Isolation, Characterization, and GC–MS Profiling of *Bacillus zhangzhouensis* NB1 with Plant Growth-Promoting and Antibacterial Activity Against *Xanthomonas* sp.

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) have emerged as promising alternatives to chemical inputs for sustainable crop protection. A total of 21 rhizospheric bacterial isolates were systematically evaluated for key plant growth-promoting (PGP) attributes, including indole-3-acetic acid (IAA) production, hydrogen cyanide (HCN) synthesis, phosphate solubilization (PS), siderophore production (SP), ammonia (NH_3) generation, and hydrolytic enzyme activities (amylase, protease, and cellulase). Among these, isolate NB1 exhibited a broad spectrum of PGP traits, demonstrating consistent positive responses across all tested parameters. Quantitatively, NB1 produced 36.4 ± 1.2 µg mL^{-1} of IAA and showed a high phosphate solubilization index of 4.2. Molecular characterization based on partial 16S rRNA gene sequencing indicated that isolate NB1 shared 99.78% sequence similarity with *Bacillus zhangzhouensis*. The antimicrobial efficacy of its extracellular crude extract was confirmed against *Xanthomonas* sp., producing inhibition zones of up to 16 mm. The chemical profiling of extracellular metabolites using gas chromatography–mass spectrometry (GC–MS) revealed presence of bioactive compounds such as N-acetyltyramine, pyrrolo [1,2-a] pyrazine derivatives, and fatty acid esters, which are known for their antimicrobial properties.The application of *B. zhangzhouensis* (NB1) demonstrated enhanced antagonistic activity against phytopathogenic bacteria. This formulation highlight its potential as an effective biocontrol strategy under in vitro conditions.

Keywords: Phosphate solubilisation, *Bacillus zhangzhouensis*; GC –MS profiling, N-acetyltyramine, Pyrrolo [1,2-a] pyrazine, Fatty acid esters, *Xanthomonas* sp

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INTRODUCTION

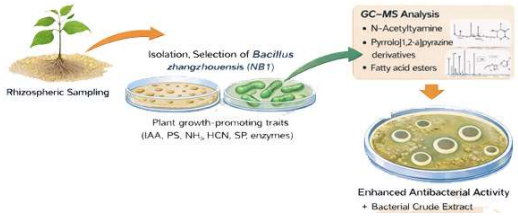
Xanthomonas is a Gram-negative bacterium belonging to the class Gammaproteobacteria, known to infect more than 400 plant species, including economically important crops such as rice, wheat, citrus, tomato, pepper, cabbage, banana, and bean[1].Species of the genus *Xanthomonas* are responsible for numerous economically important bacterial diseases affecting a wide range of agricultural crops, including rice, citrus, tomato, pepper, cabbage, banana, and bean. These pathogens can cause significant reductions in crop yield and quality, leading to substantial economic losses worldwide. These diseases can significantly reduce crop productivity and market value, posing a major challenge to sustainable agriculture worldwide. Consequently, the development of environmentally friendly and effective strategies for the management of *Xanthomonas*-associated diseases has become an important area of research [2].The continued occurrence of *Xanthomonas*-associated diseases, including bacterial leaf blight of rice, bacterial spotf tomato and pepper, black rot of crucifers, and bacterial leaf streak of cereals, in major crop-producing regions highlights the necessity for alternative and environmentally friendly disease management strategies beyond conventional chemical control measures [3].

Current management approaches primarily rely on the application of copper-based bactericides and the removal of infected plant material [4]. The use of induced systemic resistance (ISR) elicitors, in combination with copper formulations such as copper hydroxide and copper oxychloride, has been reported to effectively reduce fruit blemishes and premature fruit drop[5]. However, excessive dependence on copper-based compounds and synthetic agrochemicals has contributed to environmental contamination, the emergence of resistant pathogen strains, and deterioration of soil health, thereby necessitating the development of more sustainable alternatives. PGPR offer an eco-friendly strategy by enhancing plant growth

through multiple mechanisms, including phytohormone production, nutrient solubilization, siderophore (SP) secretion, and the production of hydrolytic enzymes. In addition, PGPR can suppress phytopathogens through direct antagonistic interactions or by inducing systemic resistance in plants[6]. Among these, *Bacillus* sp. has gained particular attention due to its ability to form endospores, metabolic adaptability, and well-documented biocontrol efficacy against a wide range of plant pathogens[7].

Indole-3-acetic acid (IAA) production plays a key role in modulating root system architecture, thereby enhancing nutrient and water uptake efficiency. Phosphate solubilization (PS) increases the availability of phosphorus for plant utilization, while siderophore (SP)-mediated chelation of iron and manganese restricts the accessibility of these essential micronutrients to competing phytopathogens[8]. In addition, hydrolytic enzymes such as cellulases, proteases, and amylases contribute to the degradation of pathogen cell walls, enabling direct antagonistic activity [9].Although *Bacillus zhangzhouensis* has been reported as an environmental *Bacillus* sp. with metabolic versatility and potential applications in biotechnology, studies exploring its role as a PGPR and biocontrol agent remain limited. Recent reports have indicated its occurrence in soil and rhizosphere environments, along with traits associated with enzyme production and antimicrobial potential, suggesting its prospective utility in sustainable agriculture [10].

This study aimed to (i) isolate and evaluate the functional attributes of a potent PGPR strain from the rhizosphere, (ii) identify the isolate based on 16S rRNA gene sequencing, (iv) analyze the bioactive metabolites present in the crude extract through GC–MS, and (v) determine the antibacterial efficacy of the *B. zhangzhouensis* crude extract against *Xanthomonas* sp.



MATERIALS AND METHODS

2.1. Sample collection, isolation, and identification of PGPR

Rhizospheric soil samples were collected from five distinct agricultural fields in Namakkal District, Tamil Nadu, India. A total of five samples were obtained from the root zones (0–15 cm depth) of healthy crop plants using sterile spatulas. The samples were transported to the laboratory in sterile polyethylene bags at 4 °C and processed within 24 h to minimize alterations in the native microbial community . For isolation, 10 g of each soil sample was aseptically suspended in 90 mL of sterile distilled H_2O and serially diluted up to 10^{-5}. Subsequently, 0.1 mL aliquots from appropriate dilutions were spread onto nutrient agar (NA), King's B (KB) medium, and Jensen's nitrogen-free medium to facilitate the growth of diverse rhizobacterial populations. The inoculated plates were incubated at 28 ± 2 °C for 48–72 h. Morphologically distinct colonies were selected and purified through repeated streaking on NA plates . Pure cultures were maintained on NA slants at 4 °C for short-term storage and preserved at −20 °C in 20% glycerol for long-term use . Preliminary identification of the isolates was performed based on biochemical characteristics following standard protocols described in *Bergey's Manual of Determinative Bacteriology* [11]. The obtained results were compared with established biochemical profiles of reference genera to assign tentative identification.

2.2. Isolation of *Xanthomonas* sp.

The Strain of *Xanthomonas* sp. used in this study was procured from Royal Bioresearch. Upon acquisition, the culture was revived and maintained under laboratory conditions using yeast extract dextrose calcium carbonate (YDC) agar medium composed of yeast extract (10 gL^{-1}), dextrose (20 gL^{-1}), calcium carbonate (20 gL^{-1}), and agar (15 gL^{-1}). The cultures were incubated at 28 °C for 48–72 h to ensure optimal growth and viability. Periodic subculturing was performed to maintain active cultures, and stocks were preserved under appropriate storage conditions.

2.3. Screening for PGP traits

All isolates were assessed for key PGPR traits using standard qualitative assays. IAA production was determined using Salkowski's method. NH_3 production was evaluated using Nessler's reagent following established protocols, whereas HCN production was detected using picric acid–sodium carbonate impregnated filter paper . PS activity was examined on Pikovskaya's agar medium based on halo zone formation around colonies. Nitrogen fixation potential was qualitatively assessed using nitrogen-free medium (NFM), and SP production was evaluated on Chrome Azurol S (CAS) agar plates. Hydrolytic enzyme activities, including amylase, protease, and cellulase, were screened using agar plate assays according to standard procedures. The formation of clear zones surrounding the colonies after incubation was considered indicative of positive enzymatic activity [12].

2.4. Molecular identification of the isolate NB1

The isolate NB1, which exhibited the highest overall PGP potential, was subjected to molecular identification based on 16S rRNA gene sequencing. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany), and DNA quality was assessed using a NanoDrop 2000 spectrophotometer and agarose gel electrophoresis. The 16S rRNA gene was amplified using universal primers 27F and 1492R under standard PCR conditions. Amplified products were purified and sequenced bidirectionally by Eurofins Genomics (India). The resulting sequences were assembled and analyzed using BLASTn against the NCBI GenBank database. Phylogenetic analysis was performed using the Maximum Likelihood method in MEGA12 with 1000 bootstrap replicates. Species-level identification was interpreted based on the 98.65% 16S rRNA gene sequence similarity threshold [13].

2.5. GC-MS analysis

The crude methanolic extract of isolate NB1 was subjected to chemical profiling using GC-MSto identify bioactive compounds. The analysis was performed using an Agilent 7890B gas chromatograph coupled with an Agilent 5977A Mass Selective Detector (MSD) equipped with an HP-5MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness; Agilent Technologies, USA). Helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The GC oven temperature was initially set at 60 °C (held for 2 min), then increased to 280 °C at a rate of 10 °C min^{-1} with a final hold of 10 min, resulting in a total run time of 35 min. The injector temperature was maintained at 250 °C, and samples were introduced in split mode with a split ratio of 10:1. Mass spectrometric detection was carried out under electron ionization at 70 eV, with a scanning range of m/z 50–550. The ion source and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively. Compounds were tentatively identified by comparing their mass spectra with those available in the NIST 2020 spectral database and Wiley 11 library, and further validated through comparison with previously reported metabolites from *Bacillus* sp. Only peaks with a match factor of ≥80% were considered, and their corresponding retention times, peak areas, molecular weights, and molecular formulas were recorded[14].

2.6. Antagonistic assay against *Xanthomonas* sp.

The antagonistic activity of isolate NB1 was evaluated against two strains of *Xanthomonas* sp. using the agar well diffusion method, following a modified protocol . The pathogen cultures were grown in nutrient broth (NB) for 24 h at 28 ± 2 °C, and the cell suspensions were adjusted to approximately 1 x 10^8 CFU mL^{-1} (OD_{600} ≈ 0.1). These suspensions were evenly spread onto nutrient agar (NA) plates using sterile cotton swabs to obtain a uniform bacterial lawn. The test isolate NB1 was cultured in NB for 48 h under shaking conditions (150 rpm). The cultures were centrifuged at 10,000 x g at 4 °C for 15 min, and the supernatant was collected, filtered, and used as the crude extract. Wells of 6 mm diameter were aseptically made in the agar plates, and 50 µL of the crude extract at concentrations of 2.5, 5.0, 7.5, and 10.0 mg mL^{-1} were dispensed into each well. Streptomycin (10 µg mL^{-1}) was used as the positive control, while methanol served as the negative control. The plates were incubated at 28 ± 2 °C for 24 h, and antimicrobial activity was determined by measuring the diameter of the inhibition zones, including the well diameter, expressed in millimeters (mm)[15].

RESULTS

3.1. Isolation and identification of PGPR

A total of 21 bacterial isolates were recovered from the collected rhizospheric soil samples. Based on preliminary biochemical characterization, the isolates were tentatively assigned to the genera *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp., and *Azospirillum* sp. Among these, *Bacillus* sp. accounted for the highest proportion (38.10%) of the total isolates, followed by *Pseudomonas* sp. and *Azotobacter* sp., each representing 19.05%. *Rhizobium* sp. and *Azospirillum* sp. were comparatively less abundant, each comprising 9.52% of the isolates.

3.2. Isolate-specific distribution of PGP traits

Individual isolates are presented in **(Table 1)**, revealing considerable variability in PGP traits. Isolates belonging to *Rhizobium* sp., *Azotobacter* sp., and *Bacillus* sp. demonstrated multiple beneficial attributes, including IAA production, nutrient mobilization, and hydrolytic enzyme activities. In contrast, certain isolates of *Bacillus* sp. and *Pseudomonas* sp. exhibited a comparatively limited functional profile, primarily associated with phytohormone production and specific enzymatic activities. Among all isolates, *Bacillus* sp. NB1 exhibited all nine evaluated PGP traits, indicating its superior functional potential. Additionally, *Rhizobium* sp.2, *Azotobacter* sp. 1, and *Bacillus* sp. Group B4 also displayed broad-spectrum PGP characteristics.

3.3. Comparative distribution of PGP traits across bacterial genera

The distribution of key plant growth-promoting (PGP) traits varied considerably among the isolated bacterial genera **(Figure 1)**. *Rhizobium* sp. isolates exhibited complete positivity (100%) for IAA production, NH_3 generation, nitrogen fixation (NF), SP production, and hydrolytic enzymes such as

amylase and protease, while moderate activity was observed for PS (50%) and cellulase (50%). *Azospirillum* sp. isolates showed consistent PS activity (100%) and partial IAA production (50%), along with moderate enzymatic activities for amylase and cellulase (50% each). Among *Bacillus* sp., Group A isolates demonstrated high IAA production (75%), NH_3 generation (50%), and notable amylase and protease activities (75% each), whereas nutrient acquisition traits such as PS and NF were less frequent (25% each). Group B isolates followed a similar pattern but exhibited relatively higher cellulase activity (75%) and moderate HCN production (25%). *Pseudomonas* sp. isolates displayed strong enzymatic potential, with 75% of strains producing amylase and protease; however, their nutrient mobilization capacity was limited, with low PS activity (25%) and no evidence of nitrogen fixation. *Azotobacter* sp. isolates showed 100% IAA production, moderate NH_3 generation (25%), high PS activity (75%), and detectable SP production (25%), along with variable hydrolytic enzyme activities. Notably, the *Bacillus* sp. NB1 isolate exhibited 100% positivity across all evaluated traits, including phytohormone production, nutrient mobilization, SP production, and hydrolytic enzyme activities, highlighting its highly versatile functional profile and potential as a broad-spectrum bioinoculant.

3.4. 16S rRNA sequence-based identification of the isolate NB1

The potent isolate NB1 was subjected to molecular identification based on partial 16S rRNA gene sequencing. BLASTn analysis against the NCBI GenBank database revealed 99.78% sequence similarity with *Bacillus zhangzhouensis*, with 100% query coverage and significant alignment scores, indicating a close phylogenetic relationship with this species **(Figure 2)**. Phylogenetic analysis using the Maximum Likelihood method further supported this assignment, with isolate NB1 clustering closely with *B. zhangzhouensis* within the *Bacillus* clade **(Figure 3).** The topology of the phylogenetic tree demonstrated a stable association of NB1 with reference sequences of *B. zhangzhouensis*, supporting its identification as *Bacillus zhangzhouensis* based on 16S rRNA gene sequence analysis.

3.5. GC-MS analysis

The GC–MS analysis of the methanolic crude extract of *B. zhangzhouensis* NB1 revealed a complex profile of metabolites distributed across retention times of 10.33–26.19 min **(Figure 4 and Table 2)**. A total of 42 compounds were identified based on spectral matching (≥80%) with standard libraries. Among these, major compounds included N-acetyltyramine (16.07%), diketopiperazine derivatives such as pyrrolo[1,2-a] pyrazine-1,4-dione analogues, and phenolic compounds including 2,4-bis(1-phenylethyl) phenol. Additionally, fatty acid methyl esters such as methyl stearate and hexadecanoic acid methyl ester were detected in moderate proportions.

Several of the detected metabolites have previously been reported to possess antimicrobial and related biological activities. Diketopiperazine derivatives have been associated with antimicrobial and quorum-sensing inhibitory properties, whereas phenolic compounds and fatty acid esters have been reported to exhibit antimicrobial effects in different microbial systems. Therefore, these metabolites may collectively contribute to the antibacterial activity observed in the crude extract; however, their individual roles remain to be experimentally validated.

ANTAGONISTIC ACTIVITY

3.6. *B. zhangzhouensis* NB1 crude extract against *Xanthomonas* sp.

Two strains obtained from a research laboratory produced characteristic mucoid, bright yellow, circular colonies on YDC medium and were designated as *Xanthomonas* sp. isolate 1 and isolate 2. The crude extract of *B. zhangzhouensis* NB1 exhibited a concentration-dependent inhibitory effect against both *Xanthomonas* isolates **(Table 3 and Figure 5 (A, B)**. *Xanthomonas* sp. 1 showed inhibition zones ranging from 10 mm at 2.5 mg mL^{-1} to 16 mm at 10 mg mL^{-1}, comparable to the standard antibiotic streptomycin (16 mm). In contrast, *Xanthomonas* sp. 2 exhibited lower sensitivity, with inhibition observed only at higher concentrations, producing zones of 10 mm and 12 mm at 7.5 mg mL^{-1} and 10 mg mL^{-1}, respectively. No inhibition was observed in the methanol control, confirming the specificity of the antimicrobial activity.

DISCUSSION

4.1. Diversity and frequency of isolated PGPR traits

The recovery of 21 rhizospheric bacterial isolates, with a clear predominance of *Bacillus* sp., is consistent with previous reports where *Bacillus* sp. dominates due to their ecological adaptability, endospore-forming capability, and efficient utilization of root exudates under diverse environmental conditions . The widespread occurrence of IAA production among the isolates is noteworthy, as microbial IAA plays a crucial role in modulating root architecture, promoting lateral root development, and enhancing nutrient uptake efficiency. Similarly, PS activity observed in several isolates represents an important trait, as the secretion of organic acids and phosphatases facilitates the conversion of insoluble phosphorus into plant-available forms, thereby improving nutrient acquisition . In addition, hydrolytic enzymes such as cellulases and proteases contribute to biocontrol by degrading structural components of phytopathogens, ultimately reducing their virulence and infectivity [16]. Previous studies have demonstrated that *Bacillus* isolates possessing combined traits such as IAA production, phosphate solubilization, and hydrolytic enzyme activity can significantly enhance plant growth and contribute to disease suppression in crops such as tomato. This multifunctional capability supports both direct plant growth promotion and indirect biocontrol mechanisms, highlighting the potential of the potent isolate identified in the present study as a promising candidate for bioinoculant development in sustainable and eco-friendly agricultural systems [17].

4.2. Molecular identification

The 16S rRNA gene-based identification indicates that isolate NB1 is closely affiliated with *B. zhangzhouensis*, supporting its placement within the *Bacillus* genus. Members of *B. zhangzhouensis* have been increasingly reported from soil and rhizosphere environments and are recognized for their metabolic versatility and adaptability to diverse ecological conditions[18]. Such traits are particularly relevant for PGPR, as they enable effective colonization and persistence in the rhizosphere. The phylogenetic clustering of NB1 with *B. zhangzhouensis* further suggests that the observed functional attributes, including plant growth promotion and antimicrobial activity, may be linked to inherent genetic and metabolic characteristics associated with this group. Previous studies on closely related *Bacillus* sp.have demonstrated their ability to produce diverse bioactive metabolites, enzymes, and secondary compounds that contribute to both plant growth enhancement and pathogen suppression[19]. Although 16S rRNA gene sequencing provides reliable genus-level resolution, further genome-based analyses such as whole-genome sequencing or average nucleotide identity (ANI) could offer deeper insights into strain-level differentiation and functional potential. Nonetheless, the present findings establish *B. zhangzhouensis* NB1 as a promising candidate with potential applications in sustainable agriculture and biocontrol strategies.

4.3. GC-MS analysis

GC–MS analysis of the crude extract of *B. zhangzhouensis* NB1 revealed a diverse profile of secondary metabolites, including N-acetyltyramine, pyrrolo[1,2-a]pyrazine derivatives, and fatty acid esters. Several of these compounds have previously been documented in *Bacillus* sp. and are associated with antimicrobial activity. N-acetyltyramine has been implicated in quorum-sensing interference and antimicrobial effects against phytopathogenic microorganisms[20]. Pyrrolo[1,2-a]pyrazine derivatives, belonging to the diketopiperazine group, are commonly detected in *Bacillus* sp. and *Pseudomonas* sp. and have been associated with antimicrobial and antibiofilm activities. Fatty acid esters identified in the extract are also recognized for their antimicrobial properties, potentially through disruption of microbial membrane integrity. The coexistence of these metabolite classes suggests that the antibacterial activity observed against *Xanthomonas* sp. may be mediated through multiple mechanisms, including quorum-sensing disruption, membrane destabilization, and direct antimicrobial action. Comparable metabolite profiles have been documented in biocontrol-active *Bacillus* isolates, where similar compounds were associated with the suppression of phytopathogens such as *X. oryzae* and *X. campestris* in different crop systems[21]. These findings support the potential role of *B. zhangzhouensis* NB1 as a source of bioactive metabolites with relevance in sustainable plant disease management.

4.4. Antagonistic assays-crude extract activity and mode of action

The crude extract of *B. zhangzhouensis* NB1 exhibited dose-dependent antibacterial activity against *Xanthomonas* sp., with inhibition zones ranging from 10 to 16 mm. The GC–MS profile of the extract supports a multi-mechanistic mode of action, involving compounds associated with quorum-sensing interference (e.g., N-acetyltyramine), membrane destabilization (fatty acid esters), and disruption of cell wall integrity and biofilm formation (pyrrolo[1,2-a]pyrazine derivatives). Similar antagonistic effects have been reported in *B. velezensis* against *X. campestris*, where the

combined action of lipopeptides and diketopiperazines contributed to effective pathogen suppression under both in vitro and in planta conditions [22].

Previous studies have demonstrated that microbial consortia can play a significant role in plant disease suppression and soil health improvement. For instance, co-inoculation of *F. mosseae* with *Bacillus* sp. has been shown to mitigate the disease cycle of soybean root rot caused by *F. oxysporum* by reducing harmful soil metabolites such as ethylbenzene and styrene. This reduction was associated with enhanced soil quality, increased microbial activity, and elevated levels of plant defense enzymes, including β-1,3-glucanase, chitinase, and phenylalanine ammonia-lyase, ultimately alleviating rhizosphere stress in plants . In the context of the present study, the crude extract of *B. zhangzhouensis* NB1 demonstrated notable antibacterial activity. The observed effects represent a comparative evaluation under the given experimental conditions, and no direct attribution to individual components is implied[23].

Despite the promising antibacterial activity observed in the present study, the efficacy of the *B. zhangzhouensis* NB1-derived formulation was evaluated only under in vitro conditions. Therefore, further greenhouse and field studies are required to validate its effectiveness against *Xanthomonas* sp. under natural conditions and to assess its potential for practical agricultural applications.

CONCLUSION

In this study, a potent PGPR was successfully isolated and characterized from rhizospheric soil, exhibiting multiple functional traits including cellulase and amylase production, along with IAA production, PS, and SP secretion. The isolate, identified as *B. zhangzhouensis* based on 16S rRNA gene sequence analysis (99.78% similarity), demonstrated significant antibacterial activity against *Xanthomonas* sp. GC–MS profiling of the crude extract revealed the presence of diverse bioactive metabolites associated with antimicrobial and plant growth-promoting functions, providing a mechanistic basis for the observed antagonistic activity. The application of *B. zhangzhouensis* metabolites exhibited enhanced antibacterial efficacy, which may be attributed to improved stability, increased interaction with target cells, and sustained release of bioactive compounds and also used for sustainable crop disease management, with potential to reduce reliance on chemical inputs while supporting plant growth. However, further studies involving detailed taxonomic validation, metabolite-specific characterization, field-level evaluation, and environmental safety assessment are necessary to advance its application in agricultural systems.

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Table 1: PGP traits of bacterial isolates from rhizospheric soil.

S. No.	Organisms	PGP traits								
		IAA	NH_3	HCN	PS	NF	SP	Amylase	Protease	Cellulase
1	*Rhizobium* sp.1	+	+	-	-	+	+	+	+	-
2	*Azospirillum*sp.1	+	-	-	+	-	-	-	-	+
3	*Bacillus* sp. Group A1	+	-	-	-	-	-	+	+	-
4	*Pseudomonas* sp.1	+	-	-	+	-	-	+	-	-
5	*Bacillus* sp. Group A 2	+	+	-	-	-	-	+	+	-
6	*Bacillus* sp. GroupB 1	+	-	-	+	-	-	-	-	+
7	*Rhizobium* sp. 2	+	+	-	+	+	+	+	+	+
8	*Azot*	+	-	-	+	+	-	+	+	-

	obacters sp. 1									
9	*Bacillus* sp. GroupB 2	-	+	-	+	-	-	-	-	+
1	*Azotobacters* sp. 2	+	-	-	+	-	-	+	+	-
1	*Bacillus* sp. Group A 3	+	+	-	-	-	-	-	-	-
1	*Azotobacters* sp.3	+	-	-	-	-	+	+	+	-
1	*Pseudomonas* sp.2	+	-	-	-	-	-	-	+	-
1	*Bacillus* sp. Group A 4	-	-	-	+	-	-	+	+	-
1	*Bacillus* sp. Group B 3	+	-	+	-	-	-	+	+	+
1	*Pseudomonas* sp.3	+	-	-	-	-	-	+	+	+
1	*Bacillus* sp. Group B 4	+	+	-	+	+	-	+	+	+
1	*Bacillus* sp. NB1	+	+	+	+	+	+	+	+	+
1	*Azotobacter* sp. 4	+	+	-	-	-	-	+	+	-
2	*Azospirillum* sp. 2	-	-	-	+	-	-	+	+	-
2	*Pseudomonas* sp. 4	-	-	-	-	-	-	+	+	-

Table 2: Bioactive compounds identified in the methanolic extract of *Bacillus zhangzhouensis* by GC–MS analysis.

S. No.	RT (min)	Compound Name	Molecular Formula	Molecular Weight (g mol⁻¹)	Peak Area (%)
1	10.3345	1,4:3,6-Dianhydro-α-D-glucopyranose	$C_6H_8O_4$	144.13	0.77
2	10.4364	Methane, tris(methylthio)-	$C4H_{10}S_3$	154.31	4.05
3	11.2339	Indole	C_8H_7N	117.15	2.49
4	16.5723	Cyclo(L-alanyl-L-leucyl)	$C_9H_{16}N_2O_2$	184.24	0.56
5	16.6634	2,5-Piperazinedione, 3-methyl-6- (1-methylpropyl)	$C_{10}H_{18}N_2O_2$	198.27	0.90
6	16.8637	3,6-Diisopropylpiperazine-2,5-dione	$C_{11}H_{20}N_2O_2$	212.29	1.24
7	17.6284	4-Dibutylamino-2-en-1-ol	$C_{12}H_{25}NO$	199.33	0.63
8	17.7121	N-Acetyltyramine	$C_{10}H_{13}NO_2$	179.22	16.07
9	17.7995	3,6-Diisopropylpiperazine-2,5-dione	$C_{10}H_{18}N_2O_2$	198.27	1.30
10	17.8614	Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210.27	2.60
11	18.2620	3-Isopropylidene-tricyclo[4.3.1.1(2,5)]undecan-10-one	$C_{14}H_{20}O$	204.31	1.79
12	18.3858	7-(6-Methylpyridin-2-yl)heptane-1,6-diol	$C_{13}H_{21}NO_2$	223.31	0.62
13	18.484	7,9-Di-tert-butyl-1-	$C_{17}H_{24}O_3$	276.37	2.96

S.No.	RT (min)	Compound Name	Molecular Formula	Molecular Weight (g mol^{-1})	Peak Area (%)
	1	oxaspiro(4,5)deca-6,9-diene-2,8-dione			
14	18.5606	Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210.27	0.89
15	18.6338	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	0.95
16	18.7307	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226.32	1.02
17	18.7937	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226.32	0.47
18	18.8446	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226.32	3.21
19	18.8956	Hexahydro-3-(1-methylpropyl)pyrrolo[1,2a]pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210.27	3.43
20	18.9612	1,2-Benzenedicarboxylicacid, butyloctyl ester	$C_{20}H_{30}O_4$	334.45	0.87
21	19.1204	Menthol,1-(butyn-3-one-1-yl)-(1S,2S,5R)-	$C_{14}H_{22}O_2$	222.32	0.97
22	19.9007	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334.45	0.56
23	20.0537	Di-n-butyl phthalate	$C_{18}H_{26}O_4$	302.40	1.64
24	20.4068	Phthalic acid, dec-2-ylpentyl ester	$C_{23}H_{36}O_4$	376.53	0.70
25	20.5816	Methyl stearate	$C_{19}H_{38}O_2$	298.51	0.89
26	21.408	Phthalic acid, cyclohexylpentyl	$C_{19}H_{26}O_4$	318.41	0.33
	2	ester			
27	22.0892	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$	312.50	0.85
28	22.2786	N-[5-(2,5-Dioxopyrrolidin-1-yl)pentyl]-N-hydroxyacetamide	$C_{11}H_{18}N_2O_4$	242.28	0.79
29	22.5371	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	244.29	0.49
30	22.6245	Phthalic acid, dec-2-ylisobutyl ester	$C_{22}H_{34}O_4$	362.50	0.59
31	22.8830	Hexanedioic acid, dioctyl ester	$C_{22}H_{42}O_4$	370.57	1.04
32	23.0214	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	362.50	4.32
33	23.1197	Phthalic acid, decylneopentyl ester	$C_{23}H_{36}O_4$	376.53	4.90
34	23.2545	Phenol, 2,4-bis(1-phenylethyl)-	$C_{22}H_{22}O$	302.41	1.79
35	23.4220	Phthalic acid, decylneopentyl ester	$C_{23}H_{36}O_4$	376.53	3.08
36	23.6987	Phenol, 2,4-bis(1-phenylethyl)-	$C_{22}H_{22}O$	302.41	3.83
37	23.7497	Phthalic acid, dec-2-ylpentyl ester	$C_{23}H_{36}O_4$	376.53	8.67
38	24.3542	1,2-Benzenedicarboxylic acid, monononyl ester	$C_{17}H_{24}O_4$	292.38	1.49
39	25.6469	1,4-Benzenedicarboxylicacid,bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.56	2.76
40	25.8727	Di(3,7-dimethyl-1-octyl)phthalate	$C_{28}H_{46}O_4$	446.67	0.61

S. No.	RT (min)	Compound Name	Molecular Formula	Molecular Weight (g mol⁻¹)	Peak Area (%)
41	25.9128	Di(3,7-dimethyl-1-octyl)phthalate	$C_{28}H_{46}O_4$	446.67	0.48
42	26.1968	Didecan-2-yl phthalate	$C_{28}H_{46}O_4$	446.67	12.85

Table 3: Antagonistic activity of crude extract from *B. zhangzhouensis* against *Xanthomonas* sp.

S.No.	Different conc. of extract (mg)	Zone of inhibition in mm	
		Xanthomonas sp. 1	*Xanthomonas* sp. 2
1	Crude Extract		
	2.5	10±0.5	-
	5	12±1.1	-
	7.5	14±1.0	10±1.0
	10	16±1.6	12±1.1
3	Std antibiotic Streptomycin	-	16±1.5
4	Methanol	-	-

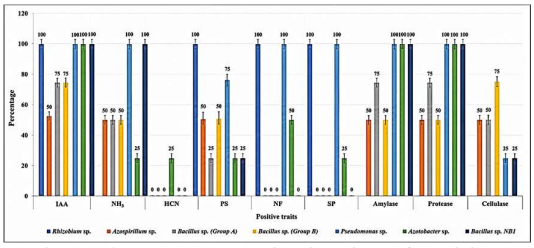

Figure 1: Percentage distribution of positive plant growth-promoting traits among different bacterial isolates

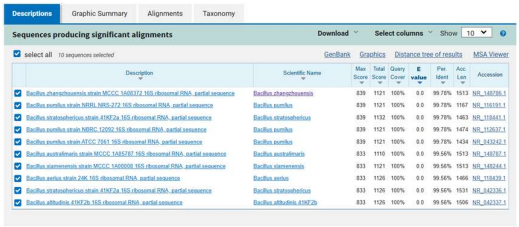

Figure 2: BLASTn analysis showing significant sequence alignments of 16S rRNA gene indicating closest similarity to *B. zhangzhouensis*

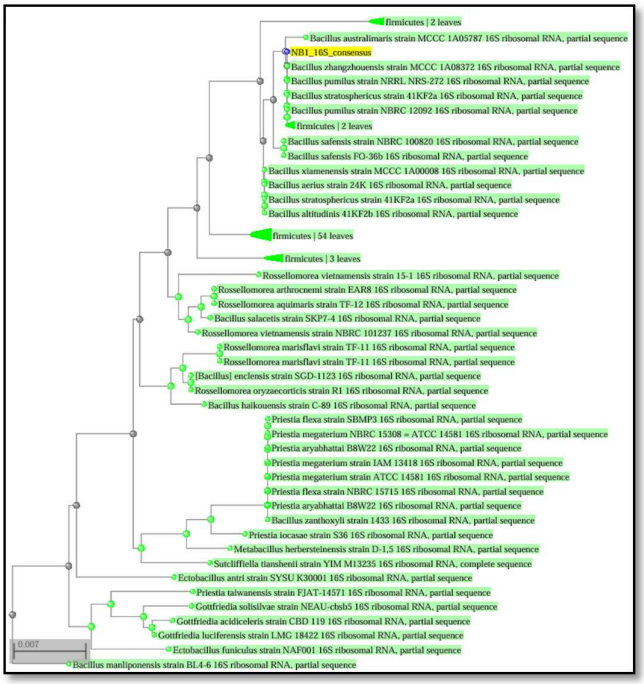

Figure 3: Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of the isolated strain to *B. zhangzhouensis* and related taxa

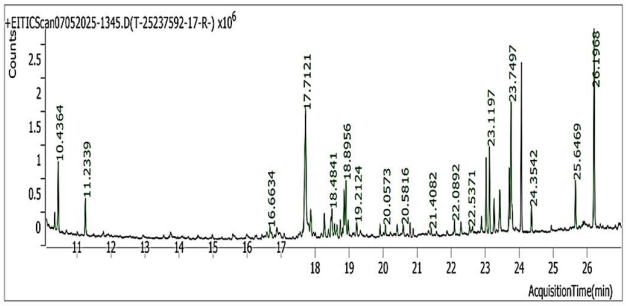

Figure 4: GC–MS chromatogram showing the profile of bioactive compounds produced by *B. zhangzhouensis* with prominent peaks at different retention times

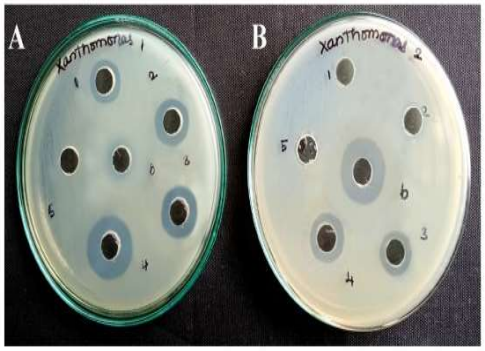

Figure 5: Antibacterial activity of *B. zhangzhouensis* crude extract against *Xanthomonas* sp.

A and B: Antimicrobial activity of extract of *B. zhangzhouensis* (1: 2.5 mg, 2: 5 mg, 3: 7.5 mg, 4: 10 mg, 5: Methanol, 6: Streptomycin).