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Beneficial Endophytic Bacteria Associated with Medicinal Plant Vernonia anthelmintica flowers: Characterization and Biological activities

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Abstract

Endophytic bacteria associated with medicinal plants are a vital component of the plant microbiome and represent a valuable biological resource. This study investigates the diversity and biological activities of endophytic bacteria isolated from the flowers of *Vernonia anthelmintica*, a medicinal plant native to China. The research focuses on evaluating the cytotoxic, antimicrobial, antioxidant, and antidiabetic properties of natural products derived from these bacteria, as well as their effects on melanin synthesis and tyrosinase activity in B16 cells. A total of 32 bacterial strains were isolated and cultured, of which eight crude extracts exhibiting antimicrobial activity were selected for further analysis. These isolates were identified as *Bacillus paranthracis* XJB-1, *Bacillus safensis* XJB-51, *Bacillus pumilus* XJB-30, *Bacillus halotolerans* XJB-36, *Bacillus subtilis* XJB-57, *Streptococcus lutetiensis* XJB-76, *Priestia megaterium* XJB-41), *Paenibacillus alvei* XJB-13). Among them, *P. megaterium* XJB-41 demonstrated the strongest pharmacological potential, warranting further investigation to optimize its culture conditions for enhanced bioactive compound production. The optimal growth conditions for *P. megaterium* XJB-41 were determined to be LB and Nutrient Broth (NB) media, with peptone as the carbon source and yeast extract as the nitrogen source, under a 24-hour incubation period. These conditions significantly enhanced both bacterial growth and metabolite yield. Moreover, two secondary metabolites: cyclo(D-leu-L-pro) [1] and 2-benzoxazolone [2] were isolated for the first time from the ethyl acetate fraction of *P. megaterium* XJB-41. This strain shows significant promise as a natural source for the development therapeutic agents targeting vitiligo, cancer, and infectious diseases.

Introduction

Endophytic microorganisms, including bacteria and fungi, reside within plant tissues over extended periods without causing visible harm to their hosts [1]. They are characterized by their ability to colonize the intercellular spaces during part or all of a plant`s life cycle [2]. These microorganisms often form symbiotic relationships with their host plants, contributing to enhanced growth, increased resistance to pathogens, and improved tolerance to environmental stresses. In return, plants offer endophytes a protected habitat and a steady supply of nutrients [3]. Endophytic microbiomes, which remain asymptomatic within plant tissues, have gained attention as promising sources of novel secondary metabolites with diverse biological activities [4]. These pharmacologically important compounds can be extracted and isolated from cultured endophytes under optimized growth conditions [5]. The ability of endophytes to produce such compounds is closely linked to their biosynthesis of natural compounds and phytohormones [6]. Notably, many secondary metabolites produced by endophytic bacteria are structurally similar to those found in thier host plants [7]. The structurally diverse and biologically active metabolites generated by endophytes offer great potential in modern medicine and the pharmaceutical industry, particularly for the treatment of life-threatening and chronic diseases [8]. Endophytic bacteria are known to produce a wide range of antimicrobial agents, including antibiotics, bacteriocins, and lipopeptides, which can inhibit the growth of various pathogens: bacteria, fungi, and viruses, thus protecting host plants and applications in agriculture and human health. Moreover, endophytic bacteria represent a rich reservoir of bioactive compounds with antimicrobial, anticancer, antioxidant, and other

therapeutic properties. In recent years, interest in exploring the chemical diversity and biological activities of endophytic microorganisms has grown significantly [9–11]. Numerous studies have reported the isolation, characterization, and pharmacological activities of endophytic microorganisms. For instance, the bacterial species *Pseudomonas aeruginosa*, isolated from the leaves of *Anredera cordifolia*, was found to produce antibiotics and exhibited antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus mirabilis* [12].

Zhao et al. [13] reported the isolation of an endophytic *Streptomyces* species capable of producing novel peptide-type compounds with significant cytotoxic activity against the human cancer cell lines Hep3B2.1-7 and H1299. Numerous bacterial endophytes are known to produce secondary metabolites with antimicrobial properties. Among them, several species within the genus *Bacillus* have demonstrated particularly strong antimicrobial activity. Endophytic bacteria associated with plants are considered promising candidates for the development of new antibiotic agents targeting human pathogens [14]. Previous studies have shown that *Bacillus* species exhibit notable antimicrobial activity against a wide range of pathogenic microorganisms. For example, *B. subtilis* YRL02, *B. licheniformis* YRL03, and *B. subtilis* YRL07 have shown moderate antifungal activity against plant pathogens such as *Phytophthora capsici*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pythium ultimum* [15]. Christina et al. [16] also reported that several plant-associated endophytic bacteria exhibit significant antimicrobial potential. In our previous study, we focused on the isolation, identification, and biological activities of endophytic bacteria associated with the roots of *Vernonia anthelmintica*. The results revealed that both the crude extracts and pure secondary metabolites produced by these endophytes exhibited significant antimicrobial, antioxidant, and antidiabetic activities, along with effects on melanin synthesis and tyrosinase activity in B16 cells [14, 17, 18]. To further explore the biodiversity and pharmacological potential of endophytic bacteria in the aerial parts of *V. anthelmintica*, it is essential to investigate those associated with its aerial parts. In the present study, we isolated and molecularly identified endophytic bacteria from the flowers of *V. anthelmintica* and evaluated their antimicrobial, cytotoxic, antidiabetic, and antioxidant activities, as well as their influence on melanin content in B16 cells. Our findings may contribute to the development of new pharmacological agents and provide a foundation for future therapies targeting a range of diseases. Given their diverse metabolic capabilities, endophytic microorganisms are attracting growing interest in both research and industry as a valuable resource for discovering novel bioactive compounds and promoting environmentally sustainable agricultural practices.

Materials and Methods

Isolation of endophytic bacteria

Before surface sterilization, the flowers were thoroughly rinsed under running tap water to remove any surface debris [14]. They were then briefly rinsed with sterile distilled water and subjected to surface sterilization by immersion in 99.9% ethanol for 2 minutes, followed by a 2-minute treatment with 10% sodium hypochlorite (NaClO). After sterilization, the flowers were rinsed five times with sterile distilled

water and air-dried on a sterile paper towel for 5 minutes. Fresh sterile flowers, weighing approximately 5–10 grams, were cut into small pieces and ground using a sterile mortar and pestle. One gram of the homogenized tissue was transferred into plastic tubes containing 9 mL of sterile phosphate-buffered saline (PBS) (20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and vortexed for 1 minute using a Vortex Biosan B-1. The resulting suspension was serially diluted (10^1 to 10^5) and plated onto Nutrient Agar (NA) (composition: 5 g/L peptone, 3 g/L yeast extract, 5 g/L agar, and 5 g/L NaCl; pH 7.4) and Luria-Bertani (LB) medium (composition: 10 g/L peptone, 5 g/L yeast extract, 5 g/L agar, and 5 g/L NaCl). The incubated plates were incubated at 28°C for four days. Distinct bacterial colonies, characterized by differences in shapes, colors, and textures, were selected and streaked onto fresh nutrient agar plates to obtain pure cultures. These plates were incubated for an additional 72 hours to confirm the purity of the isolates. Pure colonies exhibiting diverse morphologies were subsequently used for DNA extraction.

Identification of endophytic bacteria

All isolated bacterial strains were cultured in 250 mL Erlenmeyer flasks containing 50 mL of fresh Nutrient Broth (NB) and incubated at 28°C for 24 hours with shaking at 200 rpm. Genomic DNA was extracted using previously described protocols with slight modifications. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GAAAGGAGGTGATCCAGCC-3') (Sigma-Aldrich, St. Louis, MO, USA), according to the method described by Weisburg et al. [19]. PCR amplification was carried out using a PTC-200 thermal cycler (Bio-Rad Laboratories, USA). The resulting 16S rRNA gene sequences were compared to existing sequences in the NCBI database using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov) for taxonomic identification. Phylogenetic relationships were inferred using the neighbor-joining method [20, 21], and evolutionary distances were calculated with the Maximum Composite Likelihood method [22]. The robustness of the phylogenetic tree was constructed with bootstrap analysis with 500 replicates. Branch lengths were proportional to evolutionary distances, which were calculated and expressed as the number of base substitutions per site. A total of 40 nucleotide sequences were included in the analysis, with ambiguous positions removed using the pairwise deletion approach. The final alignment comprised 1,604 nucleotide positions. All evolutionary analyses were conducted using MEGA X software [23].

Preparation of bacterial crude extract

Endophytic bacterial isolates were initially cultured in LB liquid medium (10 g/L peptone, 5 g/L yeast extract, and 5 g/L NaCl) and incubated at pH 7.0 and 28°C for 48 hours with shaking at 160 rpm. Following this initial growth phase, seed cultures were transferred to 1 L Erlenmeyer flasks containing 500 mL of fresh LB broth and incubated for an additional 24 hours under the same conditions. During this fermentation period, the endophytic bacteria produced bioactive natural products in the culture medium. After incubation, the cultures were centrifuged at 8000 rpm for 15 minutes at 4°C to separate the supernatant. The cell-free supernatant was subsequently extracted with ethyl acetate using a

separatory funnel, and the organic phase was concentrated by rotary evaporation to yield the crude extract. The crude extract was stored at 4°C for further chemical and biological analyses.

Biological activities of endophytic bacteria

The total crude extracts obtained from all selected bacterial strains were dissolved in DMSO and evaluated for antimicrobial, antioxidant, antidiabetic, and cytotoxic activities, as well as for their effects on melanin content and tyrosinase activity.

Antimicrobial activity

An *in vitro* antimicrobial assay was performed to evaluate the crude extracts of endophytic bacteria against three pathogenic microorganisms: the Gram-positive bacterium *Staphylococcus aureus* (ATCC 6538), the Gram-negative bacterium *Escherichia coli* (ATCC 11229), and the fungus *Candida albicans* (ATCC 10231). The antimicrobial activity was assessed using the paper disk diffusion method, following protocols described in previous studies [24, 25]. The zone of inhibition was determined by measuring the diameter of the clear area around each disk where microbial growth was inhibited.

Melanin content assay and tyrosinase activity

Cell Culture

Murine B16 melanoma cell lines (B16F10) were obtained from the Chinese Academy of Sciences (CAS), China. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Life Technologies, Waltham, MA, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 U/mL of penicillin G, along with 100 mg/mL of streptomycin (Gibco-BRL, Grand Island, NY, USA). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO2.

Melanin Measurement and Tyrosinase Activity Assay

The effects of natural products synthesized by bacterial endophytes associated with *V. anthelmintica* flowers on melanin synthesis and tyrosinase activity were evaluated using the method described by Rustamova et al. [26, 27].

MTT assay

To evaluate anticancer activity, secondary metabolites from crude ethyl acetate extracts, along with pure compounds isolated from endophytic bacteria, were evaluated using the MTT assay ([3-(4,5-dimethylthiazolyl)-2,5-diphenyl tetrazolium bromide]), following the methodology described by Rustamova et al. [24, 26, 28, 29] with some slight modifications. The assay was conducted on MCF-7 (breast cancer), HeLa (cervical cancer), and HT-29 (colon cancer) cell lines. MCF-7 and HeLa cells were

obtained from the Shanghai Cell Bank (Shanghai, China), while HT-29 cells were sourced from Procell Life Science & Technology Co., Ltd. (Wuhan, China). Doxorubicin (DOX), purchased from BBI Inc. (Shanghai, China), was used as the positive control.

Antidiabetic Activity (PTP-1B Inhibition Assay)

To investigate the *in vitro* inhibitory activity of protein tyrosine phosphatase 1B (PTP1B) by crude extracts from bacterial endophytes, the evaluation was conducted using previously established methods [14], with a known PTP1B enzyme inhibitor as the positive control.

Antioxidant activity (DPPH radical scavenging activity)

To assess the antioxidant potential of crude extracts from endophytic bacteria, the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated following the methodology described in our previous study [30]. A 100-µL aliquot of the sample (at a concentration of 50 mg/mL) was mixed with 100 µL of a freshly prepared 0.2 mM DPPH solution in each well of a 96-well microplate. The microplate was incubated in the dark at room temperature for 30 minutes. Absorbance was then measured at 517 nm, and the inhibition rate (IR) was calculated. Ascorbic acid was used as the positive control. The inhibition rate for both the positive control and the samples was determined using the following formula:

$$IR\,(\%) = 1 - \left(\frac{A_0 - A_1}{A_0} \right) \times\ 100$$

Where A_0 refers to the absorbance of the blank, and A_1 denotes the absorbance of the test sample.

Optimization of culture condition

We investigated the effects of various culture media, incorporating different carbon and nitrogen sources as well as varying incubation times, on the growth and production of bioactive secondary metabolites by the most active endophytic bacterial strain. The bioactive strain *Priestia megaterium* XJB-41 was cultured in multiple media formulations, including: LB broth (peptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L), Nutrient Broth (NB) (peptone 5 g/L, yeast extract 3 g/L, NaCl 5 g/L), N-free broth (K_2HPO_4 0.1 g/L, KH_2PO_4 0.4 g/L, $MgSO_4$ 0.2 g/L, NaCl 0.1 g/L, $CaCl_2$ 0.02 g/L, $FeCl_3$ 0.01 g/L, $NaMoO_4$ 0.001 g/L, and glucose 10 g/L), De Man, Rogosa, and Sharpe (MRS) broth (peptone 10 g/L, yeast extract 0.4 g/L, beef extract 10 g/L, glucose 20 g/L, C_2H_9NaO5 0.5 g/L, tween 80 1 mL, K_2HPO_4 0.2 g/L, and $MgSO_4$ 0.02 g/L), Tryptone Soy Broth (TSB) (tryptone 15 g/L, soya peptone 5 g/L, NaCl 5 g/L), and Tryptic Soy Dextrose Broth (TSD) (tryptone 17 g/L, soytone 3 g/L, dextrose 2.5 g/L, NaCl 5 g/L, K_2HPO_4 2.5 g/L). The medium in which *P. megaterium* XJB-41 exhibited the highest growth and production of bioactive compounds was selected as the optimal medium for further experiments.

The effect of cultivation time on the secondary metabolite production of endophytic bacteria

Incubation time is a critical factor for the growth of endophytic microorganisms and the production of secondary metabolites [31]. To evaluate the optimal incubation period for the production of bioactive secondary metabolites, cultures were inoculated at 1% (v/v) and incubated at pH 7.0 and 30°C for time intervals ranging from 24 to 240 hours.

The extracts were obtained using ethyl acetate, and the yield of the produced metabolites was calculated (Table 1).

The effect of pH, temperature and shaking speed on syntheses of secondary metabolites

To determine the optimal pH for metabolite production, cultures were inoculated at 1% (v/v) and incubated at 30°C for 48 hours under five different pH conditions: 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 using 1 M NaOH and 0.5 M HCl (Table 1.). The synthesis of secondary metabolites by the most active endophytic bacteria and fungi in each pH-adjusted sample was subsequently assessed. To determine the optimal temperature for metabolite production, cultures were inoculated at 1% (v/v) and incubated at pH 7.0 for 48 hours at five different temperatures: 20, 25, 30, 35, 40 and 45°C. The production of secondary metabolites was then determined using the method described by Kothari and Pathak et al. [32]. To determine the optimal shaking speed for metabolite production, cultures were inoculated at 1% (v/v) and incubated at pH 7.0 and 30°C for 48 hours, with shaking speeds ranging from 120 to 190 rpm. Their impact on growth phases and secondary metabolite synthesis was evaluated.

Table 1
Effect of pH, shaking speed and temperature for growth and produced secondary metabolites by the most-active endophytic bacteria *P. megaterium* XJB-41

pH		**Temperature**		**Shaking speed**	
pH	**mg/mL**	**^0C**	**mg/mL**	**rpm**	**mg/mL**
6	145	20	229	120	155
6.5	232	25	392	130	189
7	395	30	401	140	229
7.5	398	35	348	150	392
8	335	40	174	160	402
8.5	234	45	112	170	348
9	116			180	175
				190	111

Scanning Electron Microscopy analysis of endophytic bacteria

Scanning electron microscopy (SEM) was carried out on the most active isolate, *P. megaterium* XJB-41, following the procedure described by Nongkhlaw et al. [33]. After incubation, the bacterial cells was gold-coated using an iron sputter coater (Hitachi E-1045, Japan). Morphological characteristics, including cell shape, colony diameter, and surface features, were observed using a Zeiss Supra 55 VP scanning electron microscope (Germany).

General procedures

Fractionation of the crude extract was performed using a CombiFlash system (CombiFlash RF, Teledyne ISCO, Inc., USA) equipped with an ODS-A 120C column (90 mL, 50 μm). Analytical HPLC was conducted using a DIONEX UltiMate 3000 system (Thermo, Waltham, MA, USA), while preparative HPLC was performed on a SHIMADZU LC-20A system (Shimadzu Corporation, Kyoto, Japan), combined with the columns: Xselect® HSS T3 on C18, 4.6 × 250 mm, 5 μm (Waters, USA); XSELECT CSHTM Prep C18, 5 μm (Waters Co., Milford, MA, USA). UV spectra were acquired on SHIMADZU UV-2550 UV- visible Spectrophotometer (Shimadzu Corporation, Japan). NMR spectra were acquired on MR-400 or VNMRS600 spectrometers (Varian, Palo Alto, USA) operating at 400 or 600 MHz for ^{1}H NMR and 100 or 150 MHz for ^{13}C NMR, respectively, using TMS as the internal standard. The carbon signal of the solvent (CD_3OD, 49.00 ppm) was used as a reference for ^{13}C NMR chemical shifts.

Isolation and purification of secondary metabolites of P. megaterium XJB-41

The endophytic bacterium *P. megaterium* XJB-41 exhibited stronger biological activities compared to other bacterial isolates, prompting further investigation of its chemical composition. From a pure culture of *P. megaterium* XJB-41, a total of 3.2 grams of crude extract was obtained by extracting 15 liters of the bacterial culture with ethyl acetate (EtOAc). The crude extract was fractionated using flash chromatography on an ODS-A 120c (octadecylsilyl silica gel) column. A gradient elution solvent system of methanol (MeOH) and water (H_2O), ranging from 10:90 to 100:0, was employed to obtain fractions F1 through F10. Fraction F4 (800 mg) was further chromatographed on a Sephadex LH-20 column, eluted with chloroform-methanol (1:1), and purified using preparative HPLC (SHIMADZU, XSELECT CSH™ Prep C18, 5 μm) with a gradient elution solvent system of acetonitrile (MeCN) and water (H_2O) from 60:40 to 100:0 (v/v) over 40 minutes at a flow rate of 3.0 mL/min, yielding Compounds **1** and **2** (42 mg and 138 mg, respectively). The purification of additional secondary metabolites from the ethyl acetate extract of *P. megaterium* XJB-41 is ongoing, and further experiments are in progress.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism. One-way analysis of variance (ANOVA) with subcolumn analysis was performed based on the replicates. Tukey's post hoc test was used to assess

statistical significance, with a p-value of less than 0.05 considered significant. Correlation coefficient (ρ) were calculated using the Pearson`s method.

Results and Discussion

Isolation and identification of endophytic bacteria

Bacterial endophytes are increasingly recognized as valuable natural sources of novel bioactive compounds, with numerous beneficial strains have been isolated from medicinal plants. *V. anthelmintica* is a highly valued medicinal plant spice in China, India, and Pakistan, traditionally used to treat vitiligo, skin diseases, inflammation, and various gastrointestinal conditions [34]. This study investigates the biodiversity and molecular identification of endophytic bacteria associated with the flowers of *V. anthelmintica* growing in China (Fig. 1). Eight bacterial strains were selected for molecular identification based on distinct colony morphologies such as color, shape, size observed on LB and NA media. Phylogenetic analysis (Fig. 2) and 16S rRNA gene sequencing (Supplementary information) revealed that the isolates belong to four genera and eight species: *Bacillus* (*B. paranthracis* XJB-1, *B. safensis* XJB-51, *B. pumilus* XJB-30, *B. halotolerans* XJB-36, and *B. subtilis* XJB-57), *Streptococcus* (*S. lutetiensis* XJB-76), *Priestia* (*P. megaterium* XJB-41), and *Paenibacillus* (*P. alvei* XJB-13). (Table 2). All isolated strains have been deposited in GenBank under the accession numbers MW820295, MW876153, MW876142, MW876144, MW876165, MW876162, MW876148, and MW876134.

Table 2
Qequence similarities of the endophytic bacteria isolated from the flowers of *V. anthelmintica* were compared with sequences available in GenBank.

Isolated strains sequences deposited in GenBank		**Closest match among bacteria (16S rRNA genes) (GenBank)**		
Strains	**Accession number**	**Species**	**Source**	**ID%**
XJB-1	MW820295	*B. paranthracis*	Flowers	99,93
XJB-51	MW876153	*B. safensis*	Flowers	100
XJB-30	MW876142	*B. pumilus*	Flowers	99,86
XJB-36	MW876144	*B. halotolerans*	Flowers	100
XJB-57	MW876165	*B. subtilis*	Flowers	99,93
XJB-76	MW876162	*S. lutetiensis*	Flowers	100
XJB-41	MW876148	*P. megaterium*	Flowers	100
XJB-13	MW876134	*P. alvei*	Flowers	99,93

Antimicrobial activity of endophytic bacteria

Endophytic bacteria, particularly those associated with ethnopharmacologically significant medicinal plants, have garnered significant scientific interest due to their ability to produce unique bioactive metabolites with diverse pharmacological properties. These microorganisms can synthesize secondary metabolites both *in vitro* and within the intracellular environment of their host plants, making them promising natural sources of antimicrobial agents. Numerous bacterial species, particularly those within the *Bacillus* genus, are well known for their producing secondary metabolites with potent antimicrobial activity [35]. In this study, ethyl acetate extracts from selected bacterial strains were evaluated for their *in vitro* inhibitory activity against the pathogenic bacteria *E. coli*, *S. aureus*, and the fungus *C. albicans*. The *Bacillus* species exhibited moderate to high antimicrobial activity against all three pathogens. Notably, the strains *S. lutetiensis* XJB-76 and *P. alvei* XJB-13 demonstrated strong antibacterial activity against *S. aureus*, with inhibition zones of 16 mm and 13 mm, respectively. These strains also showed moderate antifungal activity against *C. albicans*, with inhibition zones of 10 mm and 11 mm, respectively. Furthermore, the ethyl acetate extract of *P. megaterium* XJB-41 exhibited significant antibacterial activity against *E. coli* and *S. aureus*, with inhibition zones of 16 mm and 17 mm, respectively, indicating its superior potency compared to the other strains (Table 3).

Table 3
In vitro antimicrobial activity (ZOI) of crude extracts of endophytic bacteria from medicinal plant *V. anthelmintica*

Sample	**Sample concentration**	**Sample amount (µL)**	***C. albicans* (mm ZOI)**	***E. coli* (mm ZOI)**	***S. aureus* (mm ZOI)**
Ampicillin	312.5 µg/mL	20		12.5	
Ampicillin	4.88 µg/mL	20			10
Amphotericin B	5 µg/mL	20	15		
B. paranthracis XJB-1	50 µg/mL	20	10	13	14
B. safensis XJB-51	50 µg/mL	20	11	12	16
B. pumilus XJB-30	50 µg/mL	20	10	15	14
B. halotolerans XJB-36	50 µg/mL	20	8.5	12	15.5
B. subtilis XJB-57	50 µg/mL	20	11	14	15
S. lutetiensis XJB-76	50 µg/mL	20	10	8	16
P. megaterium XJB-41	50 µg/mL	20	9.5	16	17
P. alvei XJB-13	50 µg/mL	20	11	9.5	13

Melanin synthesis and tyrosinase activity evaluation of endophytic bacteria

Melanin biosynthesis is essential for skin pigmentation, and its impairment is centrel to the development of vitiligo a condition marking by the loss of epidermal pigmentation due to melanocyte destruction and decreased melanin production. The complex process is primarily regulated by tyrosinase enzymes. Clinically, vitiligo presents as irregular white patches on the skin resulting from impaired melanogenesis [36]. In humans, melanin pigments that determine skin color, are synthesized in melanocytes, with tyrosinase playing a key regulatory role. Natural products from the medicinal plant *V. anthelmintica* have been reported to activate tyrosinase and promote melanin synthesis in B16 cells [18, 37], attracting significant interest for their potential in treating pigmentation disorders. Previous studies have also shown that crude extracts and pure compounds derived from endophytic microorganisms associated with *V. anthelmintica* can modulate melanin production and tyrosinase activity [26]. In this study, the effects of bioactive compounds produced by endophytic bacteria isolated from *V. anthelmintica* flowers were assessed for their ability to stimulate melanin synthesis and enhance tyrosinase activity. The objective was to identify potential natural products with anti-vitiligo properties. Total extracts from all isolated endophytic strains exhibited stronger melanogenic activity than the positive control, 8-methoxypsoralen (8-MOP) at 50µg/mL, which sowed 129.9 ± 4.179% activity. The observed melanin synthesis activity ranged from 131.01 ± 7.5% to 232.07 ± 3.592% (Table 4). Notably, four strains demonstrated the highest melanin stimulating activity at 50µg/mL: *B. halotolerans* XJB-36 197.91 ± 4.226%, *B. subtilis* XJB-57, 176.18 ± 17.5%, *S. lutetiensis* XJB-76 178.30 ± 3.592% and *P. megaterium* XJB-41 232.07 ± 3.592% (Table 5 and Fig. 3A). Further investigation revealed a dose-dependent increase in melanin synthesis upon treatment with crude extracts from theses four strains (Fig. 3B). At concentrations of 1, 10, 50µg/mL, melanin synthesis levels were as follows: *P. megaterium* XJB-41: 145.4 ± 8.02%, 172.06 ± 2.821%, and 232.07 ± 3.592%, *B. halotolerans* XJB-36: 162.8 ± 7.48%, 179.1 ± 6.09%, and 197.91 ± 4.226%, *S. lutetiensis* XJB-76: 121.4 ± 12.04%, 149.06 ± 1.56%, and 178.30 ± 3.592% and *B. subtilis* XJB-57: 119.2 ± 7.50%, 134.1 ± 5.05%, and 176.18 ± 17.5%. For comparison, 8-MOP (50µM) exhibited a melanin synthesis activity of 129.9 ± 4.179. Additionally, thyrosinase activity in B16 cells was measured. All four bacterial extracts induced a concentration-dependent increase in tyrosinase activity, as shown in Fig. 3C.

Table 4
Effects of melanin synthesis of secondary metabolites in B16 cells treated with same concentrations

Experimental group	**Concentration (n = 3)**	**Relative melanin content (%)**
NC)	NC	100.0 ± 3.465
8-MOP-50μM	50 μM	129.9 ± 4.179
B. paranthracis XJB-1	50 μg/mL	126.02 ± 6.537
B. safensis XJB-51	50 μg/mL	131.01 ± 7.5
B. pumilus XJB-30	50 μg/mL	148.53 ± 16.57
B. halotolerans XJB-36	50 μg/mL	197.91 ± 4.226
B. subtilis XJB-57	50 μg/mL	176.18 ± 17.5
S. lutetiensis XJB-76	50 μg/mL	178.30 ± 3.592
P. megaterium XJB-41	50 μg/mL	232.07 ± 3.592
P. alvei XJB-13	50 μg/mL	163.85 ± 15.68

Table 5
Effects of melanin synthesis and tyrosinase activity of secondary metabolites in B16 cells treated with various concentrations

Experimental group	Concentration (n = 3)	Relative melanin content (%)	Relative tyrosinase activity (%)
NC	50 μM	100.0 ± 5.193	100.0 ± 5.193
8-MOP	50 μM	132.0 ± 2.818	124.1 ± 3.172
P. megaterium XJB-41	1μg/mL	145.4 ± 8.02	122.02 ± 3.546
	10μg/mL	172.06 ± 2.821	137.18 ± 6.35
	50μg/mL	232.07 ± 3.592	183.84 ± 4.41
B. halotolerans XJB-36	1μg/mL	162.8 ± 7.48	129.67 ± 4.56
	10μg/mL	179.1 ± 6.09	134.9 ± 4.166
	50μg/mL	197.91 ± 4.226	168.40 ± 2.878
S. lutetiensis XJB-76	1μg/mL	121.4 ± 12.04	120.74 ± 8.287
	10μg/ mL	149.06 ± 1.56	125.26 ± 5.18
	50μg/ mL	178.30 ± 3.592	154.9 ± 3.26
B. subtilis XJB-57	1μg/ mL	119.2 ± 7.50	113.9 ± 5.334
	10μg/ mL	134.1 ± 5.05	116.12 ± 9.5
	50μg/ mL	176.18 ± 17.5	131.7 ± 3.266

Note

*Compared with blank control group (NC), P < 0.05; **Compared with blank control group (NC), P < 0.01; ***Compared with blank control group (NC), P < 0.001; ****Compared with blank control group (NC), P < 0.0001.

Cytotoxic activity of endophytic bacteria

In recent years, bioactive natural products derived from endophytic microorganisms have emerged as valuable resources in medicinal chemistry and pharmacology, particularly due to their significant cytotoxic effects against various human cancer cell lines. Numerous endophytic microorganisms associated with medicinal plant species have been shown to produce cytotoxic compounds [38]. For example, natural products isolated from the endophytic fungus *Aspergillus versicolor* exhibited strong cytotoxic activity against the mouse lymphoma L5178Y cell line [39]. Jian Xiao reported novel anticancer metabolites from *Botryosphaeria dothidea* KJ-1 that displayed potent cytotoxic activity against HCT 116 cancer cells. Similarly, four novel natural compounds produced by *A. terreus* have been documented to

possess anticancer properties [40]. Endophytic fungi continue to be a rich source of anticancer compounds. For example, metabolites from *Chaetomium globosum* demonstrated *in vitro* cytotoxicity against the human hepatoma cell line HepG-2 [41], while *Paramyrothecium roridum*, isolated from the fresh roots of *Morinda officinalis*, exhibited cytotoxic activity against SF-268, NCI-H460, and HepG-2 cell lines. Li et al. [42] reported that the endophytic fungus *Peniophora incarnata* displayed cytotoxic activity against A375, MCF-7, and HL-60 human cancer cells. In our previous work, endophytic fungi isolated from the medicinal plant *V. anthelmintica* demonstrated significant anticancer activity against MDA-MB-231, HeLa, and HT-29 [24, 26] cell lines. In the present study, we evaluated the cytotoxic effects of crude extracts from eight endophytic bacterial strains against three human cancer cell lines: HT-29, MCF-7, and HeLa. Among them, metabolites from *P. megaterium* XJB-41 and *B. halotolerans* XJB-36 exhibited the most potent cytotoxic activity. The IC_{50}values for *P. megaterium* XJB-41 were 8.62 ± 1.15 µg/mL (HT-29), 13.7 ± 0.33 µg/mL (MCF-7), and 11.92 ± 1.52 µg/mL (HeLa). For *B. halotolerans* XJB-36, the IC_{50}values were 13.086 ± 0.15 µg/mL (HT-29), 11.054 ± 1.04 µg/mL (MCF-7), and 17.13 ± 0.81 µg/mL (HeLa). Doxorubicin (DOX) was used as a positive control (Table 6). Crude extracts from the remaining bacterial strains displayed moderate cytotoxic activity against all tested cancer cell lines.

Table 6
Cytotoxic activity of secondary metabolites of crude extracts of bacterial endophytes associated with *V. anthelmintica*.

Samples	Cell lines		
	IC_{50} (µg/mL)		
	HT-29	MCF-7	HeLa
B. paranthracis XJB-1	36.5 ± 0.01	34.9 ± 0.22	32.3 ± 0.98
B. safensis XJB-51	21.2 ± 0.2 ± 3.20	Not active	36.69 ± 1.59
B. pumilus XJB-30	34.66 ± 1.45	48.32 ± 1.34	39.6 ± 0.13
B. halotolerans XJB-36	13.086 ± 0.15	11.92 ± 1.52	17.13 ± 0.81
B. subtilis XJB-57	38.44 ± 1.22	43.07 ± 0.14	Not active
S. lutetiensis XJB-76	28.98 ± 1.03	23.84 ± 0.76	17.18 ± 0.74
P. megaterium XJB-41	8.62 ± 1.15	13.7 ± 0.33	11.054 ± 1.04
P. alvei XJB-13	34.31 ± 1.20	37.6 ± 0.90	29.18 ± 0.08
DOX	0.82 ± 0.041	0.17 ± 0.006	33.11 ± 0.005

Antidiabetic activity endophytic bacteria

Diabetes mellitus is one of the most prevalent systemic diseases globally, posing a significant health threat due to its increasing incidence and associated mortality rates. Historically, both natural products

and synthetic compounds have been employed as antidiabetic agents for managing this condition [43]. Among natural sources, microbial bioactive secondary metabolites have gained attention due to their structural diversity and therapeutic potential in the treating diabetes mellitus. Several studies have highlighted the antidiabetic poten have been explored by various researchers [44]. For example, the marine fungus *Scedosporium apiospermum* F41-1 produces bioactive alkaloid derivatives that may exhibit antidiabetic effects [45]. Additionally, Gao et al. isolated polyketide compounds from *Pestalotiopsis neglecta*, which exhibited moderate antidiabetic activity by targeting SHP1, CDC25B, and PTP1B [46]. In the current study, crude extracts from endophytic bacteria were evaluated for their antidiabetic activity using *in vitro* PTP1B inhition assays. The results indicated that crude extracts from *B. pumilus* XJB-30, *B. subtilis* XJB-57, *P. megaterium* XJB-41 and *P. alvei* XJB-13 exhibited strong PTP1B inhibitory activity, with IC_{50} values of 3.83 ± 0.39, 7.098 ± 1.26, 6.07 ± 1.54 and 9.18 ± 1.067 µg/mL respectively. Meanwhile, the endophytic strains *B. paranthracis* XJB-1, *B. safensis* XJB-51, *B. halotolerans* XJB-36 and *S. lutetiensis* XJB-76 demonstrated moderate activity, with IC_{50} values of 11.04 ± 0.31, 16.5 ± 1.02, 13.74 ± 0.57 and 16.02 ± 0.34 µg/mL respectively. A known PTP1B inhibitor was used as a positive control (Table 7).

Table 7
Antidiabetic activity of secondary metabolites of crude extracts of bacterial endophytes associated with *V. anthelmintica*.

Sample	IC_{50} (µg/mL)
B. paranthracis XJB-1	11.04 ± 0.31
B. safensis XJB-51	16.5 ± 1.02
B. pumilus XJB-30	3.83 ± 0.39
B. halotolerans XJB-36	13.74 ± 0.57
B. subtilis XJB-57	7.098 ± 1.26
S. lutetiensis XJB-76	16.02 ± 0.34
P. megaterium XJB-41	6.07 ± 1.54
P. alvei XJB-13	9.18 ± 1.067
PTP1B inhibitor	1.59 ± 0.40

Antioxidant activity of endophytic bacteria

Natural products isolated from endophytic microorganisms have been used for decades in traditional medicine, pharmacology, and medicinal chemistry to develop a wide range of therapeutic agents for various diseases. Endophytic bacteria, in particular, are recognized for producing natural products with antioxidant properties. For example, the endophyte *Glutamicibacter halophytocola* KLBMP has been

reported to produce antioxidant compounds [47]. Additionally, several species within the genus *Bacillus* have exhibited significant antioxidant activity [48]. In our previous research, we investigated the antioxidant potential of secondary metabolites produced by endophytes associated with the roots of *V. anthelmintica* [24]. In the present study, we evaluated the antioxidant activity of total ethyl acetate extracts from endophytic bacteria isolated from the flowers of *V. anthelmintica*. The DPPH radical scavenging activities of the crude ethyl acetate extracts from *B. pumilus* XJB-30, *P. megaterium* XJB-41 and *P. alvei* XJB-13 were 26.765 ± 2.25, 25.65 ± 1.49 and 37.21 ± 0.10 μg/mL respectively. Furthermore, *B. paranthracis* XJB-1, *B. halotolerans* XJB-36 and *B. subtilis* XJB-57 showed moderate antioxidant activity, with IC_{50} values of 56.18 ± 1.08, 52.11 ± 0.7 and 47.14 ± 0.15, respectively (Table 8). Ascorbic acid was used as a standard reference compound in the assays.

Table 8
Antioxidant activity of secondary metabolites of crude extracts of bacterial endophytes associated with *V. anthelmintica*.

Sample	IC_{50} (μg/mL)
B. paranthracis XJB-1	56.18 ± 1.08
B. safensis XJB-51	121.28 ± 0.26
B. pumilus XJB-30	26.765 ± 2.25
B. halotolerans XJB-36	52.11 ± 0.7
B. subtilis XJB-57	47.14 ± 0.15
S. lutetiensis XJB-76	133.054 ± 5.06
P. megaterium XJB-41	25.65 ± 1.49
P. alvei XJB-13	37.21 ± 0.10
Vitamin C	5.87 ± 0.52

The effect of culture condition on the secondary metabolite production of endophytic bacteria

The impact of various physicochemical parameters - such as pH, temperature, incubation time and different carbon and nitrogen sources on the production of bioactive secondary metabolites by endophytes has been extensively documented. For example, Yi et al. [31] identified the optimal culture conditions for the growth and antifungal activity of the endophytic strain BT4. Their study revealed that the bacterium achieved maximum growth and produced antifungal metabolites after 48 hours of incubation, with an optimal pH 7.5. Starch and yeast extracts were determined to be the most effective carbon and nitrogen sources, respectively. Similarly, Deka et al. [49] investigated the influence of culture conditions on the synthesis of bioactive natural compounds by the endophyte *Geosmithia pallida*.

Optimizing the growth and secondary metabolite production of endophytic bacteria requires meticulous control of environmental and nutritional factors. Incubation time, in particular, is critical parameter that influences both bacterial growth and the yield of natural products. The relationship between incubation time and metabolite production can be understood by examining key factors, such as the point during the stationary phase when metabolite biosynthesis reaches its peak. This timing varies depending on the bacterial species and the specific compound of interest. Therefore, regular sampling and analysis of the culture are essential for determining the optimal harvesting time to maximize yield. The following is a comprehensive overview of the culture conditions that influencing the growth and secondary metabolite production of the highly active endophytic bacterium *P. megaterium* XJB-41. Among all the isolated bacterial strains, *P. megaterium* XJB-41 exhibited the most significant biological activities, including strong antimicrobial activity against *S. aureus* (inhibition zone of 17 mm), potent cytotoxic effects against HT-29, MCF-7, and HeLa cell lines (IC_{50} values of 8.62 ± 1.15 µg/mL, 13.7 ± 0.33 µg/mL, and 11.05 ± 1.04 µg/mL, respectively), and a substantial increase in melanin content (232.07 ± 3.59%) in B16 cells compared to other strains. Based on these findings, we optimized the culture conditions to enhance both cell growth and the production of bioactive secondary metabolites. The impact of incubation time on bacterial growth and metabolite yield is illustrated in Fig. 4. A. A. *P. megaterium* XJB-41 reached its maximum growth at 48 hours, with secondary metabolite production reaching 430 mg/L. Optimal metabolite production was observed in LB medium (420 mg/L) and NB (390 mg/L), as showen in Fig. 4. B. Various microorganisms, including endophytic bacteria are well known for their ability to synthesize bioactive secondary metabolites. Notably, members of the *Bacillus* genus, such us *B. velezensis* Ea73 [50], *B. velezensis* D1 [51] and *B.cereus* [52] have been reported to produce a wide range of natural bioactive compounds.

Effect of the initial pH and temperature

The initial pH of the culture medium significantly influences the synthesis of secondary metabolites by endophytic bacteria. The effect of initial pH on the production of secondary metabolites by *P. megaterium* XJB-41 shown in Fig. 5. The initial pH of the medium prior to autoclaving was set to 6, and the impact of varying pH levels (ranging from 6 to 9) on metabolite production was investigated. Optimal production bioactive secondary metabolites was observed at pH 7 and 7.5, yielding 395 mg/mL and 398 mg/mL, respectively (Fig. 5.). In contrast, significantly lower yields were recorded at pH 6 (145 mg/mL) and pH 9 (116 mg/mL). In comparison, previously reported *Bacillus* species required broader pH ranges for optimal metabolite production, spanning from 7.0 up to pH 10.2. For example, *B. subtilis* BZR demonstrated a high metabolite titer at an optimal pH 8.0 [53]. Similarly, Kiesewalter et al. identified optimal pH conditions for *B. subtilis* that promote growth and the production of natural products [54]. The initial pH of the culture medium is a crucial factor influencing bacterial growth, enzyme activity, nutrient availability, regulatory mechanisms, and the overall metabolic profile. Optimizing pH conditions can significantly enhance the production of valuable secondary metabolites for pharmaceutical, medicinal chemistry, and industrial applications. In addition to pH, the temperature stability of secondary metabolites produced by endophytic bacteria varies depending on the specific metabolite and bacterial strain. Some endophytic bacteria, particularly those adapted to higher temperatures, may produce

metabolites with enhanced thermal stability. For instance, the optimal temperature for the production of bioactive secondary metabolites by *B. subtilis* was reported to be 24^{o}C [55], while other *Bacillus* species exhibited maximal secondary metabolites production at 37^{o}C [56]. In the present study, the cultivation temperature for *P. megaterium* XJB-41 was tested within a range of 20^{o}C to 45^{o}C. The highest yields of natural products were recorded at 25^{o}C and 30 ^{o}C, producing 392 mg/mL and 401 mg/mL, respectively. Furthermore, a significant decrease in metabolite production was observed at 40^{o}C (175) and 45^{o}C and (112 mg/mL). Therefore, cultivation temperature is also critical factor affecting both the quantity and stability of secondary metabolites production.

Effect of shaking speed

Shaking speed plays a critical role in microbial cultivation processes, significantly influencing both microbial growth and synthesis of natural bioactive. Optimizing shaking speed is essential for maximizing metabolite yield, ensuring experimental reproducibility, and maintaining the physiological health of the microbial culture. Shaking speed directly affects the level of dissolved oxygen in the culture medium. Increased shaking speeds improve oxygen transfer, thereby preventing oxygen limitation and supporting higher microbial growth rates. Previous studies have demonstrated that shaking speeds in the range 200–250 rpm enhance oxygen transfer effeciency, leading to elevated production of bioactive metabolites. In this study, the shaking speed was optimized to enhance both the growth and natural compound production of the most active endophytic bacterium, *P. megaterium* XJB-41. Shaking speeds ranging from 120 to 190 rpm were tested. Optimal production of secondary metabolites was achieved at 150 rpm and 160 rpm, yielding 392 mg/mL and 402 mg/mL, respectively. In contrast, lower the shaking speeds of 120 and 130 rpm significantly reduced metabolite production, yielding only 155 mg/mL and 189 mg/mL. Although shaking speeds above 170 rpm slightly increased metabolite production, the bioactive compounds formed colloidal suspensions in the fermented medium, potentially complicating downstream purification and processing (Fig. 5. and Table 9).

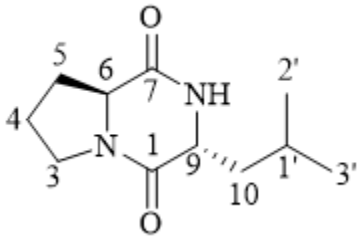
Shaking speed is a fundamental parameter in microbial cultivation that significantly influences both microbial growth rates and the synthesis of natural compounds. These bioactive compounds possess substantial potential in pharmacology and pharmaceutical medicinal chemistry. In the present study, we successfully optimized the culture conditions for the production of bioactive natural compounds by the highly active endophytic bacterium *P. megaterium* XJB-41 in nutrient broth (NB) medium. The optimal conditions identified were a pH 7.5, an incubation period of 48 hours, shaking at 160 rpm, and a temperature of 30°C (Table 9.).

Table 9
Optimal culture condition for growth and produced secondary metabolites by the most-active endophytic bacteria *P. megaterium* XJB-41

Culture medium	**Incubation condition**			
NB medium	pH	Time (hours)	Shaking speed (rpm)	Temperature (^{0}C)
Amount of secondary metabolites	7.5	48	160	30
	398 mg/mL	430 mg/L	402 mg/mL	401 mg/mL

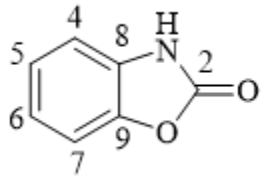
Chemical composition of P. megaterium XJB-41.

The most bioactive bacterial strain, *P. megaterium* XJB-41, was isolated for the first time from the flowers of the Chinese medicinal plant *V. anthelmintica*. The total ethyl acetate extract of this strain demonstrated notable biological activities, including potent antimicrobial and cytotoxic effects, along with a significant impact on melanin synthesis and tyrosinase activity in murine B16 cells. These promising bioactivities prompted further investigation into the chemical composition of the ethyl acetate extract to identify the specific compounds responsible. To the best of our knowledge, this is the first report describing the chemical constituents of the endophytic bacterium *P. megaterium* XJB-41 isolated from *V. anthelmintica* flowers. Two secondary metabolites were identified from this strain when cultivated in nutrient broth (NB) medium: cyclo(D-Leu-L-pro [57] and 2-benzoxazolone [58], both reported here for the first time from *P. megaterium* XJB-41.



Structure of compound 13 (Cyclo(D-Leu-L-Pro)

^{1}H NMR (400 MHz, CD_3OD, ppm, *J*/Hz): 4.26 (1H, t, *J* = 5.2 Hz, H-9), 4.12 (1H, m, H-6), 3.50 ~ 3.52 (2H, m, H_2-3), 2.30 (1H, m, H-5), 1.97 ~ 2.07 (2H, m, H-4, 1'), 1.85 ~ 1.96 (3H, m, H-4, H-5, H-10), 1.52 (1H, m, H-10), 0.96 (6H, d, *J* = 4.4 Hz, H_3-2', 3'). ^{13}C NMR (100 MHz, CD_3OD, ppm): 172.7 (C-1), 168.8 (C-7), 60.2 (C-6), 54.6 (C-9), 46.4 (C-3), 39.4 (C-10), 29.7 (C-5), 25.7 (C-1'), 23.6 (C-4), 23.3 (C-2'), 22.2 (C-3').The above data are basically consistent with the data reported in the literature for Cyclo(D-Leu-L-Pro [57].



Structure of compound 14 (2-Benzoxazolone)

2-benzoxazolone is yellow needle crystal. (−) HRMS *m/z* 134.0236 $[M-H]^-$ ($[M-H]^-$ the calculated value is 134.0248), determine the molecular formula$C_7H_5NO_2$。^{1}H NMR (400 MHz, CD_3OD, ppm, *J*/Hz): 7.20 (1H, d, *J* = 8.0 Hz, H-4), 7.16 (1H, ddd, *J* = 8.0, 8.0, 1.2 Hz, H-2), 7.11 (1H, dd, *J* = 8.0, 1.2 Hz, H-7), 7.08 (1H, m, H-6). ^{13}C NMR (100 MHz, CD_3OD, ppm): 157.2 (C-2), 145.4 (C-9), 131.6 (C-8), 125.1 (C-5), 123.4 (C-7), 110.8 (C-6), 110.6 (C-4).The above data are basically consistent with the data of 2-benzoxazolone reported in the literature [58].

Antimicrobial and cytotoxic activity of secondary metabolites of P. megaterium XJB-41.

As mentioned earlier, the total extract of *P. megaterium* XJB-41 exhibited significantly stronger biological activity compared to extracts from other strains. Consequently, we decided to isolate and purify the individual compounds from the ethyl acetate extract in order to assess their antimicrobial and cytotoxic activities. This targeted approach allowed us to identify the specific compounds responsible for the bioactvities observed in the total extract. Both isolated metabolites (**1** and **2**) demonstrated potent antimicrobial activity against *E. coli* and *S. aureus*, with inhibition zones measuring 13, 11.5, 16, and 14 mm, respectively (Table 10). Additionally, these compounds exhibited moderate antifungal activity against *C. albicans* with inhibition zones 9 and 11 mm, respectively. Furthermore, both compounds displayed significant cytotoxic activity against the HeLa cancer cell line, with IC_{50} values of 15.7 µg/mL ± 0.05 and 11.6 µg/mL ± 1.27, respectively (Table 11). Endophytic microorganisms are known to produce a diverse array of secondary metabolites with significant antimicrobial properties. For example, a stigmasterol derivative (22E,24R)-stigmasta-5,7,22-trien-3-β-ol, was isolated from a culture of the endophytic fungus *A. terreus*, obtained from the roots of *Carthamus lanatus*. This compound demonstrated potent antimicrobial activity against methicillin-resistant *S. aureus* and *C. neoformans*, with IC_{50} values of 0.96 µg/mL and 4.38 µg/mL, respectively [59]. Moreover, the total extract of *A. terreus* demonstrated significant antifungal activity against mucormycosis-causing fungi, including *Rhizopus oryzae*, *M. racemosus*, and *S. racemosum*, with inhibition zones of 20 mm, 37 mm, and 18 mm, respectively [60]. Many natural compounds derived from plants or microbial sources undergo biotransformation in the body, which can enhance or activate their biological properties. Some of these transformed metabolites have demonstrated anticancer activity. For example, flavonoids and polyphenols from fruits and vegetables are metabolized into active compounds with antioxidant and anticancer effects. Natural compound panaxatriol, which, when incubated with a culture of *A. flavus* Link AS 3.3950 synthesized four novel biotransformation products: 24β-hydroxy-20(R)-panaxatriol, 24α-hydroxy- 20(R)-panaxatriol, 23β-hydroxy-20(R)-panaxatriol and 15β-hydroxy-20(R)-panaxatriol. These

metabolite inhibited selective cytotoxicity against K562/ADR cancer cell lines in a dose- and time-dependent manner [61]. Overall, the exploration of natural products and their anticancer potential presents a promising avenue in cancer research. This multidisciplinary approach integrating pharmacology, biochemistry, and molecular biology offers opportunities to develop novel therapies with improved efficacy and safety profiles.

Table 10
Antimicrobial activity of secondary metabolites **1** and **2**

Sample	Sample concentration	Sample amount (µL)	*C. albicans* (mm)	*E. coli* (mm)	*S. aureus* (mm)
Ampicillin	10 mg/mL	5		18.5	
Ampicillin	1 mg/mL	5			20
Amphotericin B	5 mg/mL	20	18		
1	50 µM	20	9	13	16
2	50 µM	20	11	11.5	14

Table 11
Cytotoxic activity of secondary metabolites **1** and **2**

Samples	Cell lines		
	IC_{50} (µg/mL)		
	HT-29	MCF-7	HeLa
1	21.70 ± 0.04	32.56 ± 3.7	15.7 ± 0.05
2	33.5 ± 0.01	29.9 ± 0.22	11.6 ± 1.27
DOX	0.82 ± 0.041	0.17 ± 0.006	0.11 ± 0.005

Scanning Electron Microscopy analysis of endophytic bacteria

Scanning Electron Microscopy (SEM) is a powerful technique for analyzing the surface morphology and composition at high resolution, making it particularly suitable for studying endophytic bacteria. In this study, SEM was employed to visualize the morphological structure of the colonizing bacterial strain *P. megaterium* XJB-41. The surface morphology of this highly bioactive endophytic bacterium was observed with distinct structural features and varying sizes (Fig. 6). Based on preliminary screening results, which showed significantly stronger biological activities compared to the other isolates, the crude extracts from *P. megaterium* XJB-41 were selected for further study. Overall, SEM proved to be an invaluable tool for characterizing the surface morphology of endophytic bacteria. Accurate sample

preparation and the use of appropriate imaging parameters were essential for obtaining high-quality images that contribute to a deeper understanding of these microorganisms (Fig. 7).

Conclusion

This study highlights the symbiotic relationship between the medicinal plant *V. anthelmintica* and its diverse community of endophytic bacteria. The genera *Bacillus, Priestia*, and *Streptococcus* were identified as promising sources of pharmacologically active agents, demonstrating broad-spectrum antimicrobial, antitumor, and anti-vitiligo activities. Among them, the endophytic bacterial strain *P. megaterium* XJB-41 exhibited particularly strong biological activity. For the first time, this study successfully optimized the production of natural bioactive compounds from *P. megaterium* XJB-41, achieving a yield of 430 mg/L under optimized culture conditions. Furthermore, two pure compounds were isolated from this highly active strain on a larger scale, supporting their potential for future pharmaceutical development.

Declarations

Author contributions Nigora Rustamova conducted the experiments, lab work and prepared the manuscript draft. Ahmidin Wali and Niu Litao plant collected, Jakhongir Movlanov, Kakhramon Davranov and Abulimiti Yili analyzed data and revised the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors

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Figures

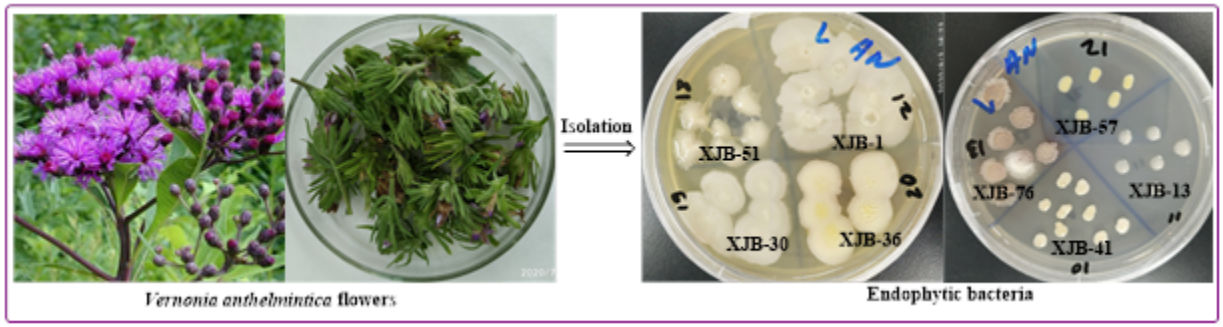


Figure 1

Isolation of endophytic bacteria associated with flowers of *V. anthelmintica*

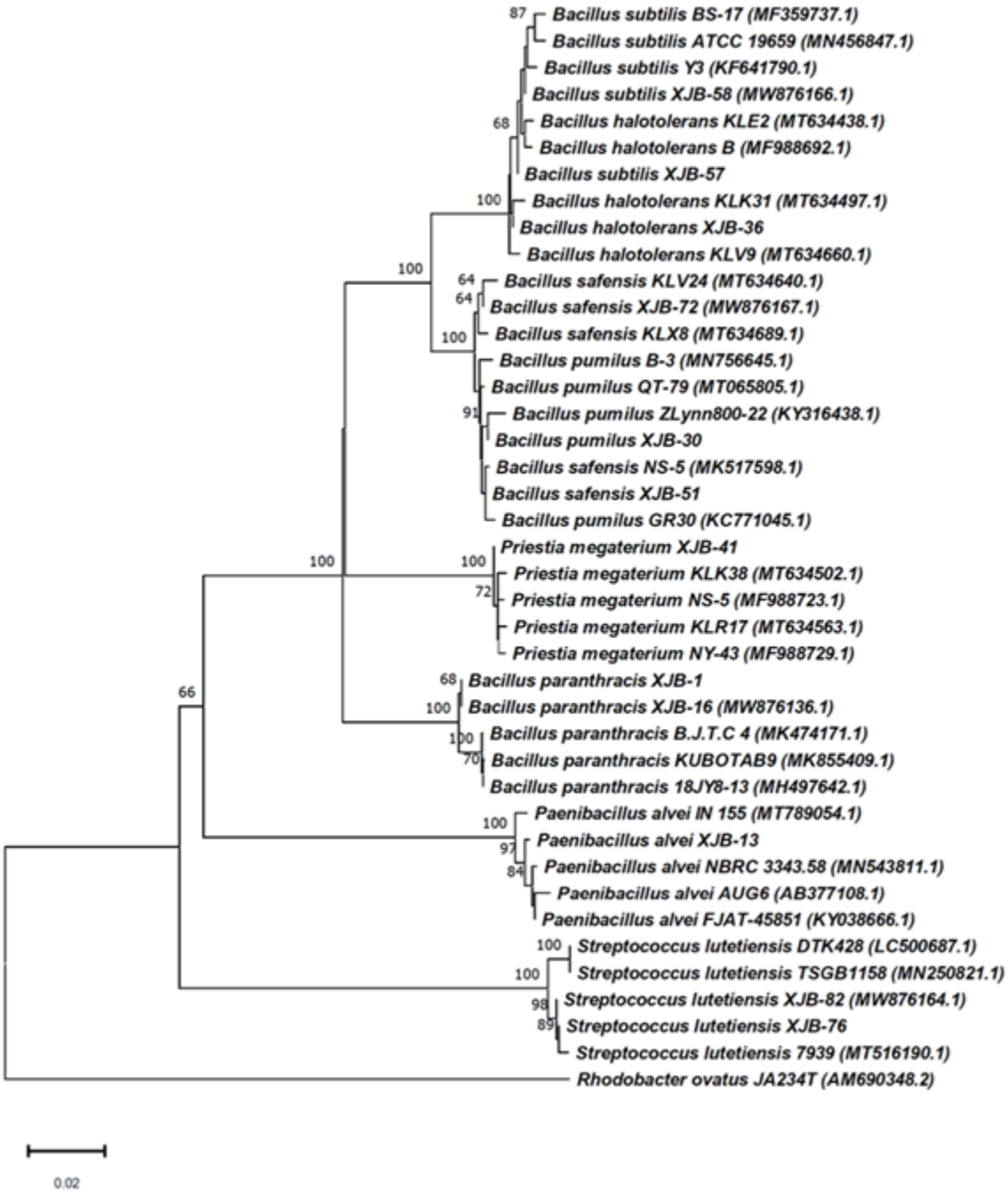


Figure 2

The phylogenetic tree of endophytic bacteria isolated from *V. anthelmintica*, and their closest relatives from GenBank GenBank of NCBI.

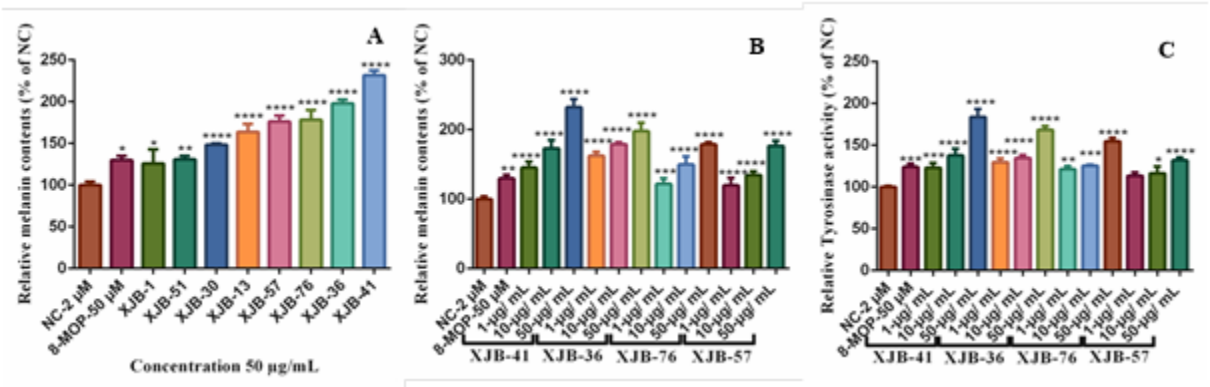


Figure 3

(A) Effect of secondary metabolites from the crude ethyl acetate extracts of endophytic bacteria on melanin content and tyrosinase activity in B16 melanoma cells. (B and C) melanin synthesis and tyrosinase activity of cells treated with different concentrations of crude extracts

Note: *Compared with blank control group (NC), P<0.05; **Compared with blank control group (NC), P<0.01; ***Compared with blank control group (NC), P<0.001; ****Compared with blank control group (NC), P<0.0001.

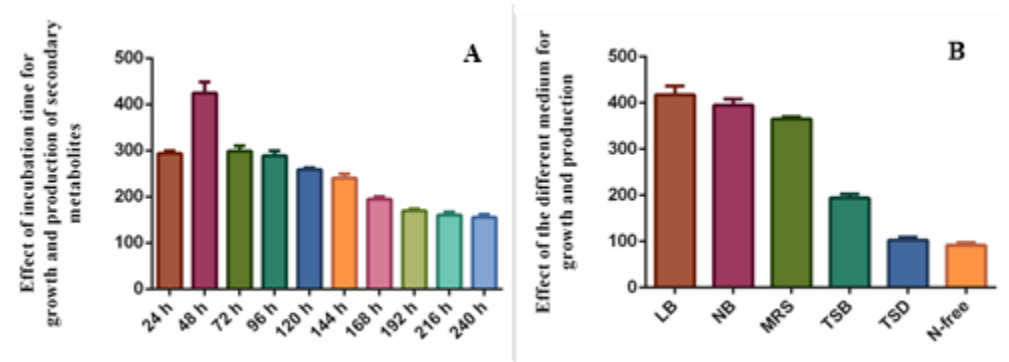


Figure 4

The effect of incubation time (A) and culture media (B) for growth and production secondary metabolites by most active endophytic bacteria

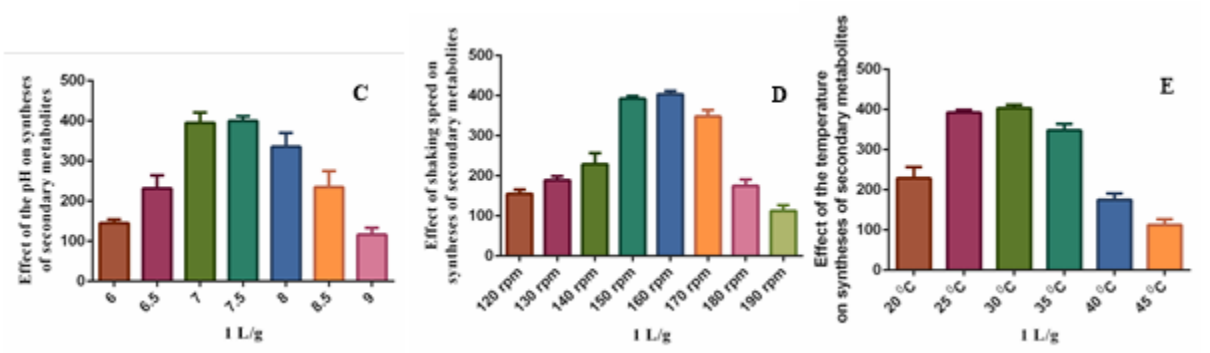


Figure 5

Effect of pH (C), shaking speed (D) and temperature (E) for growth and produced secondary metabolites by the most-active endophytic bacteria *P. megaterium* XJB-41

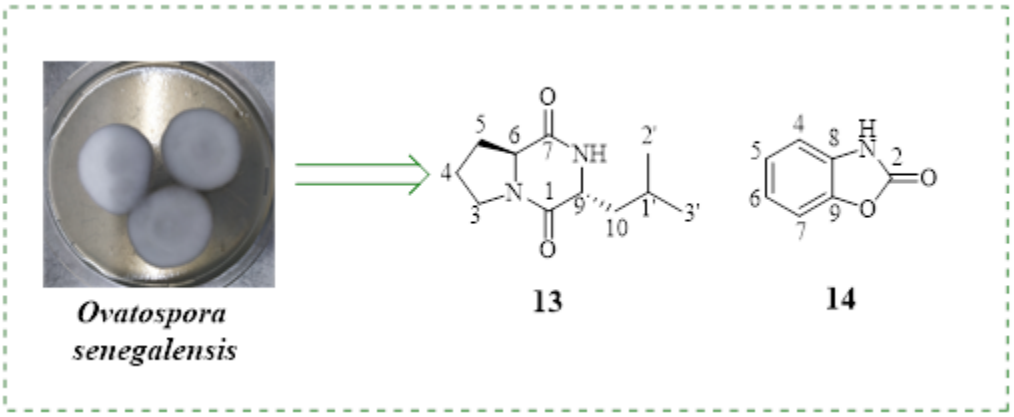


Figure 6

Chemical composition of *P. megaterium* XJB-41.

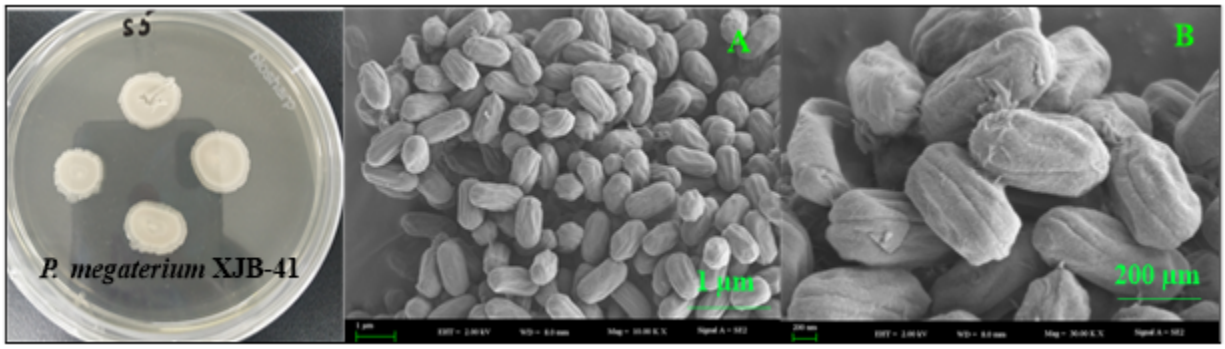


Figure 7

Morphological characterization of most active endophytic bacteria *P. megaterium* XJB-41 by SEM from a plate cultured with agar medium. Scale bars= 1 (A) and 200 (B) μm respectively.

Supplementary Files

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