

Research Article  
Open Access



CrossMark

- <sup>1</sup> Pharmaceutical Research Laboratory, Department of Biotechnology Research, Kyaukse, Mandalay Division, Myanmar.  
<sup>2</sup> Cell Culture Laboratory, Department of Biotechnology Research, Kyaukse, Mandalay Division, Myanmar.  
<sup>3</sup> Mycology Laboratory, Department of Biotechnology Research, Kyaukse, Mandalay Division, Myanmar.  
<sup>4</sup> Microbiology Laboratory, Department of Biotechnology Research, Kyaukse, Mandalay Division, Myanmar.

\* To whom correspondence should be addressed: [thesumoe@dbi.gov.mm](mailto:thesumoe@dbi.gov.mm)

**Editor:** Morad Mokhtar, *University Mohammed VI Polytechnic, BenGuerir, Morocco.*

**Reviewer(s):** Sakchai Hongthong, *Division of Chemistry, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand.*

Danielle Silveira Santos, *Federal University of Rio de Janeiro, Instituto de Biodiversidade e Sustentabilidade, Macaé 27965-045, Brazil.*

Sudarshna Negi, *Department of Basic Sciences, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan 173230, Himachal Pradesh, India.*

**Received:** October 10, 2025

**Accepted:** December 17, 2025

**Published:** December 27, 2025











**Citation:** Moe TS, Thida M, Khaing MP, Ei SL, Mon WW, Win ZK, Htay NN, Aung T, Tun TT, Lin Z. Bioprospecting endophytic *Bacillus pumilus* PhS 6-1 from *Phyllanthus acidus*: A source of antimicrobial and plant growth-promoting compounds. 2025 Dec. 27;8:bs202510

**Copyright:** © 2025 Moe TS *et al.*. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and supplementary materials.

**Competing interests:** The authors declare that they have no competing interests.

## Bioprospecting endophytic *Bacillus pumilus* PhS 6-1 from *Phyllanthus acidus*: A source of antimicrobial and plant growth-promoting compounds

The Su Moe<sup>\*1</sup> , Mya Thida<sup>2</sup> , Myat Phyu Khaing<sup>3</sup> , Shun Lai Ei<sup>4</sup> , Wai Wai Mon<sup>4</sup> , Zar Kyi Win<sup>1</sup> , Nwe Nwe Htay<sup>1</sup> , Thandar Aung<sup>1</sup> , Theint Theint Tun<sup>1</sup> , Zarni Lin<sup>1</sup> 

### Abstract

*Bacillus pumilus*, PhS 6-1, was isolated from the stem of *Phyllanthus acidus* (L.) Skeels. In the present work, the metabolite extracted from this endophyte was investigated for antibacterial, antioxidant, antiglycation, and cytotoxic activities. The extract showed antibacterial activity against clinically important bacteria: *Escherichia coli* (11.33 ± 0.58 mm), *Enterococcus faecalis* (20.67 ± 1.15 mm), *Staphylococcus aureus* (19.33 ± 1.15 mm), *Pseudomonas aeruginosa* (12.33 ± 1.53 mm), *Bacillus cereus* (18.00 ± 0.00 mm), and multidrug-resistant *S. aureus* (17.33 ± 0.58 mm). The MIC values for all tested bacterial species ranged from 12.5 to 25 mg/mL, while the MBC values ranged from 50 to >100 mg/mL. The extract exhibited mild antioxidant activity with a percentage inhibition of 12.77 ± 3.34 and showed no antiglycation activity. The total phenolic content was 39.39 ± 1.06 mg gallic acid equivalents (GAE)/g. The hemolytic assay revealed low hemolytic activity (18.06 ± 2.9% lysis), indicating good biocompatibility. PhS 6-1 also showed strong antagonistic effects against phytopathogenic fungi, particularly *Pythium* sp. and *Rhizoctonia solani*. Plant growth-promoting capabilities, such as nitrogen fixation and potassium solubilization, were observed, highlighting its potential in sustainable agriculture. GC-MS profiling of the ethyl acetate extract identified twelve compounds, including 2,3-butanediol, nonane, undecane, 1-tridecene, 1-pentadecene, 1-octadecene, 1-nonadecene, 1-(+)-ascorbic acid 2,6-dihexadecanoate, behenic alcohol, 9-octadecenoic acid, N-tetracosanol, and 17-pentatriacontene. Finally, sequencing of the 16S rRNA gene and BLAST analysis confirmed PhS 6-1 as *Bacillus pumilus* with 99.93% identity.

**Keywords:** *Bacillus pumilus*, *Phyllanthus acidus*, endophyte, antimicrobial, metabolite profiling

### Introduction

Endophytes are symbiotic microbes residing within plant tissues, typically establishing non-pathogenic associations with their hosts, and have attracted significant attention for their potential applications in agriculture and biotechnology. Endophytes can positively impact plants by assisting in nutrient acquisition, modulating plant growth processes, increasing tolerance to environmental challenges, suppressing the virulence of pathogens, enhancing plant defense mechanisms, and inhibiting the growth of competing plants [1]. They often exhibit beneficial traits, such as nitrogen fixation; zinc, potassium, and phosphorus solubilization; phytohormone and siderophore synthesis; and the ability to act as biocontrol agents against diverse plant pathogens, making them valuable in the agricultural sector [2].

While endophytic bacteria are well known for their growth-promoting capabilities, emerging research indicates that they can also produce secondary metabolites with antimicrobial and antitumor activities, thereby offering potential solutions to various plant and human health challenges [3]. One particular endophytic strain, *Bacillus pumilus*, appears to be a strong candidate owing to its versatility and adaptability. *Bacillus pumilus* is a spore-forming Gram-positive bacterium that is common across diverse ecosystems, such as soil, water, sediment, and plants [4]. This species has also been isolated

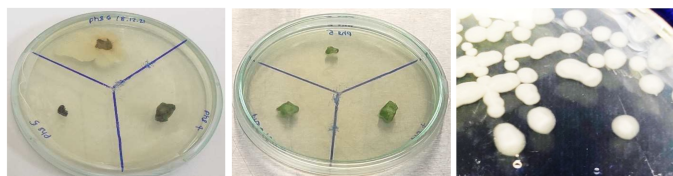
from multiple plant species and has exhibited diverse plant-beneficial properties, including plant growth-enhancing, antimicrobial, and anticancer activities [3]. On the other hand, medicinal plants have long been recognized as important therapeutic resources in the management of diverse human diseases. These plants possess many bioactive compounds, such as phenols, flavonoids, terpenoids, and alkaloids, that interact with the human body in ways that can alleviate or cure diseases [5]. *Phyllanthus acidus*, a tropical plant species, is believed to have originated in Madagascar and can be found in Asiatic countries such as Myanmar, India, Indonesia, Laos, and Malaysia. It possesses several medicinal properties, such as antioxidant, hypoglycemic, antidiarrheal, analgesic, anesthetic, and antimicrobial properties, and is exploited in traditional medicines for the treatment of hepatic diseases, respiratory disorders, rheumatism, bronchitis, asthma, inflammation, hypertension, and diabetes [6]. Therefore, the endophytes harbored by this plant might possess unique characteristics that could contribute to pharmaceutical and agricultural applications.

In this study, the endophytic *Bacillus pumilus* strain PhS 6-1, isolated from the stem of *Phyllanthus acidus* (L.) Skeels, was identified and investigated for its potential bioactivities using *in vitro* antioxidant, antiglycation, antimicrobial, cytotoxic, and plant growth-promoting assays. Moreover, the metabolites present in the ethyl acetate extract of PhS 6-1 were analyzed using GC-MS.

## Materials and Methods

### Plant sample collection and endophyte isolation

*Phyllanthus acidus* (L.) Skeel stems were obtained from the field of the Department of Biotechnology Research in Kyaukse Township, Myanmar. Surface sterilization and endophyte isolation were conducted following a previously published protocol [7]. After surface sterilization, the stem samples were cut into small fragments and placed onto tryptic soy agar (TSA) plates. The plates were incubated at 28 °C for 2–3 days until bacterial colonies became visible. Colonies observed around the stem fragments were aseptically transferred onto fresh TSA plates and incubated at 28 °C. Pure isolates were obtained through repeated subculturing. The isolated endophyte was preserved by freeze-drying and by preparing glycerol stocks, which were stored at -80 °C.



**Figure 1.** Isolation of *Bacillus pumilus* strain PhS 6-1 from *Phyllanthus acidus* (L.) Skeels and its colony morphology on TSA medium

### Biochemical, morphological and molecular identification of endophytic bacteria

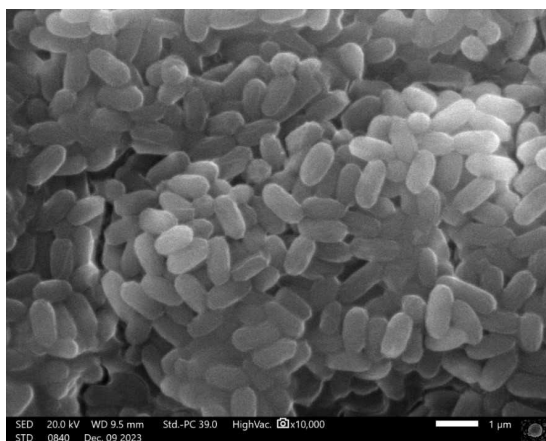
The isolated endophytic strain PhS 6-1 was characterized based on colony morphology, Gram staining, scanning electron microscopy (SEM), and a series of biochemical tests, including the catalase test, oxidase test, methyl red test, Voges-Proskauer test, indole test, gelatin liquefaction test, citrate utilization test, starch hydrolysis test, urease test, triple sugar iron (TSI) test, casein hydrolysis test, and carbohydrate fermentation tests using glucose, dextrose, mannitol, sorbitol, lactose, and sucrose. Gram staining was performed following a previously described method [8]. Bacterial cell preparation for SEM analysis was carried out according to a previously described protocol [9]. The endophytic isolate was examined for morphological characteristics using a scanning electron microscope (SEM; Shimadzu, Japan). Digital images were obtained from secondary electron signals at an accelerating voltage of 20 kV. Genomic DNA extraction for molecular analysis was performed using the FastPure DNA Isolation Mini Kit (Vazyme, China). A pure colony of the endophytic strain PhS 6-1 was used for DNA extraction. Identification was carried out by analysis of the 16S ribosomal RNA (rRNA) gene sequence. Approximately 1,500 bp of the 16S rRNA gene was amplified using the primers 16S-27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S-1492R (5'-TACGACTTAACCCCAATCGC-3'). The amplified 16S rRNA gene was sequenced by Novogene Company (Beijing, China). Sequence similarity searches were conducted using the NCBI BLAST program. Multiple sequence alignment was performed using ClustalW, and phylogenetic trees were constructed in MEGA 11 using the neighbor-joining and maximum likelihood methods [10]. The obtained sequence was deposited in the GenBank database under accession number OQ690493.

### Screening of the optimal time for bioactive metabolite production by endophytic bacteria

The incubation time of endophytic bacteria plays a critical role in maximizing the production of bioactive metabolites. Consequently, the PhS 6-1 strain was cultured in nutrient broth and incubated at 28 °C for 7 days. The broth culture was collected and filtered through a 0.45 µm syringe filter, and samples were assessed daily for antioxidant, antiglycation, and antibacterial activities to determine the optimal incubation time for bioactive metabolite production.

### Extraction of metabolites from endophytic bacteria

Metabolite extraction from PhS 6-1 was performed using a solvent partition method as described by Khan *et al.*, with slight modifications [11]. The PhS 6-1 strain was incubated in nutrient broth for 5 days in a water bath shaker at 28 °C. After incubation, the broth cultures were centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was collected, filtered through 0.45 µm membrane filters, and subjected to liquid-liquid extraction to recover metabolites. The supernatant and ethyl acetate were mixed in equal volumes in a separatory funnel, shaken vigorously,



**Figure 2.** Morphology of the endophytic bacterium *Bacillus pumilus* strain PhS 6-1, which was isolated from the stem of *Phyllanthus acidus* (L.) Skeels, as revealed by scanning electron microscopy (SEM)

and allowed to stand for phase separation. To achieve optimal extraction efficiency, this procedure was repeated three times. After separation of the aqueous and organic phases, the solvent phase containing the metabolites was concentrated to dryness using a rotary evaporator, yielding the crude ethyl acetate extract.

#### ***In vitro* DPPH free radical scavenging assay**

The free radical-scavenging activity of the extract was evaluated using the DPPH radical-scavenging assay as described by Lee *et al.* [12]. DMSO was used as the blank control, and ascorbic acid served as the standard. The inhibition rate was calculated using the following formula:

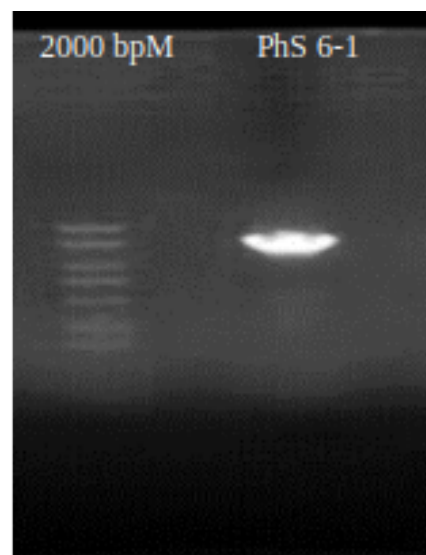
$$\text{Inhibition rate (\%)} = \left[ 1 - \frac{\text{OD of sample}}{\text{OD of control}} \right] \times 100$$

#### **Measurement of Total Phenolic Content**

Total phenolics in the PhS 6-1 extract were measured using the Folin–Ciocalteu method [13; 14; 15]. A gallic acid standard curve was prepared and used to calculate the total phenolic content (TPC) as mg of gallic acid equivalent (GAE) per g of extract. At least three replicates were performed to measure TPC. The total phenolic content was calculated using the following equation:

$$T = \frac{C \times V}{M}$$

- $T$  = Total phenolic content in mg/g of extract as GAE,
- $C$  = Concentration of gallic acid determined from the calibration curve (mg/mL),
- $V$  = Volume of the extract solution (mL),
- $M$  = Weight of the extract (g).



**Figure 3.** Gel electrophoresis analysis of the 1500 bp amplified PCR product from *Bacillus pumilus* strain PhS 6-1

#### ***In vitro* antiglycation assay**

The antiglycation activity of the PhS 6-1 extract was determined using the BSA-glucose model [15; 16; 17]. AGE inhibition was assessed by the measurement of fluorescence intensity (Excitation 340 nm, Emission 440 nm) on an Agilent Cary Eclipse spectrophotometer (MY15010005, Agilent Technology). The inhibition rate was calculated via the following formula:

$$\text{Inhibition rate (\%)} = \left[ 1 - \frac{\text{Fluorescence intensity of sample}}{\text{Fluorescence intensity of control}} \right] \times 100$$

#### ***In vitro* antimicrobial assays**

The antimicrobial activity of *PhS 6-1* was determined using an agar well diffusion assay by modifying the method described by Schlegel [18]. Pathogenic bacteria: *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and multidrug-resistant *Staphylococcus aureus*, were grown overnight in Mueller–Hinton broth at 37 °C. The resulting culture was then adjusted with normal saline to an OD<sub>600</sub> of 0.08–0.1, corresponding to roughly  $1.5 \times 10^8$  CFU/mL. Mueller–Hinton agar plates were prepared and autoclaved at 121 °C for 15 minutes for sterilization. Broth cultures of the test pathogens were spread on Mueller–Hinton agar to achieve uniform growth. Following inoculation, 8 mm diameter wells were created in the agar using a sterile cork-borer.

100 µL (0.2 g/mL) of *PhS 6-1* extract was introduced into each well labeled. Chloramphenicol (30 µg/well) served as the positive control, while 70% ethanol was used as the negative control. Plates were incubated at 37 °C overnight, after which inhibition zones were inspected and their diameters recorded to the nearest millimeter. An inhibition zone diameter of 8–12 mm

was considered low activity, 13–17 mm was considered moderate activity, and  $\geq 18$  mm was considered high activity. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a microplate-based resazurin assay adapted from Sarker *et al.* [19]. *PhS 6-1* extract was prepared in a two-fold dilution series (100 – 0.049 mg/mL), and the positive control, chloramphenicol, was tested at concentrations ranging from 128 to 0.063  $\mu\text{g/mL}$ . Bacterial inoculum was adjusted to a 0.5 McFarland standard, and bacterial inoculum (10  $\mu\text{L}$ ) was added to wells containing sample dilutions (50  $\mu\text{L}$ ), Mueller–Hinton broth (30  $\mu\text{L}$ ), and resazurin (10  $\mu\text{L}$ ). After incubation at 37 °C for 24 hrs, the MIC was recorded as the lowest concentration showing no resazurin color change. For MBC determination, aliquots from wells without color change were plated onto nutrient agar, and the MBC was defined as the lowest concentration producing no visible colonies. Each assay included growth and sterility controls, as well as a chloramphenicol reference.

#### Cytotoxicity analysis by hemolytic assay

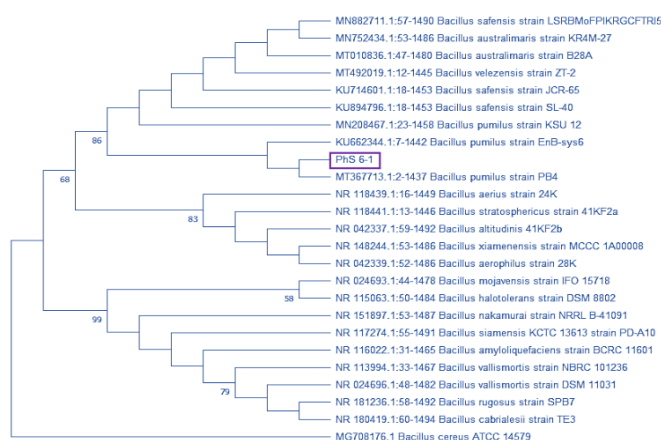
Cytotoxicity analysis of the extracts through the hemolytic assay using human red blood cells (RBCs) was carried out via the methods described by Powell *et al.* [20]. RBCs preparation was done by previously described method [21]. The RBC count was maintained at  $7.07 \times 10^8$  cells/mL for each assay. Triton X-100 (0.1%) was used as a positive control and was regarded as 100% lysis of RBCs, and PBS was used as a negative control.

#### *In vitro* antagonistic activity against plant pathogenic fungi and plant growth-promoting (PGP) assays

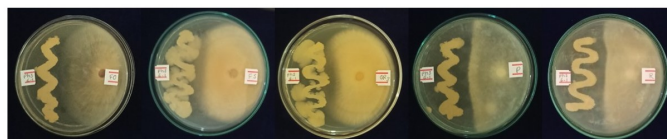
The dual culture technique was applied to evaluate the antagonistic activity of PhS 6-1 by modifying the method described by Al-Hussini *et al.* [22]. The plant pathogenic fungi *Fusarium oxysporum* (FO), *Fusarium solani* (FS), OR3, *Pythium* sp., and *Rhizoctonia solani* were cultivated on potato dextrose agar (PDA) at 28 °C. A bacterial colony was introduced at one end of a 90-mm nutrient agar plate, while a 6-mm mycelial disc from a 7-day-old culture of the fungal pathogen was placed at the opposite end. The plates were incubated at 28 °C until the fungus fully colonized the control plate. Control plates contained only a single fungal disc. Plant growth-promoting (PGP) assays were conducted to determine important plant growth-promoting traits, including nitrogen fixation activity, phosphate solubilization activity, and potassium (K) decomposition activity.

#### GC–MS analysis for metabolite profiling and statistical analysis

GC–MS was used to profile the bioactive metabolites in the ethyl acetate extract of PhS 6-1 strain using a Shimadzu GC–MS QP2010 Ultra system. Helium served as the carrier gas at 1.31 mL/min, and the analysis followed the method of Dahibhate *et al.*, with minor modifications. [23]. Shimadzu real-time software was used to operate the GC–MS system, manage chromatographic and mass-spectrometric parameters, and handle data acquisition and processing. Compound identification was performed using



**Figure 4.** Phylogenetic analysis of 16S rRNA gene sequences of *Bacillus pumilus* strain PhS 6-1 isolated from the stem of *Phyllanthus acidus* (L.) Skeels. The sequences were aligned using ClustalW in MEGA 11 software. A phylogenetic tree was constructed using the maximum likelihood method. Bootstrap values are shown as percentages based on 1000 replicates. *Bacillus cereus* MG708176.1 (ATCC 14579) was used as an outgroup.

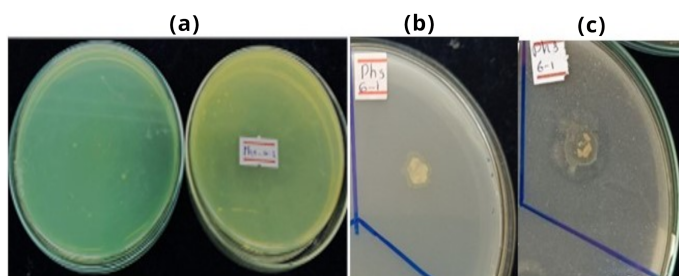


**Figure 5.** Antagonistic activity of *Bacillus pumilus* strain PhS 6-1 against the plant pathogenic fungi *Fusarium oxysporum* (FO), *Fusarium solani* (FS), OR3, *Pythium* sp. (P), and *Rhizoctonia solani* (RS).

the NIST library. All results are presented as the mean  $\pm$  standard error of the mean (SEM) from at least three independent measurements. Statistical analyses were performed using Microsoft Office 2010.

## Results and Discussion

While endophytic *Bacillus* species are known primarily for their agricultural benefits, some studies suggest that they also possess potential for medicinal applications [24]. Certain endophytic *Bacillus* species produce a variety of compounds exhibiting antimicrobial activity against bacteria, fungi, and viruses, which may contribute to the discovery of new antibiotics or antimicrobial compounds [25; 26]. Additionally, some reports indicate that compounds produced by endophytic *Bacillus* may have immunomodulatory effects, potentially influencing the immune system in ways beneficial to human health [27]. Antibacterial and antioxidant properties have also been identified in metabolites extracted from endophytic microorganisms [28; 29; 30; 31]. In this study, several endophytes were isolated from the stems of *Phyllanthus acidus* (L.) Skeels. All the isolated endophytes were screened for biological activities via daily assessment of antioxi-



**Figure 6.** Nitrogen fixation activity detection (a), phosphate solubilization (b), and K decomposition (c) of *Bacillus pumilus* strain PhS 6-1

dant, antiglycation, and antimicrobial activities for 7 days. The endophytic strain PhS 6-1 was the most promising strain and was selected for further studies (**Figure 1**). The incubation period is a critical factor influencing the production of bioactive metabolites by endophytic bacteria, and the optimal incubation time for the PhS 6-1 strain was found to be 5 days to produce its maximum bioactive metabolite.

#### Biochemical, morphological, and molecular characteristics of *Bacillus pumilus* PhS 6-1

Biochemical characterization of the *Bacillus pumilus* PhS 6-1 strain revealed that it was positive to the catalase test, Voges-Proskauer test, indole test, and casein hydrolysis test and negative to the oxidase test, methyl red test, gelatin liquefaction test, starch hydrolysis test, citrate utilization test, and urease test. The PhS 6-1 strain is alkaline in the TSI test and does not produce any gases. For the sugar fermentation tests, the PhS 6-1 strain can utilize sucrose, dextrose, glucose and mannitol and cannot use lactose or sorbitol. The isolated strain PhS 6-1 appeared as a gram-positive, rod-shaped bacterium with colonies of medium size and circular, smooth, flat, entire, off-white colonies with a moist consistency and negative KOH string reactions, which are typical characteristics of the genus *Bacillus*. Scanning electron microscopy (SEM) analysis also revealed that PhS 6-1 is a small rod-shaped bacillus (**Figure 2**).

For the molecular characterization of PhS 6-1, a BLAST search of the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) revealed that the 1500 bp long 16S rRNA gene sequence was closely related to *Bacillus pumilus*, with 99.93% similarity (**Figures 3, 4**). A phylogenetic tree was constructed with 24 homologous 16S rRNA sequences, including PhS 6-1, using *Bacillus cereus* ATCC14579 (MG708176.1) as an outgroup sequence. Phylogenetic analysis revealed that PhS 6-1 is closely related to *B. pumilus* strains PB4, EnB-sys6, and KSU 12, which share 99.93% 16S rRNA sequence similarity. The 16S rRNA gene sequence of the isolated strain PhS 6-1 was deposited at GenBank under accession number OQ690493.

#### Extraction of secondary metabolites from *Bacillus pumilus* PhS 6-1

The isolated strain PhS 6-1 was inoculated into nutrient broth, incubated for 5 days and extracted using the solvent partition

method. Following extraction, 3.6361 g of crude metabolites were obtained from 18.6 L of broth, corresponding to a yield of 0.1955 g/L. Although the yield is adequate for preliminary screening, it is considered moderate and highlights the need for process optimization to enhance metabolite production. Factors such as medium composition, incubation period, aeration, and overall fermentation conditions are likely to influence metabolite yield [32]. Systematic optimization of these parameters will be necessary to improve productivity and to facilitate the eventual scale-up of metabolite production for industrial or commercial applications.

#### *In vitro* DPPH free radical scavenging activity

In DPPH free radical scavenging assay, the extract of PhS 6-1 had mild inhibitory effect, reducing radical formation by  $12.77 \pm 3.34\%$  at 500  $\mu\text{g/mL}$  concentration. While this result suggests the presence of antioxidant compounds within the extract, it is notably lower than the potent scavenging activity displayed by ascorbic acid, the standard antioxidant, which demonstrated a  $98.04 \pm 0.95\%$  reduction in radical formation at the same concentration. These findings suggest that the PhS 6-1 extract has antioxidant potential, but enhancing its effectiveness may require further optimization of the extraction or purification of specific bioactive compounds.

#### Total phenolic content

Total phenolics present in the PhS 6-1 extract was quantified via the Folin-Ciocalteu method. A standard curve was constructed using gallic acid at concentrations ranging from 0 to 3.33 mg/L, resulting in the equation  $y = 0.7512x + 0.1043$  ( $R^2 = 0.9981$ ). The extract was found to contain  $39.39 \pm 1.06$  mg gallic acid equivalents per gram of extract. Further analyses, such as LC-MS or other chromatographic methods, could be conducted to identify and quantify specific phenolic compounds within the extract to obtain a deeper understanding of the potential health benefits of PhS 6-1.

#### *In vitro* antiglycation activity

The antiglycation activity of the extract of PhS 6-1 was evaluated via a nonenzymatic glycation reaction with a BSA-glucose model. The ethyl acetate extract of PhS 6-1 was not active in terms of antiglycation activity and had no percent inhibition. The absence of antiglycation activity in this specific assay does not necessarily imply the absence of antiglycation compounds within the extract and it could be due to the fact that the key metabolites responsible for inhibition of Advanced Glycation End products (AGEs) were present below the effective concentration in the crude extract, which aligns with the concentration-dependent nature of antiglycation activity reported by Roudbari M. *et al.* [33]. Further biological assays, such as  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory assays, glucose uptake and glucose adsorption assays, could be used to study the potential biological activities of the extracts, especially for antidiabetic potentials.

**Table 1.** Antimicrobial activity of the ethyl acetate extract of the *Bacillus pumilus* strain PhS 6-1 isolated from the stem of *Phyllanthus acidus* (L.) Skeels

Sample Name	Zone Diameter (mm)					
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>B. cereus</i>	MRSA
PhS 6-1	11.33 ± 0.58	12.33 ± 1.53	19.33 ± 1.15	20.67 ± 1.15	18.00 ± 0.00	17.33 ± 0.58
70% Ethanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Chloramphenicol	19.67 ± 1.97	12.67 ± 1.53	19.50 ± 1.76	22.83 ± 1.83	16.67 ± 2.94	19.00 ± 1.73

**Table 2.** The MIC and MBC values of the ethyl acetate extract of the *Bacillus pumilus* strain PhS 6-1 isolated from the stem of *Phyllanthus acidus* (L.) Skeels

Sample Name	Susceptibility of bacteria to tested samples											
	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>		<i>E. faecalis</i>		<i>B. cereus</i>		MRSA	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
PhS 6-1	25	>100	12.5	>100	25	50	12.5	100	12.5	>100	50	>100
70% Ethanol	0	0	0	0	0	0	0	0	0	0	0	0
Chloramphenicol	4	64	16	16	2	64	32	>64	16	>64	16	64

MIC and MBC values of PhS 6-1 are expressed in mg/mL, whereas those of chloramphenicol are expressed in µg/mL.

### Antimicrobial activity

The ethyl acetate extract of the PhS 6-1 displayed notable antimicrobial activity against a range of gram-positive and gram-negative bacteria. Notably, it demonstrated strong inhibitory effects against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and multidrug-resistant *Staphylococcus aureus*. The zone of inhibition for PhS 6-1 ranged from 11.33 ± 0.58 mm to 20.67 ± 1.15 mm, indicating its broad-spectrum antimicrobial activity (**Table 1**). The solvent control (70% ethanol) showed no inhibitory activity against any of the tested microorganisms, demonstrating its suitability for dissolving the extracts. Chloramphenicol, a broad-spectrum antibiotic, served as a positive control and exhibited significant inhibition zones against all bacteria, with values ranging from 12.67 ± 1.53 mm to 22.83 ± 1.83 mm. Determination of MIC/MBC values of PhS 6-1 against all the above tested bacterial species also revealed the remarkable antimicrobial activities of PhS 6-1 which were ranging from 12.5 to 25 mg/mL for MIC and ranging from 50 to >100 mg/mL for MBC (**Table 2**). The extract exhibited the greatest susceptibility against *P. aeruginosa*, *E. faecalis*, and *B. cereus*, each with MIC values of 12.5 mg/mL, although MBC values were generally high (≥100 mg/mL), indicating predominantly bacteriostatic rather than bactericidal effects. Activity against *E. coli* and *S. aureus* was weaker, with MICs of 25 mg/mL, and no bactericidal effect was observed at the highest concentration tested for *E. coli*.

70% ethanol used as the reconstitution solvent showed no antimicrobial activity in all tested bacteria. Therefore, it could be said that the observed effects were only attributable to PhS 6-1 rather than the solvent effect. The positive control, chloramphenicol, demonstrated strong antimicrobial activity across all the strains tested, as expected, with substantially lower MIC values (2–32 µg/mL). However, some strains such as *S. aureus*, *E.*

*faecalis*, and *B. cereus* required higher concentrations to achieve bactericidal action, highlighting known variability in chloramphenicol sensitivity [34]. Overall, these findings indicate that the ethyl acetate extract of PhS 6-1 exhibits strong antimicrobial activity and may serve as a promising source of novel antimicrobial compounds. This observation is consistent with the work of Dimkić, Stanković, and colleagues, who reported that lipopeptides from ethyl acetate extract—particularly members of the iturin family—produced by various *Bacillus* strains demonstrated notable antimicrobial potency.

### Cytotoxicity by hemolytic assay and antagonistic activity

The cytotoxicity of the extract from PhS 6-1 was evaluated to assess its potential safety for medicinal applications through hemolytic assay. The extract's cytotoxicity was compared to that of a positive control (1% Triton X-100, known to induce complete hemolysis) and a negative control (phosphate-buffered saline, PBS). The results indicated that the ethyl acetate extract of PhS 6-1 exhibited significantly lower hemolytic activity than Triton X-100. At 1000 µg/mL, the extract induced 18.06 ± 2.9% hemolysis, compared to 100% hemolysis caused by Triton X-100. In contrast, PBS, which served as a negative control, induced minimal hemolysis (3.42 ± 0.19%). These findings suggest that the ethyl acetate extract of PhS 6-1 has low cytotoxicity to the human RBCs and can be considered a safe candidate for further development as a potential therapeutic agent. The extract of PhS 6-1 demonstrated significant antifungal activity against a series of plant pathogenic fungi, including *Fusarium oxysporum* (FO), *Fusarium solani* (FS), OR3, *Phythium* sp. (P), and *Rhizotonia solani* (R) (**Figure 5**). In dual culture assays, the extract inhibited all the tested fungi, indicating its ability to suppress fungal growth. The extent of inhibition varied among the different fungal species, with *Phythium* sp. (P) and *Rhizotonia solani* (R) showing the most pronounced inhibition. These results suggest

**Table 3.** Metabolites detected in the ethyl acetate extract of *Bacillus pumilus* strain PhS 6-1

No.	Compound name	Retention time	Formula	m/z	Molecular weight	Area (%)
1	2,3-Butanediol	2.337	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	45.00	90	7.05
2	Nonane	3.411	C <sub>9</sub> H <sub>20</sub>	57.05	128	2.85
3	Undecane	4.896	C <sub>11</sub> H <sub>24</sub>	57.05	156	8.37
4	1-Tridecene	9.563	C <sub>13</sub> H <sub>26</sub>	55.05	182	3.77
5	1-Pentadecene	14.083	C <sub>15</sub> H <sub>30</sub>	55.05	210	8.79
6	1-Octadecene	18.796	C <sub>18</sub> H <sub>36</sub>	83.05	252	11.82
7	1-Nonadecene	22.590	C <sub>19</sub> H <sub>36</sub>	83.05	266	10.91
8	1-(+)-Ascorbic acid, 2,6-dihexadecanoate	25.499	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	73.00	652	15.42
9	Behenic alcohol	25.879	C <sub>22</sub> H <sub>46</sub> O	83.05	326	6.52
10	9-octadecenoic acid, E-	29.097	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	55.05	282	7.98
11	N-Tetracosanol	29.749	C <sub>24</sub> H <sub>50</sub> O	57.05	354	3.04
12	17-Pentatriacontene	31.995	C <sub>35</sub> H <sub>70</sub>	55.05	490	2.09

that the ethyl acetate extract of PhS 6-1 contains bioactive compounds with potent antifungal properties, which could be useful in the development of novel biocontrol agents for plant diseases.

#### Plant growth-promoting (PGP) activity

The nitrogen fixation activity was tested via nitrogen-free mineral media to determine the ability of PhS 6-1 to fix atmospheric nitrogen (**Figure 6 a**). The results indicated that PhS 6-1 could fix nitrogen, as a few colonies grew on nitrogen-free mineral media, demonstrating its ability to utilize atmospheric nitrogen. When the phosphate solubilization activity of PhS 6-1 was determined by using Pikovskayas medium, it was found that it was unable to solubilize the phosphate (**Figure 6 b**). The potassium (K) decomposition ability of the PhS 6-1 strain was also evaluated, and the results indicated that PhS 6-1 presented a moderate level of K-decomposing activity, with K-decomposing indices of  $0.163 \pm 0.02$ ,  $0.541 \pm 0.039$ , and  $0.57 \pm 0.083$ , respectively, after 3, 5, and 7 days of incubation (**Figure 6 c**). These results suggest that the strain has the capacity to solubilize potassium from insoluble sources. Potassium is a crucial macronutrient for plant growth and contributes to various physiological processes, such as photosynthesis, enzyme activation, and disease resistance [35]. By solubilizing potassium from insoluble sources, the PhS 6-1 strain can potentially increase plant nutrition and improve crop yields. Therefore, PhS 6-1 may have potential as a biofertilizer for improving soil fertility and plant growth. However, further research is needed to determine its effectiveness and assess the strain's effectiveness in field applications.

#### Metabolite profile of *Bacillus pumilus* PhS 6-1

Metabolite profiling of PhS 6-1 extract was performed via GC-MS analysis. The presence of several metabolites with varying peak intensities and retention times was revealed. A total

of 12 compounds were putatively identified by using the NIST library. The most dominant secondary metabolite found in the ethyl acetate extract of PhS 6-1 was 1-(+)-ascorbic acid, 2,6-dihexadecanoate, with a relative abundance of 15.42%, followed by 1-octadecene (11.82%), 1-nonadecene (10.91%), 1-pentadecene (8.79%), undecane (8.37%), 9-octadecenoic acid, E- (7.98%) and 2,3-butanediol (7.05%) (**Table 3**). The presence of these compounds suggests that the ethyl acetate extract of PhS 6-1 may exhibit multiple biological activities, including antioxidant, antimicrobial, and anti-inflammatory potentials, and these findings suggest that further investigations into the potential therapeutic applications of the extract and its individual components are needed.

#### Conclusions

This study examined the potential of the endophytic *Bacillus pumilus* PhS 6-1, which was isolated from *Phyllanthus acidus*, as a source of bioactive compounds. The results indicated that the ethyl acetate extract of PhS 6-1 displayed promising biological activities, including moderate antioxidant and strong antimicrobial activity against a series of bacteria, including multidrug-resistant strains. The strain exhibited moderate potassium-solubilizing activity, demonstrating its potential for enhancing plant nutrition. The extract also demonstrated significant antifungal activity against various plant pathogens. GC-MS analysis discovered the presence of twelve bioactive compounds. These compounds were known for their antioxidant, antimicrobial, and anti-inflammatory properties [36; 37]. While the extract showed no antiglycation activity, further investigation of its constituents may reveal potential antiglycation agents. These findings suggest that the endophytic *Bacillus pumilus* PhS 6-1 and its metabolites might have promising applications in agriculture and potentially in human health, warranting further research and development.

## Acknowledgments

The authors are grateful to Dr. Tin Myo Thant, Department of Chemistry, University of Mandalay, Ministry of Education, for GC–MS analysis, and Dr. Min Shan Htun, Yangon Technology University, for SEM analysis.

## Funding

This research work was financially supported by the Department of Biotechnology Research (DBR), Ministry of Science and Technology, Myanmar (Project Grant No. Bio/KSe-23/WH-SM/373 (14)).

## Reference

- White JF, Kingsley KL, Zhang Q, Verma R, Obi N, Dvinskikh S, et al. Endophytic microbes and their potential applications in crop management. *Pest management science*. 2019;75(10):2558-65.
- Rana KL, Kour D, Kaur T, Devi R, Yadav AN, Yadav N, et al. Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie Van Leeuwenhoek*. 2020;113(8):1075-107.
- Ek-Ramos MJ, Gomez-Flores R, Orozco-Flores AA, Rodríguez-Padilla C, González-Ochoa G, Tamez-Guerra P. Bioactive products from plant-endophytic gram-positive bacteria. *Frontiers in microbiology*. 2019;10:463.
- Dobrzyński J, Jakubowska Z, Dybek B. Potential of *Bacillus pumilus* to directly promote plant growth. *Frontiers in microbiology*. 2022;13:1069053.
- Rabelo ACS, Costa DC. A review of biological and pharmacological activities of *Baccharis trimera*. *Chemico-Biological Interactions*. 2018;296:65-75.
- Brooks R, Goldson-Barnaby A, Bailey D. Nutritional and medicinal properties of *Phyllanthus acidus* L.(Jimbilin). *International Journal of Fruit Science*. 2020;20(sup3):S1706-10.
- Strobel G, Yang X, Sears J, Kramer R, Sidhu RS, Hess W. Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*. *Microbiology*. 1996;142(2):435-40.
- Vincent J, Humphrey B. Taxonomically significant group antigens in *Rhizobium*. *Microbiology*. 1970;63(3):379-82.
- Khan MS, Gao J, Chen X, Zhang M, Yang F, Du Y, et al. Isolation and characterization of plant growth-promoting endophytic bacteria *Paenibacillus polymyxa* SK1 from *Lilium lancifolium*. *BioMed Research International*. 2020;2020(1):8650957.
- Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*. 2016;33(7):1870-4.
- Khan MS, Gao J, Chen X, Zhang M, Yang F, Du Y, et al. The endophytic bacteria *Bacillus velezensis* L1e-9, isolated from *Lilium leucanthum*, harbors antifungal activity and plant growth-promoting effects. *Journal of Microbiology and Biotechnology*. 2020;30(5):668.
- Lee SK, Mbwambo ZH, Chung H, Luyengi L, Gamez E, Mehta RG, et al. Evaluation of the antioxidant potential of natural products. *Combinatorial chemistry & high throughput screening*. 1998;1(1):35-46.
- Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*. 2005;53(10):4290-302.
- Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols*. 2007;2(4):875-7.
- THIDA M, NYEIN CM, CHAN KN, NEW MT, EI SL, MYA KM. *In Vitro* study on Antidiabetic Potential of Crude Plant Extracts by Attenuating Oxidative-Stress and Advanced Glycation End-Product. *International Journal of Pharmaceutical Research* (09752366). 2021;13(2).
- Starowicz M, Zieliński H. Inhibition of advanced glycation end-product formation by high antioxidant-leveled spices commonly used in European cuisine. *Antioxidants*. 2019;8(4):100.
- Grzegorzczuk-Karolak I, Gołąb K, Gburek J, Wysokińska H, Matkowski A. Inhibition of advanced glycation end-product formation and antioxidant activity by extracts and polyphenols from *Scutellaria alpina* L. and *S. altissima* L. *Molecules*. 2016;21(6):739.
- Schlegel HG, Zaborosch C. *General microbiology*. Cambridge university press; 1993.
- Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods*. 2007;42(4):321-4.
- Powell W, Catranis C, Maynard C. Design of self-processing antimicrobial peptides for plant protection. *Letters in applied microbiology*. 2000;31(2):163-8.
- Moe TS, Thida M, Win HH, Zar Wynn Myint K, Win ZK, Htay NN. Multi-antidiabetic Properties and Cytotoxic Activities of Selected Indigenous Myanmar Medicinal Plants. *Journal of Herbs, Spices & Medicinal Plants*. 2023;29(1):24-38.
- Al-Hussini HS, Al-Rawahi AY, Al-Marhoon AA, Al-Abri SA, Al-Mahmooli IH, Al-Sadi AM, et al. Biological control

- of damping-off of tomato caused by *Pythium aphanidermatum* by using native antagonistic rhizobacteria isolated from Omani soil. *Journal of Plant Pathology*. 2019;101(2):315-22.
23. Dahibhate NL, Dwivedi P, Kumar K. GC-MS and UHPLC-HRMS based metabolite profiling of *Bruguiera gymnorhiza* reveals key bioactive compounds. *South African Journal of Botany*. 2022;149:1044-8.
24. Tripathi A, Pandey P, Tripathi SN, Kalra A. Perspectives and potential applications of endophytic microorganisms in cultivation of medicinal and aromatic plants. *Frontiers in plant science*. 2022;13:985429.
25. Bolivar-Anillo HJ, González-Rodríguez VE, Cantoral JM, García-Sánchez D, Collado IG, Garrido C. Endophytic bacteria *Bacillus subtilis*, isolated from *Zea mays*, as potential biocontrol agent against *Botrytis cinerea*. *Biology*. 2021;10(6):492.
26. Sharma M, Mallubhotla S. Diversity, antimicrobial activity, and antibiotic susceptibility pattern of endophytic bacteria sourced from *Cordia dichotoma* L. *Frontiers in microbiology*. 2022;13:879386.
27. Tshikhudo PP, Ntushelo K, Mudau FN. Sustainable applications of endophytic bacteria and their physiological/biochemical roles on medicinal and herbal plants. *Microorganisms*. 2023;11(2):453.
28. Khan MS, Gao J, Chen X, Zhang M, Yang F, Du Y, et al. The endophytic bacteria *Bacillus velezensis* L1e-9, isolated from *Lilium leucanthum*, harbors antifungal activity and plant growth-promoting effects. *Journal of Microbiology and Biotechnology*. 2020;30(5):668.
29. Rochín-Hernández LS, Rochín-Hernández LJ, Flores-Cotera LB. Endophytes, a potential source of bioactive compounds to curtail the formation-accumulation of advanced glycation end products: A Review. *Molecules*. 2022;27(14):4469.
30. Chowdhury SP, Hartmann A, Gao X, Borriss R. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42—a review. *Frontiers in microbiology*. 2015;6:780.
31. Photolo MM, Mavumengwana V, Sitole L, Tlou MG. Antimicrobial and antioxidant properties of a bacterial endophyte, *Methylobacterium radiotolerans* MAMP 4754, isolated from *Combretum erythrophyllum* seeds. *International Journal of microbiology*. 2020;2020(1):9483670.
32. Kortam YG, El-Rahim A, Wafaa M, Barakat OS, Segai UM, Zakaria M, et al. Optimization of antibiotic production against by a mutant strain of isolated from Egyptian Hamam Pharaon hot springs. *Egyptian Pharmaceutical Journal*. 2022;21(4):502-14.
33. Roudbari M, Barzegar M, Sahari MA. Pistachio green hull and pomegranate peel extracts as two natural antiglycation agents. *Food Science & Nutrition*. 2024;12(5):3688-95.
34. Fayyaz M, Mirza IA, Ahmed Z, Abbasi SA, Hussain A, Ali S. *In vitro* susceptibility of chloramphenicol against methicillin-resistant *Staphylococcus aureus*. *J Coll Physicians Surg Pak*. 2013;23(9):637-40.
35. Hasanuzzaman M, Bhuyan MB, Nahar K, Hossain MS, Mahmud JA, Hossen MS, et al. Potassium: a vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy*. 2018;8(3):31.
36. Khan I, Javaid A. Hexane soluble bioactive components of leaf extract of quinoa. 2022.
37. Sumerta IN, Yuliani Y, Komalasari M, Purnaningsih I, Kanti A. Yeast species and bioactive-compounds of traditional rice wine originated from lombok island, Indonesia. *agriTECH*. 2022;42(1):48-54.