. safensis* P1.5S genome. *TasA* is a part of the *tapA-sipW-tasA* operon and encodes a major protein component that forms functional, amyloid-like, protease-resistant fibers on hydrophobic biofilm surfaces. TapA is an accessory protein that facilitates the assembly of TasA fibers, while *bslA (yuaB)* is a hydrophobin that coats the biofilm surface, conferring hydrophobic properties (Zhang et al. 2022). The *epsA-epsO* operon is involved in EPS biosynthesis, which is essential for bacterial cell attachment to surfaces (Cámara-Almirón et al. 2020). The *remA* gene regulates extracellular polymeric matrix biosynthesis and biofilm formation by activating transcription of the matrix biosynthesis operons, including *tapA-sipW-tasA* (Hoffmann et al. 2021). The genus *Bacillus* exhibits a complex system for regulating biofilm formation, particularly under unfavorable environmental conditions (Omer Bendori et al. 2015). The activity of the two primary biofilm-forming operons (*tapA-sipW-tasA* and *epsA-O*) is indirectly regulated by the *spo0A* gene. In general, these histidine - kinase sensors detect signaling triggered by surfactin, a cyclic lipopeptide that is considered an auto-trigger of biofilm formation in many *Bacillus* species (Omer Bendori et al. 2015; Cámara-Almirón et al. 2020). In the case of P1.5S strain, surfactin may be synthesized by an enzyme complex encoded by the *srfAA - srfAD* gene cluster, with surfactin-triggered signaling activating Spo0A, leading to the formation of its phosphorylated form, Spo0A~P.

Table 5. Genes involved in chemotaxis, motility, *quorum sensing* and biofilm formation putatively encoded by the *Bacillus safensis* P1.5S genome

NCBI Gene ID*	UniProt Gene name	UniProt Protein ID*	Identity (%)	Query coverage (%)	E-value	Total score
939600	***cheA***	P29072	71,64	99	0	1009
940124	***cheB***	Q05522	62,01	99	1e-144	627
939621	***cheC***	P40403	74,76	98	2e-100	353
939003	***cheR***	P31105	71,21	100	5e-122	380
939239	***cheV***	P37599	73,51	99	2e-148	504
940120	***cheY***	P24072	91,67	100	7e-70	638
			36,97	99	4e-22	241
			33,63	99	9e-19	129
			33,63	93	2e-15	68,6
			31,86	93	1e-14	65,9
939957	***cheW***	P39802	70,20	96	3e-64	209
938840	***tlpA***	P39216	52,83	95	0	2638

937152	***tlpB***	P39217	52,73	99	0	2651
937154	***mcpA***	P39214	53,18	100	0	2672
937155	***mcpB***	P39215	53,31	100	0	2754
936206	***mcpC***	P54576	49,01	99	0	744
936102	***yfmS***	O06477	34,75	90	2e-44	158
			34,45	87	2e-42	323
			34,36	94	1e-35	656
939302	***motA***	P28611	69,70	97	6e-119	371
939304	***motB***	P28612	58,73	96	5e-92	293
51992978	***swrAA***	O32266	83,76	100	3e-63	204
939618	***swrB***	P40405	37,91	95	2e-28	107
14768252	***swrC***	O31501	66,62	95	0	1347
8303013	***swrD***	C0H412	74,65	100	8e-31	109
936739	***flgM***	P39809	52,27	100	1e-23	89,7
			***Quorum sensing* system**			
937106	***luxS***	O34667	89.17	100	4e-96	300
937179	***comA***	P14204	71.96	100	5e-104	326
			30.88	94	5e-24	133
938866	***comP***	Q99027	45.03	100	0	799
937180	***comQ***	P33690	40.66	90	2e-60	205
			Biofilm formation			
937956	***remA***	Q7WY72	95.51	100	5e-53	174
938655	***spo0A***	P06534	86.94	100	3e-157	724
938532	***tapA***	P40949	37.06	65	1e-33	125
938545	***tasA***	P54507	62.40	90	4e-99	314
938868	***sigW***	Q45585	90.91	100	1e-113	352
			31.06	85	2e-19	82,8
938544	***sinR***	P06533	93.69	100	1e-65	211
			45.05	99	8e-23	88.6
938543	***sinI***	P23308	43.75	84	2e-10	50.4
937009	***abrB***	P08874	93.75	100	2e-54	248
			53.26	95	8e-30	108
			42.86	94	2e-22	87.4
14769325	***ymcA***	O31779	83.92	100	4e-76	243
14769122	***ylbF***	O34412	74.81	87	1e-65	213
938482	***sigD***	P10726	88.98	100	3e-154	543
936362	***sigL***	P24219	52.15	95	2e-143	449
938582	***epsA***	P71050	40.44	96	1e-57	194
938640	***epsB***	P71051	64.18	87	1e-21	101
938571	***epsC***	P71052	60.27	99	0	718
938611	***epsD***	P71053	52.52	98	5e-135	423
938633	***epsE***	P71054	61.60	96	4e-110	446
938631	***epsF***	P71055	52.80	97	1e-130	456
937071	***epsG***	P71056	62.94	100	1e-151	470
938630	***epsH***	P71057	44.44	99	5e-98	442
937256	***epsI***	P71058	59.82	92	7e-135	421
937131	***epsJ***	P71059	33.23	94	2e-49	304
938240	***epsK***	P71060	41.63	99	3e-124	397

936365	***epsL***	P71062	65.31	97	4e-89	405
936372	***epsM***	P71063	48.28	93	1e-58	196
936364	***epsN***	Q795J3	64.42	95	2e-162	502
936731	***csrA***	P33911	81.36	79	2e-29	105
938831	***bslA***	P71014	66.18	73	2e-57	191
936830	***capA/pgsA***	P96738	59.10	99	3e-149	464
936833	***capB/pgsB***	P96736	87.02	100	0	716
936840	***capC/pgsC***	P96737	81.08	99	4e-72	231
936209	***mnaA***	P39131	80.37	99	0	630
938302	***yczE***	O34927	59.07	100	8e-75	242
936293	***ecsA***	P55339	81.56	98	1e-134	824
			32.61	91	3e-29	194
939298	***ecsB***	P55340	56.78	97	2e-155	483
939774	***ecsC***	P55341	55.31	95	4e-75	244
938306	***srfAA***	P27206	53.15	94	0	6197
			38.70	99	0	5677
			32.50	86	0	2189
			35.73	99	0	7030
938303	***srfAB***	Q04747	52.12	81	0	4810
			39.30	94	0	3418
			32.82	86	0	2093
			36.08	99	0	6466
938308	***srfAC***	Q08787	51.26	99	0	2900
			36.13	82	0	1599
			33.11	81	0	1270
938300	***srfAD***	Q08788	52.34	96	2e-83	268
8303165	***slrA***	P0C8M5	52.78	69	9e-08	43.1
938581	***slrR***	P71049	65.79	99	1e-62	204
938417	***glnK***	P40758	53.86	100	4e-142	444
938965	***resE***	P35164	72.16	100	0	1435
			30.35	81	2e-59	507
937404	***lytS***	P94513	66.50	98	0	791
936777	***lytC***	Q02114	51.21	100	3e-165	1170
936150	***sigH***	P17869	91.28	100	1e-132	408

* Gene IDs and protein IDs are derived from the genome of the reference strain *Bacillus subtilis* 168.

Conclusion

The genome analysis of *Bacillus safensis* P1.5S revealed its strong potential to promote plant growth through both direct and indirect mechanisms. Our *in silico* investigation identified gene clusters related to nitrogen fixation and nitrogen metabolism. Particularly noteworthy are the genes associated with the production of two key plant hormones - indole-3-acetic acid (12 genes) and salicylic acid (7 genes) and the biosynthesis of two volatile organic compounds (acetoin and 2,3-butanediol), molecules which play significant roles in promoting plant growth. Furthermore, the genome harbors 12 biosynthetic gene clusters responsible for secondary metabolites, including lipopeptides, peptides and polyketides which exhibit antifungal, antibacterial, antibiofilm and biosurfactant properties. In addition, the presence of genes involved in the production of siderophores, hydrolytic enzymes or VOCs highlights the P1.5S

strain promising biocontrol potential. Genes linked to plant root colonization - such as those involved in chemotaxis, motility, *quorum sensing*, exopolysaccharide production, and biofilm formation - further support the strain plant promoting potential. Taken together, these genomic features underscore the potential of *Bacillus safensis* P1.5S as a promising plant-beneficial microorganism for sustainable agricultural applications. However, further greenhouse and field studies are essential to validate its efficacy under real-world conditions.

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Conflict of interest

No conflict of interest declared.

Data availability

The data underlying this article are available in GenBank under the accession JARZFW000000000, BioProject number PRJNA960951, BioSample number SAMN34340091, and Sequence Read Archive accession number SRX20079035.

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