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Genomic and functional characterization of *Bacillus* sp. B.PNR2 from extinct volcanic soil in Buriram province, Thailand

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ABSTRACT

Bacillus species are renowned for producing diverse secondary metabolites with antimicrobial and plant growth-promoting (PGP) activities. This study presents a genomic and functional characterization of *Bacillus stercoris* B.PNR2, isolated from nutrient-limited volcanic soil in Buriram Province, Northeastern Thailand. The strain exhibited antifungal activity against *Fusarium oxysporum* and *Colletotrichum* spp., along with PGP traits such as indole-3-acetic acid (IAA) production and phosphate solubilization. Whole-genome sequencing revealed a 4.11 Mb genome containing 4,283 coding sequences, *60 tRNA* genes, and 5 rRNA operons, with a G+C content of 43.83%. Genome analysis identified 7 genes associated with IAA biosynthesis, 5 genes involved in phosphate solubilization (including alkaline phosphatase and phytase), 6 genes for siderophore biosynthesis and transport (bacillibactin cluster), and 9 genes related to nitrogen metabolism (nitrate/nitrite reductases, glutamine synthetase, ammonium transporters). AntiSMASH identified 13 biosynthetic gene clusters, including fengycin, bacillaene, surfactin, bacilysin, bacillibactin, and subtilosin A, with several showing low similarity to known clusters, suggesting potential for novel metabolite production. Phylogenomic analysis placed B.PNR2 within the *B. stercoris* clade. The genome also encoded 41 antimicrobial resistance genes and 322 transporter genes, indicating adaptive and defensive capabilities. The integration of genomic and functional traits supports *B. stercoris* B.PNR2 as a promising biofertilizer and biocontrol agent.

1. INTRODUCTION

The application of beneficial microorganisms as biological control agents and biofertilizers has emerged as a sustainable alternative to synthetic agrochemicals in modern agriculture [1-3]. Among these, members of the genus *Bacillus* are particularly valued for their robustness, metabolic versatility, and ability to produce a wide array of secondary metabolites that suppress plant pathogens and promote plant growth [4]. These attributes are largely attributed to their production of lipopeptides, polyketides, ribosomally synthesized peptides, and siderophores [5,6], which exert their effects through multiple mechanisms [7], including membrane disruption, iron chelation, and modulation of plant immune responses [8,9].

Recent advances in genome sequencing and bioinformatics tools, particularly genome mining platforms such as antiSMASH, have facilitated the prediction of biosynthetic gene clusters (BGCs) involved in secondary metabolism [10]. These *in silico* approaches enable the rapid screening of microbial strains for their potential to produce bioactive compounds, before experimental validation [11-16]. Many well-characterized antimicrobial metabolites from *Bacillus*, such as surfactin, fengycin, and bacillomycin, have been identified using these tools. Nevertheless, the diversity of biosynthetic pathways in *Bacillus* sp. remains underexplored, particularly in isolates from unique or extreme ecological niches [17].

Among extreme environments, volcanic soils represent geologically unique habitats that harbor microbial communities adapted to harsh physicochemical conditions [18-20]. These microorganisms often produce rare or structurally novel secondary metabolites not typically found in conventional isolates, and many have been characterized for their potential biotechnological applications in industry, medicine, and agriculture [21]. In previous investigations, two promising *Bacillus* isolates, B.PNR1 and B.PNR2, were obtained from soil

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collected near an extinct volcano in northeastern Thailand. Both isolates demonstrated strong antifungal activities against *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), the causative agent of Fusarium wilt in tomato, by significantly reducing mycelial growth and conidial germination. Notably, they also exhibited broad-spectrum antagonistic activity against other phytopathogens, including *F. oxysporum* f. sp. *cubense*, *Sclerotium rolfsii*, *Colletotrichum musae*, and *Colletotrichum gloeosporioides* [5]. Microscopy studies confirmed that the culture filtrates of these isolates induced ultrastructural damage to fungal hyphae, suggesting the presence of potent antifungal metabolites. In addition to their antifungal activity, both B.PNR1 and B.PNR2 displayed plant growth-promoting (PGP) traits, such as the production of indole-3-acetic acid (IAA) [5,22], phosphate solubilization, and hydrolytic enzymes like amylase and cellulase. Interestingly, B.PNR2 produced significantly higher levels of IAA compared to B.PNR1, indicating its potential role in enhancing root development and nutrient uptake [5]. Furthermore, B.PNR2 showed markedly greater upregulation of genes encoding aldehyde dehydrogenase and glucose 1-dehydrogenase compared to B.PNR1 when exposed to 200 µg/L of atrazine in the culture medium [23]. While *Bacillus stercoris* strain B.PNR1 has been fully characterized through whole-genome sequencing and identified as a robust source of antimicrobial and PGP-related gene clusters, strain B.PNR2, despite its similarly promising bioactivity, remains genomically uncharacterized. A deeper understanding of B.PNR2's biosynthetic potential and mechanisms of action is warranted to evaluate its full biotechnological applicability in sustainable agriculture.

Therefore, this study aims to characterize the unique genetic elements of *Bacillus* sp. B.PNR2, positioning it as a potentially valuable microbial resource for antimicrobial development and agricultural biotechnology. Through whole-genome sequencing, comparative phylogenomics, and genome mining, we investigate its specialized biosynthetic pathways and resistance traits. This work expands our understanding of the functional diversity within environmental *Bacillus* sp. and contributes to the discovery of novel bioactive metabolites for sustainable applications.

2. MATERIALS AND METHODS

2.1. Isolation and Cultivation of *Bacillus* sp. B.PNR2

Soil samples were collected from loamy soil surrounding the extinct volcano in Buriram Province, Northeastern Thailand [5]. The isolation procedure was performed with the modified method described by Boottanun *et al.* [24]. Briefly, soil from the top 10 cm was air-dried at ambient temperature (25–28°C) for 2–3 days. To isolate thermotolerant spore-forming *Bacillus* sp., 1 g of each soil sample was suspended in 99 mL of sterile distilled water and heat-treated at 100°C for 5 min to selectively enrich for thermotolerant, spore-forming *Bacillus* species while eliminating vegetative cells of non-spore-forming bacteria. After cooling, serial 10-fold dilutions were plated on nutrient agar (NA; HiMedia) and incubated at 37°C for 24–48 h. Colonies with characteristic *Bacillus*-like morphology (opaque, flat or slightly raised with irregular edges) were subcultured and stored on NA slants at 4°C.

2.2. Genomic DNA Extraction and Quality Assessment

Genomic DNA was extracted from freshly cultured B.PNR2 grown in nutrient broth using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific) following the manufacturer's protocol for Gram-positive bacteria. DNA quality and concentration were determined using a NanoDrop spectrophotometer and 1% agarose gel electrophoresis. DNA integrity was confirmed by the presence of high-molecular-weight bands without shearing.

2.3. Whole-Genome Sequencing and Phylogenetic Analysis

High-quality genomic DNA was submitted to Macrogen Inc. (Seoul, Korea) for paired-end sequencing using the Illumina platform (2 × 150 bp). Raw reads were quality-filtered and trimmed with Trimmomatic, and the resulting high-quality reads were de novo assembled using SPAdes Genome Assembler v3.15 [25]. The phylogenetic position of *Bacillus* sp. B.PNR2 was determined using the Type (Strain) Genome Server (TYGS) [26] and the Genome BLAST Distance Phylogeny (GBDP) approach [27,28]. Phylogenetic trees were constructed based on intergenomic distances and inferred using the balanced minimum evolution method implemented in FASTME 2.1.6.1 with subtree pruning and regrafting post-processing [29]. Branch support values were estimated from 100 pseudo-bootstrap replicates. Trees were midpoint-rooted [30] and visualized using PhyD3 [31]. Strain identification was further supported by digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) analyses [32]. Closely related species, including *B. stercoris*, *B. subtilis*, *B. siamensis*, and *B. mojavensis*, were included for comparative analysis.

2.4. Genome Annotation and Subsystem Classification

The assembled genome was submitted to the Pathosystems Resource Integration Center (PATRIC) for comprehensive annotation using the RASTtk pipeline [33]. Functional categorization was performed under the "Subsystems" classification, and annotations were cross-referenced with KEGG [34], gene ontology (GO) [35], and enzyme commission (EC) databases [36,37]. Antibiotic resistance genes were detected using comprehensive antibiotic resistance database (*CARD*), *NDARO*, and *PATRIC* antimicrobial resistance (*AMR*) gene databases integrated within the PATRIC toolkit [38].

2.5. Identification of BGCs

Secondary metabolite BGCs were predicted using antiSMASH v8.0.1 [39] (https://antismash.secondarymetabolites.org) with relaxed strictness parameters. The assembled genome was submitted for BGC analysis and has been deposited in GenBank under the accession number JAQMFL000000000. Known and putative clusters were annotated based on sequence similarity to the MIBiG reference database, and only high-confidence matches (≥70% similarity) are reported in this study.

2.6. Identification of AMR and Virulence Genes

AMR determinants were identified using the k-mer-based detection tools in PATRIC and cross-referenced with the CARD [40] and ResFinder [41]. Detected resistance mechanisms included antibiotic inactivation enzymes, target protection proteins, and efflux pumps. Virulence factors were identified through searches against the VFDB, Victors, and PATRIC_VF databases [42].

2.7. *PGP* Gene Identification

Genes associated with plant growth promotion were identified from the annotated genome using the RAST server [33] and cross-referenced with KEGG [34], UniProt [43], and literature-based gene function databases. The analysis targeted genes involved in IAA biosynthesis, phosphate solubilization, siderophore production, and nitrogen metabolism. Feature IDs were linked to their corresponding locus tags in the genome

assembly for consistency across results and tables.

A schematic workflow summarizing the genomic analysis of strain B.PNR2 is presented in Figure 1. Functional assays of disease resistance and plant growth promotion were reported in our previous studies [5,22,23]. The current workflow highlights genome annotation as the central step, branching into analyses of BGCs, *AMR* genes, and *PGP* genes.

3. RESULTS

3.1. General Features of the *Bacillus* sp. B.PNR2 Genome

Whole-genome sequencing of *Bacillus* sp. B.PNR2 yielded nine contigs, with a total genome length of 4,112,807 base pairs and a GC content of 43.83%, as visualized in the circular genome map [Figure 2a]. The assembly exhibited an N50 value of 1,057,280 bp, indicating a high-quality draft genome. Genome annotation using RASTtk through the PATRIC platform identified 4,283 protein-coding sequences (CDSs), along with *60 tRNA* genes and five rRNA operons (5S, 16S, and 23S). Functional annotation revealed that 3,580 proteins (83.6%) had assigned functions, including 1,054 proteins with EC numbers, 875 with GO terms, and 769 mapped to KEGG pathways. The remaining 703 proteins (16.4%) were categorized as hypothetical. The most abundant subsystem categories were metabolism (770 genes), stress response and virulence (125 genes), and energy production (213 genes) [Figure 2b].

3.2. Phylogenetic Analysis and Taxonomic Placement

The phylogenetic tree generated using the GBDP approach on the TYGS platform reveals the evolutionary placement of *Bacillus* sp. B.PNR2 among closely related *Bacillus* species. Phylogenomic analysis clustered B.PNR2 closely with *B. stercoris* and *B. subtilis*, with a bootstrap support value of 62 [Figure 3]. dDDH and ANI values exceeded the species delineation thresholds for both strains. Specifically, ANIb, ANIm, and dDDH values were 98.53%, 98.69%, and 95.4%, respectively, when compared to *B. stercoris*, and 95.11%, 95.39%, and 89.2% when compared to *B. subtilis* [Table 1]. These results support the identification of the isolate as *B. stercoris* strain B.PNR2.

3.3. Secondary Metabolite BGCs

An antiSMASH analysis identified a total of 13 BGCs in the genome of *Bacillus* sp. B.PNR2 [Table 2]. Several BGCs showed high similarity to well-characterized secondary metabolite pathways, including fengycin (nonribosomal peptide synthetase; NRPS), bacillaene (hybrid NRPS-trans-AT PKS cluster involved in polyketide biosynthesis), bacilysin (peptide antibiotic), subtilosin A, bacillibactin (catecholate-type siderophore), and surfactin (lipopeptide). In contrast, some clusters exhibited low similarity to known pathways, such as a zwittermicin A-like NRPS–PKS hybrid cluster, plipastatin (NRPS Type I), and 1-carbapen-2-em-3-carboxylic acid (NRPS Type I). These findings highlight the extensive and diverse biosynthetic potential of strain B.PNR2.

3.4. AMR, Stress Regulators and Transporter Genes

A total of 41 *AMR-related* genes were identified using the CARD, NDARO, and PATRIC AMR databases [Table 3]. These included multiple categories including antibiotic inactivation enzymes (*FosB, ANT(6)-I* and *Vgb(A)*), antibiotic target protection and replacement proteins (*fabL* and *BcrC*), efflux transporters (*BceA/B, EbrA/B, Lmr(B),* and *YkkCD*), cell wall-targeting resistance (*MprF, PgsA,* and *GdpD*), and regulator modulating expression of antibiotic resistance genes (*BceRS, LiaRS,* and *LiaF*). In addition, a total of 322 transporter genes were identified via the transporter classification database (TCDB) annotations [Table 4].

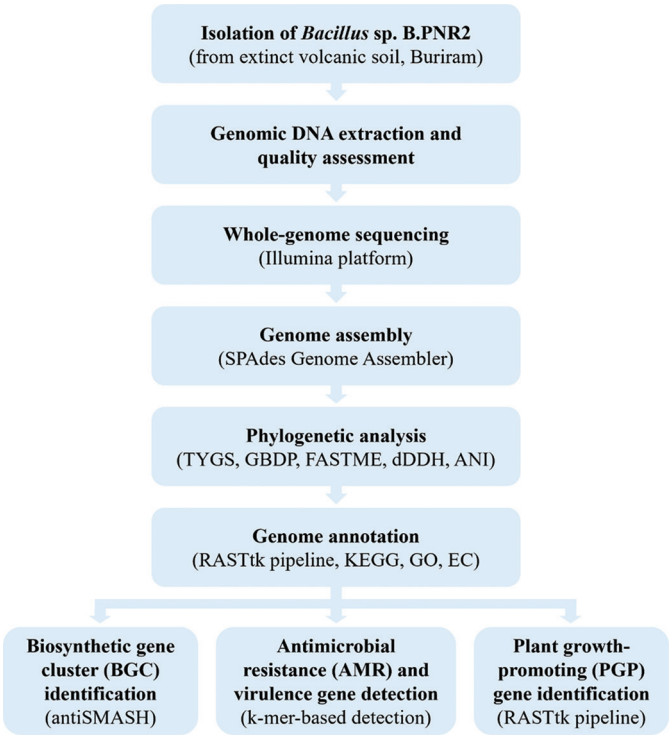

Figure 1: Workflow for genome analysis of *Bacillus* sp. B.PNR2, showing isolation, DNA extraction, Illumina sequencing, SPAdes assembly, phylogenetic analysis, and RASTtk-based annotation, followed by parallel branches for BGC detection (antiSMASH), AMR/virulence gene detection, and *PGP* gene identification.

Table 1: Comparative genomic metrics between *Bacillus* sp. B.PNR2 and closely related *Bacillus* species based on average nucleotide identity using BLAST (ANIb), average nucleotide identity using MUMmer (ANIm), digital DNA-DNA hybridization (dDDH), and genomic G+C content.

Bacterial species	ANIb (%)	ANIm (%)	dDDH (%)	GC (%)
B. stercoris	98.53	98.69	95.4	43.80
B. subtilis	95.11	95.39	89.2	43.51
B. vallismortis	90.16	90.89	65.9	43.76
B. mojavensis	86.58	87.42	79.1	43.72
B. atrophaeus	78.97	83.87	46.5	43.22
B. amyloliquefaciens	76.06	84.12	31.7	46.14
B. siamensis	76.06	84.36	32.4	45.82

3.5. *PGP* Genes

Genome analysis of *B. stercoris* B.PNR2 revealed multiple genes related to plant growth promotion, including those involved in IAA biosynthesis (e.g., tryptophan synthase, indole-3-pyruvate decarboxylase), phosphate solubilization (e.g., alkaline phosphatase, phosphatases), siderophore biosynthesis (e.g., bacillibactin, enterobactin), and nitrogen metabolism (e.g., glutamine synthetase, nitrate reductase). These genes were identified based on functional

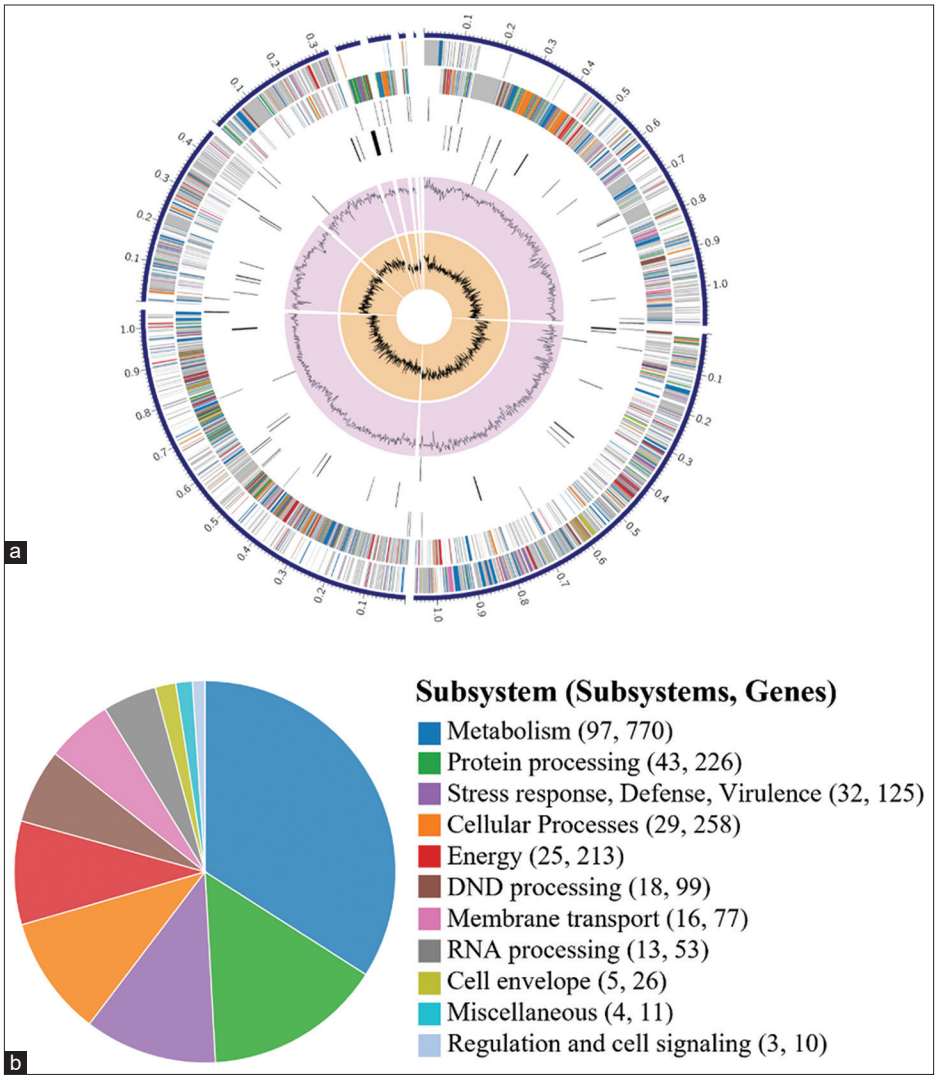

Figure 2: Genomic features and functional annotation of *Bacillus* sp. B.PNR2. (a) Circular genome map of *Bacillus* sp. B.PNR2. The genome comprises nine contigs totaling 4,112,807 bp, with a GC content of 43.83%. From the outermost to the innermost rings, the map displays: Contigs, CDSs on the forward strand, CDSs on the reverse strand, RNA genes, CDSs homologous to known antimicrobial resistance genes, CDSs homologous to known virulence factors, GC content, and GC skew. CDSs are color-coded based on their associated functional subsystems, as defined in panel B. Genome annotation was conducted using the RASTtk pipeline on the PATRIC platform. (b) Functional categorization of annotated genes in the genome of *Bacillus* sp. B.PNR2. Gene annotation was performed using the RASTtk pipeline on the PATRIC platform. The pie chart shows the distribution of genes across major functional subsystems, including metabolism, protein processing, stress response, cellular processes, and others.

Table 2: Identified biosynthetic gene clusters (BGCs) in *Bacillus* sp. B.PNR2 based on antiSMASH analysis.

Type	Contig No.	Start/from	To/Stop	Most similar known cluster	Similarity	Function	References
NRPS, betalactone	1	1	55,559	fengycin	High	Antifungal	[58]
transAT-PKS, NRPS, T3PKS, PKS-like	1	121,210	235,910	bacillaene	High	Antibacterial	[59]
Sactipeptide	2	379,707	401,318	subtilosin A	High	Antibacterial	[60]
NRP-metalophore, NRPS, terpene-precusor	2	922,310	988,536	bacillibactin	High	Antibacterial	[61]
NRPS	3	1	21,633	plipastatin	Low	Antifungal	[54]
T3PKS	3	159,654	200,751	1-carbapen-2-em-3-carboxylic acid	Low	Antibacterial	[62]
NRPS	4	178,063	243,454	surfactin	High	Antibacterial/Antifungal/ Biosurfactant	[63,64]
NRPS, T1PKS	5	58,518	139,870	zwittermicin A	High	Antibacterial/Antifungal	[52]

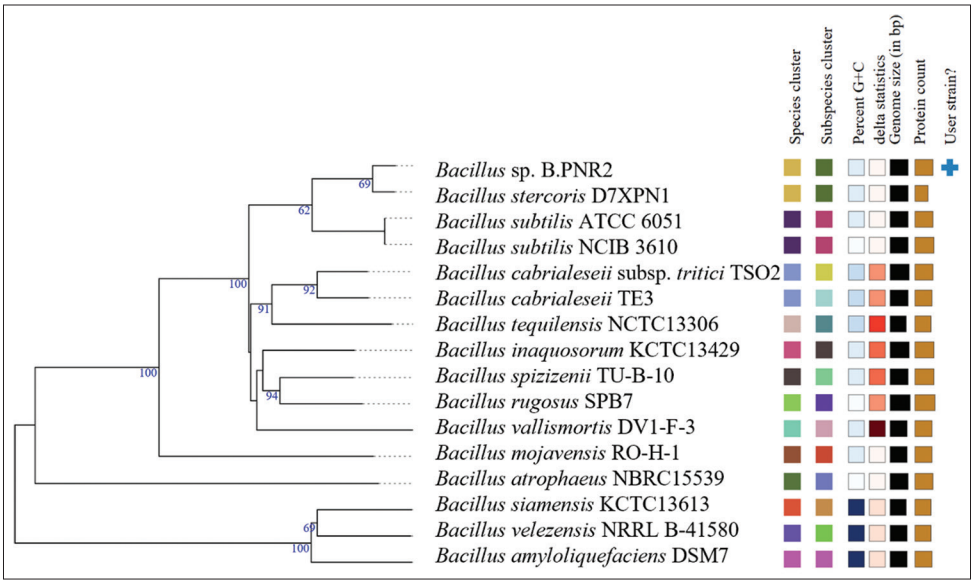

Figure 3: Phylogenomic tree depicting the evolutionary relationship of *Bacillus* sp. B.PNR2 with closely related *Bacillus* species based on genome-wide comparison using the Genome BLAST Distance Phylogeny approach implemented in the Type (Strain) Genome Server. The tree was inferred using the balanced minimum evolution method via FASTME 2.1.6.1 with subtree pruning and regrafting post-processing. Branch support values were calculated from 100 pseudo-bootstrap replicates and are shown at the nodes. The tree was midpoint-rooted for clarity.

Table 3: The specific antimicrobial resistance (AMR) genes detected in *Bacillus* sp. B.PNR2 and categorizes them by their resistance mechanisms, such as enzymatic inactivation, target modification, efflux pumps, and regulatory systems. It includes notable resistance determinants for multiple antibiotic classes.

Categorizes	Specific antimicrobial resistance (*AMR*) genes
Antibiotic inactivation enzyme	*ANT (6)-I, FosB, Vgb (A)*
Antibiotic target in susceptible species	*Alr, Ddl, dxr, EF-G, EFTu, folA, Dfr, folP, gyrA, gurB, inhA, fabI, Iso-tRNA, kasA, MurA, rho, rhoB, rpoC, S10p, S12p*
Antibiotic target modifying enzyme	*RlmA (II)*
Antibiotic target protection protein	*BcrC*
Antibiotic target replacement protein	*fabL*
Efflux pump conferring antibiotic resistance	*BceA, BceB, EbrA, EbrB, Lmr (B), YkkCD*
Gene conferring resistance via absence	*gidB*
Protein altering cell wall charge conferring antibiotic resistance	*GdpD, MprF, PgsA*
Regulator modulating expression of antibiotic resistance genes	*BceR, BceS, LiaF, LiaR, LiaS*

Table 4: The number of genes in *Bacillus* sp. B.PNR2 genome that shows homology to known antibiotic resistance genes, drug targets, transporters, and virulence factors, as identified from multiple specialized databases.

Type	Source	Number of gene
Antibiotic resistance	CARD	10
Antibiotic resistance	NCARD	2
Antibiotic resistance	PATRIC	45
Drug target	DrugBank	72
Drug target	TTD	1
Trnasporter	TCDB	322
Virulence factor	PATRIC_VF	2
Virulence factor	VFDB	1
Virulence factor	Victors	3

CARD: Comprehensive antibiotic resistance database, PATRIC: Pathosystems resource integration center, TCDB: Transporter classification database.

annotations and confirmed by KEGG and UniProt matches. A summary of *PGP-related* genes, their putative functions, and corresponding locus tags is presented in Table 5.

4. DISCUSSION

The comprehensive genomic and functional characterization of *Bacillus* sp. B.PNR2 underscores its exceptional potential as a biocontrol agent and a valuable bioresource for natural product discovery. Isolated from nutrient-poor, geothermally influenced volcanic soil in Northeastern Thailand, B.PNR2 appears well adapted to natural soil environments, as evidenced by its extensive genetic repertoire related to stress tolerance, secondary metabolite biosynthesis, and nutrient acquisition [17]. These adaptive features highlight its ecological competitiveness and suitability for application in challenging agricultural systems [44,45]. In this study, phylogenomic analysis identified B.PNR2 as a strain of *B. stercoris*, supported by high dDDH and ANI values [27,46,47]. The distinct composition of its *BGCs* and *AMR* genes further supports this classification and enhances its value as a genomic resource.

In this study, the genome annotation revealed a complex metabolic framework comprising 4,283 protein-CDSs, the majority of which were functionally characterized. Subsystem classification indicated a high abundance of genes related to metabolism, stress response, energy production, and virulence. This genetic architecture implies not only resilience in harsh soil environments but also a capacity for dynamic interactions both antagonistic and symbiotic with other soil microorganisms. A notable feature of the B.PNR2 genome

Table 5: Plant growth-promoting (PGP) genes identified in *Bacillus stercoris* B.PNR2.

PGP trait	Gene (s)/protein	Locus tag (s) (fig|3020846.6.peg.X)	Function
IAA biosynthesis	*trpA*, *trpB*, *trpE*, *trpG*	2496, 2497, 2501, 4215	Tryptophan biosynthesis enzymes; precursors for IAA production
Phosphate solubilization/ transport	*phoP*	3189	Pho regulon transcriptional regulator
	Alkaline phosphatase D (isoforms)	1050, 3819, 3923, 3492	Hydrolyzes organic phosphate esters
	3-phytase	2396	Degrades phytate to release inorganic phosphate
	pstS, *pstC*, *pstA*, *pstB*	2742–2745	Phosphate ABC transport system
	pstB (family protein)	2746	Phosphate transport ATP-binding protein
	phnP	966	Phosphonate metabolism protein
Siderophore biosynthesis	*dhbA*, *dhbC*, *dhbE*, *dhbB*, *dhbF*, *mbtH*	2115–2120	Bacillibactin biosynthesis enzymes
Siderophore uptake	*feuA*, *feuB*, *feuC*	3394–3396	Fe≥$^{+}$-siderophore transport system
	ABC-type Fe≥$^{+}$-siderophore transporters	4099–4101	Uptake of ferric-siderophore complexes
Nitrogen metabolism	*glnA*	111	Glutamine synthetase type I
	narG, *narH*, *narI*, *narJ*	1551–1554	Respiratory nitrate reductase complex
	nirB, *nirD*	3562–3563	Nitrite reductase (NADH)
	nasA, *nasB*	3564–3565	Assimilatory nitrate reductase
	nasA (nitrate transporter)	3566	Nitrate uptake

Locus tags are reported in the format fig|3020846.6.peg.X, where X is the identifier shown in the table. IAA: Indole-3-acetic acid.

is the presence of 13 BGCs identified by antiSMASH, including clusters encoding well-characterized antimicrobial compounds such as fengycin, surfactin, bacilysin, bacillaene, subtilosin A, and bacillibactin. These metabolites form a multifunctional arsenal that acts through membrane disruption, iron chelation, enzyme inhibition, and immune modulation. The co-occurrence of fengycin and surfactin clusters is particularly significant, given their synergistic antifungal activities – a hallmark of effective *Bacillus*-mediated biocontrol. Our findings align with reports that *Bacillus* species commonly combine antimicrobial metabolite production with PGP traits. Prior studies have highlighted lineage-specific adaptations and metabolite profiles supporting biocontrol activity [17,48], which is consistent with the *BGC repertoire* and *PGP* genes observed in B.PNR2.

Intriguingly, genome mining revealed several BGCs with low homology to known reference clusters, including those potentially associated with zwittermicin A-like compounds, plipastatin, and carbapenem-like metabolites. Zwittermicin A (ZmA), a linear aminopolyol antibiotic originally isolated from *B. cereus* UW85, has demonstrated broad-spectrum antimicrobial activity, including antiprotist, antibacterial (against both Gram-positive and Gram-negative bacteria), and antifungal properties [49-52]. ZmA also synergistically enhances the insecticidal activity of *Bacillus thuringiensis* toxins [53]. Likewise, plipastatin, a lipopeptide produced by *B. subtilis*, is recognized for its potent antifungal activity and holds promise as a biocontrol agent to replace synthetic fungicides in agricultural applications [54]. Carbapenems, a class of β-lactam antibiotics, are considered critically important due to their broad-spectrum efficacy and potency against both Gram-positive and Gram-negative bacteria [55]. The presence of cryptic BGCs with low similarity to known clusters suggests the potential for structurally novel compounds with unique modes of action, especially a promising avenue in the face of rising AMR. Further functional characterization of these clusters will be essential to unlocking new microbial natural products.

In terms of environmental adaptability, *Bacillus* sp. B.PNR2 possesses a diverse repertoire of *AMR* genes and transporter systems. A total of *41 AMR* genes were identified, representing a range of resistance mechanisms, including antibiotic-inactivating enzymes, target site modifications, protective proteins, and multidrug efflux systems, features that likely confer a competitive advantage in microbially rich soil environments. In addition, the presence of 322 transporter genes suggests enhanced capabilities for nutrient acquisition and detoxification, contributing to the strain's fitness under dynamic environmental conditions. Nevertheless, the presence of 41 *AMR* genes and several virulence-related factors warrants careful biosafety consideration before any field application. These elements may influence microbial community interactions and horizontal gene transfer in soil ecosystems; therefore, risk assessment and appropriate containment strategies will be essential in future agricultural deployment of B.PNR2. This finding aligns with the study by Deng *et al.* [56], who employed multi-omics analyses to investigate *Bacillus* mutant strains under environmental stress. Their results revealed coordinated changes at the genomic, transcriptomic, and proteomic levels. Despite harboring different genetic mutations, the mutants exhibited similar proteomic responses. Key metabolic pathways including the Embden-Meyerhof-Parnas glycolytic pathway, the pentose phosphate pathway, and purine biosynthesis, were significantly modulated to regulate inosine production. In addition, stress-responsive proteins involved in translation, molecular chaperoning, DNA repair, oxidative stress defense, and cell envelope stability were upregulated, enhancing the mutants' survival under extreme conditions such as those found in near-space environments. Similarly, Valencia-Marín *et al.* [57] reported that *Bacillus* species can survive in saline-stressed soils through multiple mechanisms, including the production of osmoprotectant compounds, antioxidant enzymes, exopolysaccharides, and alterations in membrane lipid composition. Additional survival strategies such as sporulation and entry into a reduced metabolic state were also noted, particularly in the context of functional interactions within the rhizosphere.

Beyond antimicrobial potential, *Bacillus* sp. B.PNR2 also harbors PGP traits, including IAA biosynthesis, phosphate solubilization, siderophore production, and nitrogen metabolism genes, consistent with phenotypes reported previously [5,22]. Taken together with the diversity of its BGCs, these features support a dual potential in crop growth promotion and pathogen suppression.

The genomic insights obtained in this study have direct implications for biotechnological applications. In agriculture, *B. stercoris* B.PNR2 could be developed into biofertilizer formulations, leveraging its IAA biosynthesis, phosphate solubilization, siderophore production, and nitrogen metabolism genes to enhance crop growth and nutrient use efficiency. As a biocontrol agent, the strain possesses diverse antimicrobial BGCs, including those encoding fengycin, surfactin, bacilysin, and bacillibactin, which provide broad-spectrum suppression of phytopathogens and can reduce dependence on chemical pesticides. In environmental biotechnology, its stress tolerance and *AMR* gene repertoire suggest resilience in contaminated or degraded soils, supporting potential use in soil remediation or reclamation programs. Furthermore, the presence of cryptic BGCs with low similarity to known clusters represents a valuable genomic resource that may yield structurally novel bioactive compounds. However, functional validation will be necessary before specific pharmaceutical applications can be established.

Taken together, these findings suggest that *Bacillus* sp. B.PNR2 holds promise as a candidate for integrated pest and nutrient management in sustainable agriculture. The genomic prediction of low-similarity BGCs also highlights its potential as a source for future antimicrobial discovery, pending experimental confirmation. Future research should prioritize functional validation of cryptic BGCs through transcriptomics, heterologous expression, and metabolite isolation. At the same time, greenhouse and field trials will be essential to confirm the biocontrol and PGP efficacy of B.PNR2 under real-world agricultural conditions. These combined efforts will advance microbial-based biotechnologies and contribute meaningfully to global initiatives in sustainable agriculture and antibiotic innovation.

5. CONCLUSION

This study provides a genomic framework for *Bacillus* sp. B.PNR2, revealing a repertoire of *BGCs* and *PGP* genes consistent with potential applications in sustainable agriculture. Phylogenomic metrics (ANI and dDDH) clearly place the isolate within *B. stercoris*. While cryptic and low-similarity BGCs indicate the possibility of novel bioactive compounds, these predictions require experimental validation through transcriptomics, metabolite purification, and activity profiling. In parallel, the detection of *AMR* genes and virulence-related factors underscores the need for biosafety assessment before field use. Overall, the genome of B.PNR2 highlights promising avenues for crop growth promotion and biocontrol, pending further functional confirmation.

6. ACKNOWLEDGMENTS

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7. AUTHORS' CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

The whole-genome assembly of *Bacillus* sp. B.PNR2 has been deposited in GenBank under accession number JAQMFL000000000.

12. PUBLISHER'S NOTE

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13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Damalas CA, Koutroubas SD. Current status and recent developments in biopesticide use. Agriculture. 2018;8(1):13. https://doi.org/10.3390/agriculture8010013
2. Thomine E, Mumford J, Rusch A, Desneux N. Using crop diversity to lower pesticide use: Socio-ecological approaches. Sci Total Environ. 2022;804:150156. https://doi.org/10.1016/j.scitotenv.2021.150156
3. Chaudhary R, Nawaz A, Khattak Z, Butt MA, Fouillaud M, Dufossé L, *et al.* Microbial bio-control agents: A comprehensive analysis on sustainable pest management in agriculture. J Agric Food Res. 2024;18:101421. https://doi.org/10.1016/j.jafr.2024.101421
4. Tyagi A, Lama Tamang T, Kashtoh H, Mir RA, Mir ZA, Manzoor S, *et al.* A review on biocontrol agents as sustainable approach for crop disease management: Applications, production, and future perspectives. Horticulturae. 2024;10(8):805. https://doi.org/10.3390/horticulturae10080805
5. Pengproh R, Thanyasiriwat T, Sangdee K, Saengprajak J, Kawicha P, Sangdee A. Evaluation and genome mining of *Bacillus stercoris* isolate B. PNR1 as potential agent for fusarium wilt control and growth promotion of tomato. Plant Pathol J. 2023;39(5):430. https://doi.org/10.5423/PPJ.OA.01.2023.0018
6. Rios-Reyes A, Gonzalez-Lozano K, Cabral-Miramontes J, Hernandez-Gonzalez J, Rios-Sosa A, Alvarez-Gutierrez P, *et al*. Exploration of plant and microbial life at "El Chichonal" volcano with

a sustainable agriculture prospection. Microb Ecol. 2025;88(1):67. https://doi.org/10.1007/s00248-025-02567-4
7. Miljaković D, Marinković J, Balešević-Tubić S. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms. 2020;8(7):1037. https://doi.org/10.3390/microorganisms8071037
8. Borriss R. Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents in agriculture. In: Maheshwari DK, editor. Bacteria in Agrobiology: Plant Growth Responses. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 41-76. https://doi.org/10.1007/978-3-642-20332-9_3
9. Beneduzi A, Ambrosini A, Passaglia LM. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet Mol Biol. 2012;35(4 (suppl)):1044-51. https://doi.org/10.1590/S1415-47572012000600020
10. Fatima A, Abbas M, Nawaz S, Rehman Y, ur Rehman S, Sajid I. Whole genome sequencing (WGS) and genome mining of *Streptomyces* sp. AFD10 for antibiotics and bioactive secondary metabolites biosynthetic gene clusters (BGCs). Gene Rep. 2024;37:102050. https://doi.org/10.1016/j.genrep.2024.102050
11. Palazzotto E, Weber T. Omics and multi-omics approaches to study the biosynthesis of secondary metabolites in microorganisms. Curr Opin Microbiol. 2018;45:109-16. https://doi.org/10.1016/j.mib.2018.03.004
12. Zhang Z, Yin L, Li X, Zhang C, Zou H, Liu C, *et al*. Analyses of the complete genome sequence of the strain *Bacillus pumilus* ZB201701 isolated from rhizosphere soil of maize under drought and salt stress. Microbes Environ. 2019;34(3):310-5. https://doi.org/10.1264/jsme2.ME18096
13. Li Z, Song C, Yi Y, Kuipers OP. Characterization of plant growth-promoting rhizobacteria from perennial ryegrass and genome mining of novel antimicrobial gene clusters. BMC Genomics. 2020;21(1):157. https://doi.org/10.1186/s12864-020-6563-7
14. Iqbal S, Vollmers J, Janjua HA. Genome mining and comparative genome analysis revealed niche-specific genome expansion in antibacterial *Bacillus pumilus* strain SF-4. Genes. 2021;12(7):1060. https://doi.org/10.3390/genes12071060
15. Ribeiro ID, Bach E, da Silva Moreira F, Müller AR, Rangel CP, Wilhelm CM, *et al*. Antifungal potential against *Sclerotinia sclerotiorum* (Lib.) de Bary and plant growth promoting abilities of *Bacillus* isolates from canola (*Brassica napus* L.) roots. Microbiol Res. 2021;248:126754. https://doi.org/10.1016/j.micres.2021.126754
16. Biessy A, Filion M. Complete genome sequence of *Bacillus pumilus* LBUM494, a plant-beneficial strain isolated from the rhizosphere of a strawberry plant. Microbiol Resour Announc. 2024;13(10):e0082524. https://doi.org/10.1128/mra.00825-24
17. Dushku E, Kotzamanidis C, Kargas A, Maria-Eleni FL, Giantzi V, Krystallidou E, *et al*. Unveiling the genetic basis of biochemical pathways of plant growth promotion in *Bacillus pumilus* and the first genomic insights into *B. pseudomycoides* as a biostimulant. Curr Res Microb Sci. 2025:100419. https://doi.org/10.1016/j.crmicr.2025.100419
18. Chen J, Xu D, Xiao Q, Zheng Y, Liu H, Li X, *et al*. Responses of soil microbial diversity, network complexity and multifunctionality to environments changes in volcanic ecosystems. J Environ Chem Eng. 2024;12(5):113334. https://doi.org/10.1016/j.jece.2024.113334
19. Fagorzi C, Del Duca S, Venturi S, Chiellini C, Bacci G, Fani R, *et al*. Bacterial communities from extreme environments: Vulcano Island. Diversity. 2019;11(8):140. https://doi.org/10.3390/d11080140
20. Li SJ, Hua ZS, Huang LN, Li J, Shi SH, Chen LX, *et al*. Microbial communities evolve faster in extreme environments. Sci Rep. 2014;4(1):6205. https://doi.org/10.1038/srep06205
21. Verma P, Yadav AN, Kumar V, Singh DP, Saxena AK. Beneficial plant-microbes interactions: biodiversity of microbes from diverse extreme environments and its impact for crop improvement. In: Plant-Microbe Interactions in Agro-Ecological Perspectives: Microbial Interactions and Agro-Ecological Impacts. Vol. 2. Springer; 2017. p. 543-80. https://doi.org/10.1007/978-981-10-6593-4_22
22. Somtrakoon K, Prasertsom P, Sangdee A, Pengproh R, Chouychai W. Potential of *Bacillus stercoris* B. PNR2 to stimulate growth of rice and waxy corn under atrazine-contaminated soil. J Arid Agric. 2024;10:20-7. https://doi.org/10.25081/jaa.2024.v10.8614
23. Sangdee A, Plaikan S, Chayapat T, Kawicha P, Somtrakoon K. Plant growth-promoting gene expression in *Bacillus stercoris* under atrazine contamination and their ability to stimulate growth of mung bean seedlings. N Z J Crop Hortic Sci. 2025;53:2165-87. https://doi.org/10.1080/01140671.2025.2455048
24. Boottanun P, Potisap C, Hurdle JG, Sermswan RW. Secondary metabolites from *Bacillus amyloliquefaciens* isolated from soil can kill *Burkholderia pseudomallei*. AMB Express. 2017;7(1):16. https://doi.org/10.1186/s13568-016-0302-0
25. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455-77. https://doi.org/10.1089/cmb.2012.0021
26. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun. 2019;10(1):2182. https://doi.org/10.1038/s41467-019-10210-3
27. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics. 2013;14(1):60. https://doi.org/10.1186/1471-2105-14-60
28. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: A database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res. 2022;50(D1):D801-7. https://doi.org/10.1093/nar/gkab902
29. Lefort V, Desper R, Gascuel O. FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol. 2015;32(10):2798-800. https://doi.org/10.1093/molbev/msv150
30. Farris JS. Estimating phylogenetic trees from distance matrices. Am Nat. 1972;106(951):645-68.
31. Kreft Ł, Botzki A, Coppens F, Vandepoele K, Van Bel M. PhyD3: A phylogenetic tree viewer with extended phyloXML support for functional genomics data visualization. Bioinformatics. 2017;33(18):2946-7. https://doi.org/10.1093/bioinformatics/btx324
32. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics. 2016;32(6):929-31. https://doi.org/10.1093/bioinformatics/btv681
33. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, *et al.* RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep. 2015;5(1):8365. https://doi.org/10.1038/srep08365
34. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27-30. https://doi.org/10.1093/nar/28.1.27
35. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, *et al*. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000;25(1):25-9. https://doi.org/10.1038/75556
36. Committee of the International Union of Biochemistry 1978: Recommendations of the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes. United States: Academic Press; 1979.
37. Schomburg I, Chang A, Ebeling C, Gremse M, Heldt C, Huhn G, *et al*. BRENDA, the enzyme database: Updates and major new

developments. Nucleic Acids Res. 2004;32(suppl_1):D431-3. https://doi.org/10.1093/nar/gkh081
38. Antonopoulos DA, Assaf R, Aziz RK, Brettin T, Bun C, Conrad N, *et al*. PATRIC as a unique resource for studying antimicrobial resistance. Brief Bioinform. 2019;20(4):1094-102. https://doi.org/10.1093/bib/bbx083
39. Blin K, Shaw S, Vader L, Szenei J, Reitz ZL, Augustijn HE, *et al.* antiSMASH 8.0: extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. Nucleic Acids Res. 2025;53:gkaf334. https://doi.org/10.1093/nar/gkaf334
40. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, *et al*. CARD 2023: Expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. Nucleic Acids Res. 2023;51(D1):D690-9. https://doi.org/10.1093/nar/gkac920
41. Florensa AF, Kaas RS, Clausen P, Aytan-Aktug D, Aarestrup FM. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microb Genom. 2022;8(1):000748. https://doi.org/10.1099/mgen.0.000748
42. Mao C, Abraham D, Wattam AR, Wilson MJ, Shukla M, Yoo HS, *et al.* Curation, integration and visualization of bacterial virulence factors in PATRIC. Bioinformatics. 2015;31(2):252-8. https://doi.org/10.1093/bioinformatics/btu631
43. The UniProt Consortium. UniProt: The universal protein knowledgebase. Nucleic Acids Res. 2016;45(D1):D158-69. https://doi.org/10.1093/nar/gkw1099
44. Saleem MH, Noreen S, Ishaq I, Saleem A, Khan KA, Ercisli S, *et al*. Omics technologies: Unraveling abiotic stress tolerance mechanisms for sustainable crop improvement. J Plant Growth Regul. 2025;44:4165-87. https://doi.org/10.1007/s00344-025-11674-y
45. Taheri P, Puopolo G, Santoyo G. Plant growth-promoting microorganisms: New insights and the way forward. Microbiol Res. 2025;297:128168. https://doi.org/10.1016/j.micres.2025.128168
46. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol. 2018;68(1):461-6. https://doi.org/10.1099/ijsem.0.002516
47. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci. 2009;106(45):19126-31. https://doi.org/10.1073/pnas.0906412106
48. Xiang L, Zhou Z, Wang X, Jiang G, Cheng J, Hu Y, *et al*. Surfactin, bacillibactin and bacilysin are the main antibacterial substances of *Bacillus subtilis* JSHY-K3 that inhibited the growth of VpAHPND (the main pathogen of acute hepatopancreatic necrosis disease in shrimp). Aquac Rep. 2025;42:102745. https://doi.org/10.1016/j.aqrep.2025.102745
49. Silo-Suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, Handelsman J. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Appl Environ Microbiol. 1994;60(6):2023-30. https://doi.org/10.1128/aem.60.6.2023-2030.1994
50. Silo-Suh LA, Stabb EV, Raffel SJ, Handelsman J. Target range of zwittermicin A, an aminopolyol antibiotic from *Bacillus cereus*. Curr Microbiol. 1998;37(1):6-11. https://doi.org/10.1007/s002849900328
51. Rogers EW, Dalisay DS, Molinski TF. (+)-Zwittermicin A: assignment of its complete configuration by total synthesis of the enantiomer and implication of D-serine in its biosynthesis. Angew Chem Int Ed. 2008;47(42):8086-9. https://doi.org/10.1002/anie.200801561
52. Kevany BM, Rasko DA, Thomas MG. Characterization of the complete zwittermicin A biosynthesis gene cluster from *Bacillus cereus*. Appl Environ Microbiol. 2009;75(4):1144-55. https://doi.org/10.1128/AEM.02518-08
53. Broderick NA, Goodman RM, Handelsman J, Raffa KF. Effect of host diet and insect source on synergy of gypsy moth (Lepidoptera: Lymantriidae) Mortality to *Bacillus thuringiensis* subsp. *kurstaki* by Zwittermicin A. Environ Entomol. 2003;32(2):387-91. https://doi.org/10.1603/0046-225X-32.2.387
54. Vahidinasab M, Lilge L, Reinfurt A, Pfannstiel J, Henkel M, Morabbi Heravi K, *et al*. Construction and description of a constitutive plipastatin mono-producing *Bacillus subtilis*. Microb Cell Fact. 2020;19(1):205. https://doi.org/10.1186/s12934-020-01468-0
55. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: Past, present, and future. Antimicrob Agents Chemother. 2011;55(11):4943-60. https://doi.org/10.1128/aac.00296-11
56. Deng A, Wang T, Wang J, Li L, Wang X, Liu L, *et al*. Adaptive mechanisms of *Bacillus* to near space extreme environments. Sci Total Environ. 2023;886:163952. https://doi.org/10.1016/j.scitotenv.2023.163952
57. Valencia-Marin MF, Chávez-Avila S, Guzmán-Guzmán P, del Carmen Orozco-Mosqueda M, de Los Santos-Villalobos S, Glick BR, *et al.* Survival strategies of *Bacillus* spp. in saline soils: Key factors to promote plant growth and health. Biotechnol Adv. 2024;70:108303. https://doi.org/10.1016/j.biotechadv.2023.108303
58. Sur S, Romo TD, Grossfield A. Selectivity and mechanism of fengycin, an antimicrobial lipopeptide, from molecular dynamics. J Phys Chem B. 2018;122(8):2219-26. https://doi.org/10.1021/acs.jpcb.7b11889
59. Patel PS, Huang S, Fisher S, Pirnik D, Aklonis C, Dean L, *et al*. Bacillaene, a novel inhibitor of procaryotic protein synthesis produced by *Bacillus subtilis*: Production, taxonomy, isolation, physico-chemical characterization and biological activity. J Antibiot. 1995;48(9):997-1003. https://doi.org/10.7164/antibiotics.48.997
60. Shelburne CE, An FY, Dholpe V, Ramamoorthy A, Lopatin DE, Lantz MS. The spectrum of antimicrobial activity of the bacteriocin subtilosin A. J Antimicrob Chemother. 2007;59(2):297-300. https://doi.org/10.1093/jac/dkl495
61. Chakraborty K, Kizhakkekalam VK, Joy M, Chakraborty RD. Bacillibactin class of siderophore antibiotics from a marine symbiotic *Bacillus* as promising antibacterial agents. Appl Microbiol Biotechnol. 2022;106(1):329-40. https://doi.org/10.1007/s00253-021-11632-0
62. Tichy E, Luisi B, Salmond G. Crystal structure of the carbapenem intrinsic resistance protein CarG. J Mol Biol. 2014;426(9):1958-70. https://doi.org/10.1016/j.jmb.2014.02.016
63. Das P, Mukherjee S, Sen R. Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*. J Appl Microbiol. 2008;104(6):1675-84. https://doi.org/10.1111/j.1365-2672.2007.03701.x
64. Zhen C, Ge XF, Lu YT, Liu WZ. Chemical structure, properties and potential applications of surfactin, as well as advanced strategies for improving its microbial production. AIMS Microbiol. 2023;9(2):195. https://doi.org/10.3934/microbiol.2023012

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