



Isolation and characterization of an endophytic *Streptomyces* sp. YBRX0 from *Croton tonkinensis* and assessment of its bioactive potential

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ABSTRACT

The rise of antimicrobial resistance and emerging infectious diseases necessitates the discovery of novel antibacterial agents. Endophytic actinomycetes, especially *Streptomyces* species colonizing medicinal plant tissues, constitute a reservoir of structurally diverse bioactive metabolites that remains insufficiently characterized. This study reports the isolation and comprehensive characterization of an endophytic actinomycete, designated strain YBRX0, recovered from the root tissues of the Vietnamese medicinal plant *Croton tonkinensis* Gagnep. Strain identity was established through an integrated approach combining colony morphology, scanning electron microscopy, physiological and biochemical profiling, and 16S rRNA gene phylogenetic analysis. Bioactivity screening of the ethyl acetate fermentation extract employed agar well diffusion to assess antibacterial spectrum, with inhibitory potency quantified by broth microdilution. Antioxidant capacity was measured via DPPH radical scavenging, and secondary metabolite composition was profiled by GC-MS. The integrated characterization placed strain YBRX0 unambiguously within the genus. At 1 mg/mL, the ethyl acetate extract demonstrated broad-spectrum activity against both Gram-positive and Gram-negative pathogens, with MIC values ranging from 8 to 16 µg/mL. Antioxidant screening yielded an IC₅₀ of 197.26 ± 8.87 µg/mL. GC-MS tentatively identified Pyrrolopyrazine diketopiperazines and Benzeneacetamide as principal constituents. Collectively, these findings indicate that the crude extract of *Streptomyces* sp. YBRX0 exhibited notable antibacterial activity despite being an unfractionated mixture, supporting its potential as a promising source of bioactive metabolites for further bioprospecting and compound isolation efforts targeting drug-resistant pathogens.

1. Introduction

The filamentous soil bacteria of the genus *Streptomyces* are among the most pharmaceutically productive genus within the bacterial domain, having supplied the majority of antibiotic scaffolds currently in clinical use, along with a diverse array of antifungal, anti-inflammatory, antiviral, and antioxidant natural products.^{1,2} However, the therapeutic relevance of these contributions is increasingly compromised by the accelerating spread of multidrug-resistant (MDR) pathogens, a trend driven by decades of inappropriate antimicrobial use and the consequent selection of resistance determinants across phylogenetically diverse bacterial populations.^{3–7} Sustaining the antibiotic discovery

pipeline, therefore, demands access to microbial reservoirs whose biosynthetic potential has not been fully exploited by existing screening platforms. In recent years, attention has broadened beyond soil isolates to include endophytic *Streptomyces* strains, those residing within plant tissues, given accumulating evidence that host-plant associations can stimulate the production of unique secondary metabolites with improved or novel biological activities.^{1,8–13} This shift is particularly relevant for medicinal plants, whose complex chemical environments may selectively enrich for endophytes capable of producing bioactive compounds aligned with the therapeutic properties traditionally attributed to the host species.

The co-evolutionary dynamics between endophytic *Streptomyces* and

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; GC-MS, Gas chromatography-mass spectrometry; MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; MRSA, Methicillin-resistant *Staphylococcus aureus*; ISP, International Streptomyces Project; SFM, Soy Flour Mannitol.

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their plant hosts are thought to exert selective pressure on microbial biosynthetic machinery, favoring the expression of metabolic pathways that are typically not activated in free-living isolates. This ecological intimacy may thus expand the chemical space accessible through conventional screening approaches. Nevertheless, despite growing appreciation of their biotechnological value, the actinomycete endophyte communities associated with the majority of medicinal plant species, particularly those native to tropical Asia, remain poorly inventoried, and the bioactive outputs of most isolates have yet to be systematically characterized. One such example is the tropical shrub *Croton tonkinensis* Gagnep. (Euphorbiaceae), a plant native to Vietnam, renowned for its complex phytochemical profile. Previous phytochemical investigations have identified essential oils,¹⁴ triterpenoids,¹⁵ and especially diterpenoids in *C. tonkinensis*.^{16,17} Ent-kaurane diterpenoids are the primary constituents among these, showing a variety of biological activities including antibacterial,^{18–20} anti-inflammatory,^{21,22} anti-urolithic,²³ and anti-cancer effects.^{19,21,24} Despite its medicinal value, the natural supply of these bioactive metabolites is threatened by the plant's slow growth and the inefficiency of traditional extraction techniques, leading to ecological pressure and population decline. Consequently, there is an urgent need to develop sustainable biotechnological alternatives to secure these valuable compounds.

Despite thorough phytochemical profiling of *C. tonkinensis*, the diversity and therapeutic potential of its associated endophytic actinomycetes remain largely uncharacterized. To bridge this gap, this research was designed to isolate and evaluate endophytic actinomycetes from this medicinal species, followed by a screening of their antimicrobial and antioxidant properties. Furthermore, Gas Chromatography–Mass Spectrometry (GC–MS) utilized to examine the fermentation broth, providing initial evidence of the metabolites underlying the observed biological activities. This comprehensive investigation underscores the value of endophytes as sustainable bio-factories and promotes their utilization in future biotechnological and pharmaceutical innovations.

2. Materials and methods

2.1. Sample collection and isolation of endophytic actinomycetes from *Croton tonkinensis* Gagnep

2.1.1. Sample collection

Healthy tissues (leaves, stems, roots) of the medicinal plant *C. tonkinensis* Gagnep. were randomly sampled from various sites in Viet Cuong, Yen Bai (21.658° N, 104.851° E). Each specimen was individually sealed in sterile plastic bags and transported to the laboratory for processing within 96 h. To eliminate surface debris, the samples underwent a rigorous cleaning protocol, including washing under running tap water followed by an ultrasonic bath treatment. Subsequently, the plant materials were left to air-dry at room temperature for 48 h before isolation.

2.1.2. Surface sterilization of plant organs

A sequential chemical decontamination procedure was applied to all plant segments, following protocols adapted from previously described methods.^{11,12,25} Immersion in 15% NaClO (5 min) was followed by neutralization in 2% Na₂S₂O₃ (2 min), with three intermediate rinses in sterile distilled water to remove residual oxidizing agents. Subsequent treatment with 70% ethanol (7 min) and three final washes completed the sterilization sequence. To confirm sterilization efficacy, 100 µL of the final rinse water was plated onto SFM agar and incubated alongside the isolation plates; the absence of growth confirmed effective surface decontamination, supporting the endophytic origin of recovered isolates. Processed samples were air-dried under laminar flow conditions until no surface moisture remained, before endophyte recovery.

2.1.3. Isolation of actinomycetes

The sterilized samples were homogenized using an industrial blender and distributed onto SFM agar (composition per liter: 20.00 g Mannitol, 20.00 g Soy flour, and 20.00 g agar). The isolation medium was fortified with nystatin (50 mg/L), nalidixic acid (25 mg/L), and K₂Cr₂O₇ (50 mg/L) to restrict the proliferation of Gram-negative bacteria and fungi. Additionally, polyvinyl pyrrolidone (2%) and tannase (0.005%) were incorporated into the isolation medium to promote colony growth.⁸ The culture plates were incubated at 30 °C for 6 to 10 weeks. Select and purify representative colonies by subculturing them onto ISP2 agar medium. The purified isolates were preserved in glycerol (20%, v/v) at –20 °C.

2.1.4. Identification based on morphological characteristics

The characterization of the isolates' phenotypic, morphological, and biochemical features was conducted using the standardized protocols established by Shirling and Gottlieb (1966)²⁶ and Waksman (1961).²⁷ Morphological investigations concentrated on the coloration of aerial and substrate mycelium, in addition to the release of diffusible pigments, were carried out using various media from the ISP media (ISP1–ISP7). The production of melanoid pigments was specifically evaluated through cultivation on ISP6 and ISP7 media. Additionally, the physiological versatility of the strain was investigated under varying conditions: temperatures (4–45 °C), pH levels (3–12), and salinity (0–10% w/v NaCl). Further biochemical profiling adhered to the criteria outlined in Bergey's Manual of Systematic Bacteriology,²⁸ with color classifications determined according to Prauser (1964).²⁹

2.1.5. Morphological characterization using Scanning Electron Microscopy (SEM)

The morphology of the actinomycete strain was further examined using scanning electron microscopy (SEM). A representative colony was sampled and mounted onto a silicon (Si) wafer. The sample was initially fixed with 2.5% (v/v) glutaraldehyde for 10 min, followed by a dehydration series in ethanol at escalating levels of ethanol (70%, 80%, 90%, and 100%) for a duration of 10 min each. Finally, the specimens were carbon-coated, and their morphological features were observed using a scanning electron microscope (SEM, model JSM-IT200).³⁰

2.1.6. Fermentation and extraction of bioactive compounds

2.1.6.1. Fermentation process. The activation of the isolated actinomycete strain was performed by inoculating a pure colony into ISP2 broth and incubating on a rotary shaker at 150 rpm for 2 days at 30 °C. Subsequently, the fermentation process was initiated by adding a 5% (v/v) inoculum of the activated culture into production medium (ISP2 broth) and incubating at 150 rpm and 30 °C for 5 days.

2.1.6.2. Extraction of bioactive compounds. Following a 5-day fermentation period, the culture broth was centrifuged (6000 rpm, 10 min, 4 °C) to eliminate cellular debris. The clarified supernatant was subsequently subjected to triple extraction with ethyl acetate (EtOAc) at a 1:1 (v/v) ratio,³¹ 15 min each time. The combined organic phases were then concentrated to dryness using a rotary vacuum evaporator under controlled conditions (45 °C, 50 rpm, 150 mmHg). The resulting crude extract served as the total metabolite pool for further bioactivity evaluations. For comparative purposes, a negative control was established by processing uninoculated ISP2 medium under identical fermentation and extraction parameters.

2.1.7. Antibacterial activity assay

2.1.7.1. Test microorganisms. The pathogenic microorganisms, including *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 33519,

Pseudomonas aeruginosa ATCC 9027, *Bacillus cereus* ATCC 1306, *Rhodococcus equi* ATCC 6939, and *Enterococcus faecalis* ATCC 29212, were acquired from the collection of the School of Life Sciences, Hanoi University of Science and Technology.

2.1.7.2. Preliminary antibacterial screening. The antibacterial potential of strain YBRX0 was evaluated using the agar well diffusion technique.³² Microbial suspensions, adjusted to a concentration of 10^8 CFU/mL, were uniformly spread across Mueller-Hinton Agar (MHA) plates. Wells (8 mm in diameter) were then loaded with 20 μ L of the ethyl acetate extract (1 mg/mL dissolved in DMSO). Gentamicin and Ampicillin (100 μ g/mL) were employed as positive controls, while pure DMSO acted as the negative control. After a 24-h incubation at 37 °C, the inhibition zone sizes were measured. Antibacterial effectiveness was deemed significant when the diameter of the inhibition zone was at least 10 mm.

2.1.8. Evaluation of antioxidant activity

2.1.8.1. DPPH radical scavenging assay. Radical-scavenging capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) spectrophotometric method³³ with minor modifications. The dried EtOAc extract was dissolved in methanol and mixed with 600 μ L of the DPPH solution. Following a 30-min period of incubation in the absence of light at room temperature, the optical density at 517 nm was recorded in triplicate on a UV-Vis spectrophotometer. L-ascorbic acid (Vitamin C) served as the positive control. The percentage of radical scavenging inhibition (I%) was calculated using the following formula:

$$I\% = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the negative control, and A_1 is the absorbance of the test sample.

2.1.8.2. Determination of biomass. The biomass of the *Streptomyces* strain was determined using the dry-weight method until a stable mass was reached. Specifically, the microbial biomass was harvested after the fermentation period by centrifugation at 6,000 rpm and 4 °C for 10 min. The collected biomass was then dried at 105 °C until its weight remained unchanged. The results were expressed as grams of dry biomass per liter of culture medium (g/L).

2.1.8.3. Minimum Inhibitory Concentration (MIC). The MIC was determined using the broth microdilution method.³³ Inhibitory concentrations were established by serial two-fold dilution of the EtOAc extract in Mueller-Hinton Broth (MHB) within 96-well microplates, generating a final concentration range of 6.00–20.00 μ g/mL per well. Each well received 10 μ L of standardized bacterial inoculum (10^8 CFU/mL), and plates were maintained for 24 h at 30 °C prior to visual inspection. Reference antibiotics (Gentamicin and Ampicillin) were included as positive controls, and solvent-only wells (DMSO) acted as the negative control to account for any solvent-associated growth inhibition.

2.1.9. Molecular identification of the isolate based on 16S rRNA sequences

2.1.9.1. DNA extraction. Genomic DNA was obtained by mechanical lysis. Cell pellets were resuspended in denaturing buffer (1% SDS, 100 mM NaCl, 4% Triton X-100). Three alternating cycles of vortexing with glass beads in chloroform: isoamyl alcohol (24:1) and ice-cooling were applied to maximize cell disruption while preventing thermal degradation of nucleic acids. DNA was precipitated by co-treatment with isopropanol/ethanol at – 20 °C, then the pellet was washed with 70% ethanol to eliminate residual salts. After purification, DNA was taken up in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

2.1.9.2. PCR amplification and sequencing. The 16S rRNA gene was amplified via PCR using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-

3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR products were verified by 1% (w/v) agarose gel electrophoresis and sequenced by Apical Scientific (Singapore). The resulting sequences were analyzed with NCBI BLAST to identify closely related species. Multiple sequence alignment was carried out with CLUSTALW software,³⁴ then the evolutionary relationships among the aligned sequences were determined using the Neighbor-Joining algorithm as implemented in MEGA 11.³⁵

2.1.9.3. Analysis of fermentation broth components by GC–MS. The ethyl acetate extract was examined using GC–MS system (Agilent 5977B GC/MSD) equipped with a DB-5 ms capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). The following temperature program was established: 50 °C (2 min), increased to 180 °C at 10 °C/min (held for 1 min), then to 280 °C at 16 °C/min (held for 5.75 min). Mass spectra were recorded in full scan mode (50–550 amu).

2.2. Data analysis

All experimental procedures were performed in at least triplicate to ensure reproducibility. Preliminary data processing and descriptive statistical analyses were conducted using Microsoft Excel 2021. To determine significant differences among treatment means, one-way ANOVA, along with Tukey's post hoc test, was conducted using SPSS software. A significance level was set at $p < 0.05$.

3. Results

3.1. Isolation and identification of endophytic Actinomycetes

The strain YBRX0 was isolated from root samples collected in Viet Cuong, Yen Bai (21.658° N, 104.851° E), using SFM (Soy Flour Mannitol) medium at 30 °C. The isolate was initially identified based on its ability to produce spores and to form substrate and aerial mycelia. Physiological and biochemical assays indicated that YBRX0 exhibits normal growth, produces abundant spores, and is Gram-positive. Morphological characteristics of the isolate, including colony morphology, microscopic observation, and scanning electron microscopy (SEM) analysis, are presented in Fig. 1.

The strain is capable of pigment production, displaying various colors across its mycelial systems (Table 1). Specifically, it produces an orange pigment on ISP3 medium and a black pigment on ISP6 medium (Fig. 2). Furthermore, biochemical tests revealed that the strain can degrade several substrates, including starch, gelatin, casein, and urea (Table 2).

The findings from the analysis of 16S rRNA gene sequences indicated that strain YBRX0 has a high similarity (99.14%) to the reference sequences of the genus *Streptomyces* (Fig. 3). The sequence has been deposited in the database under the accession number PZ289836.

3.2. Biological activities of Streptomyces strain YBRX0

Strain YBRX0 exhibited significant antibacterial activity against a range of pathogenic bacteria, including *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33519, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus cereus* ATCC 1306, *Rhodococcus equi* ATCC 6939, and *Enterococcus faecalis* ATCC 29212 (Table 3, Fig. 4). Furthermore, strain YBRX0 exhibited moderate activity as an antioxidant, exhibiting an IC₅₀ value of 197.26 ± 8.87 μ g/mL.

3.3. Minimum inhibitory concentration (MIC)

The ethyl acetate extract inhibited the growth of all tested pathogenic bacteria at the following MIC values (μ g/mL): *E. coli* 14;

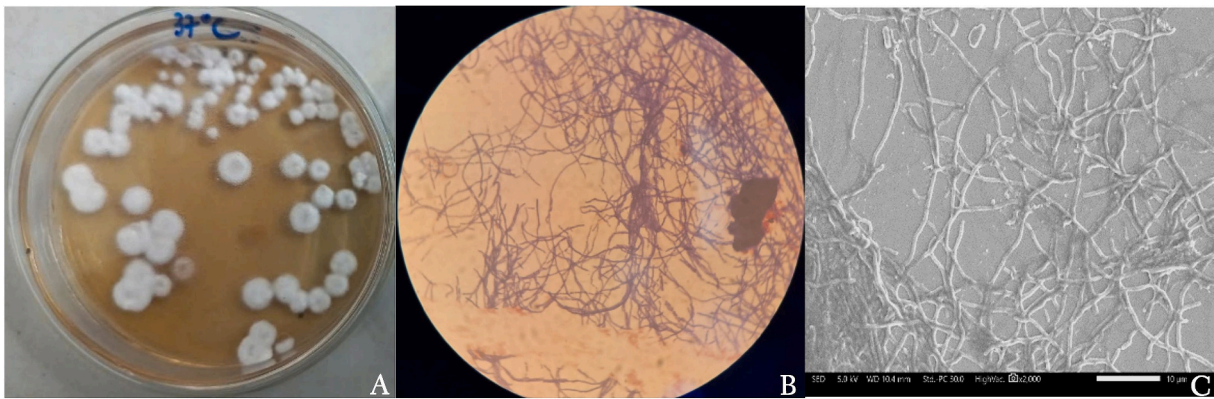


Fig. 1. Morphological characteristics of the actinomycete strain *Streptomyces* YBRX0: (A) Growth on ISP2 medium, (B) Microscopic image of spore chain at 100× magnification, (C) SEM image (at 2000× magnification).

Table 1
Morphological characteristics of the *Streptomyces* YBRX0 strain on different media.

Character	ISP1	ISP2	ISP3	ISP4	ISP5	ISP6	ISP7
Growth	+++	+++	+++	++	++	++	+++
Color of substrate mycelium	White	White	White	White	Beige	White	Beige
Color of aerial mycelium	Orange	Orange	Orange	White	White	Brown	White
Soluble pigments produced	–	–	Orange	–	–	Black	–

Note: Growth intensity: + (moderate), ++ (strong), +++ (abundant), and – (no growth).

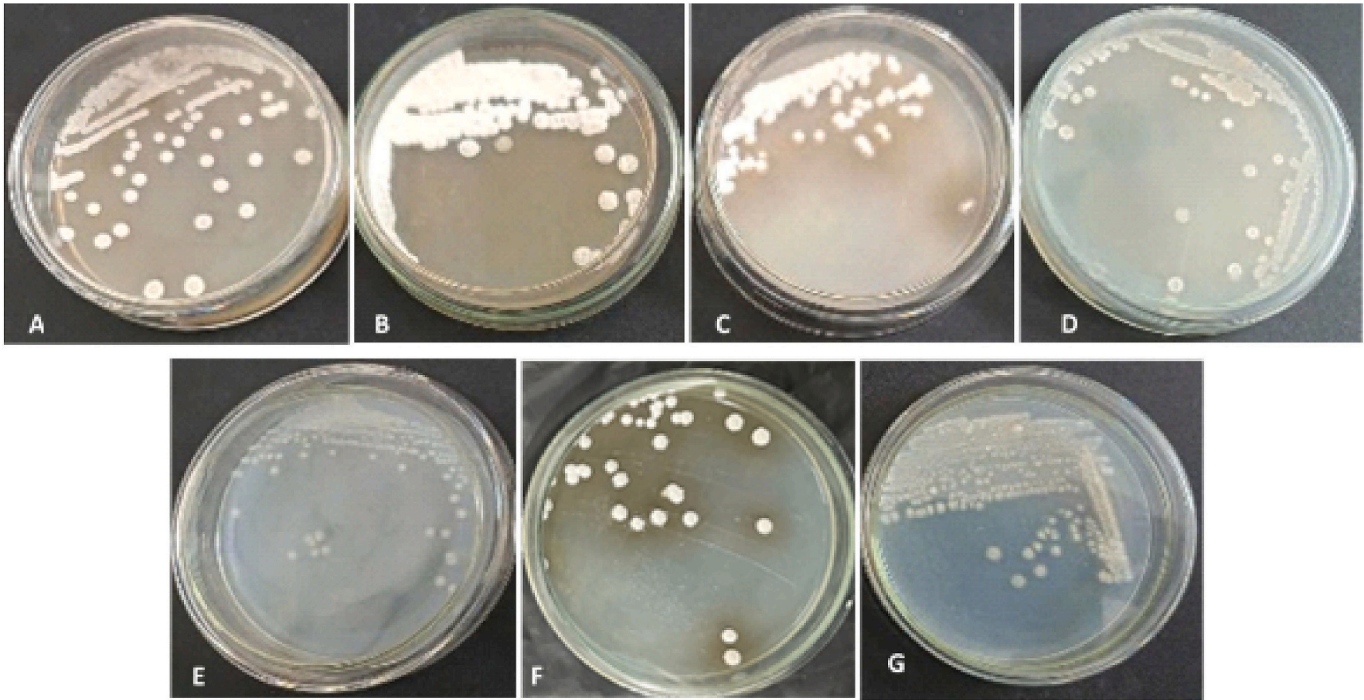


Fig. 2. Morphological characteristics of *Streptomyces* strain YBRX0 cultured on various media (A–G: ISP1–ISP7 respectively).

P. aeruginosa 12; *S. typhimurium* 16; *E. faecalis* 10; *B. cereus* 8; MRSA 8; and *R. equi* 10 (Table 4). These results demonstrate that the extract from strain YBRX0 showed notable inhibitory activity across all tested strains, with particularly low MIC values recorded against *B. cereus* and MRSA.

3.4. Analysis of fermentation product profiles from *Streptomyces* strain YBRX0

A total of 12 distinct compounds were identified in the bioactive ethyl acetate extract of *Streptomyces* strain YBRX0 by GC–MS analysis (Fig. 5 and Table 5). The major constituents identified from the GC–MS profile include: Pyrrolo[1,2-*a*]pyrazine-1,4-dione,hexahydro-3-

Table 2Physiological and Biochemical Characteristics of *Streptomyces* strain YBRX0.

Biochemical Characteristics	Results
Starch hydrolysis	+
Casein hydrolysis	+
Gelatin hydrolysis	+
Utilization of Glucose	+++
Utilization of Lactose	+
Utilization Saccharose	++
Utilization Citrate	–
H ₂ S production	–
Urease test	–
Mannitol	++
Catalase	++
Action on Skimmed milk	–
Physiological Characteristics	
Gram-staining	+
Melanoid pigment production on ISP6	+++
Melanoid pigment production on ISP7	–
Temperature (°C): 20	+
Temperature (°C): 30	+++
Temperature (°C): 45	–
Temperature (°C): 55	–
pH	5–12
NaCl tolerance (W/V)	0–3

Note: +: Positive; –: Negative.

(phenylmethyl)-,Pyrrolo[1,2-*a*]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- and Benzeneacetamide. The remaining compounds are listed in Table 5.

4. Discussion

4.1. Isolation of endophytic Actinomycetes

To date, there is a distinct lack of published literature regarding the isolation of endophytic actinomycetes from *C. tonkinensis* or the characterization of their health-promoting bioactive metabolites. Existing studies on the Euphorbiaceae family indicate a predominance of the genus *Streptomyces*, followed by other genera such as *Micromonospora*, *Microbispora*, and *Nocardiopsis*,^{36–38} with root tissues typically harboring 50–70% of the total endophytic population.^{37–39} Consistent with these

ecological patterns, the present study successfully isolated *Streptomyces* sp. YBRX0 from the roots of *C. tonkinensis* using SFM medium at 30 °C, followed by a comprehensive identification through phenotypic, biochemical, and molecular analyses.

4.2. Cultural, Morphological, Biochemical Characteristics, and Molecular Identification of the *Streptomyces* Strain

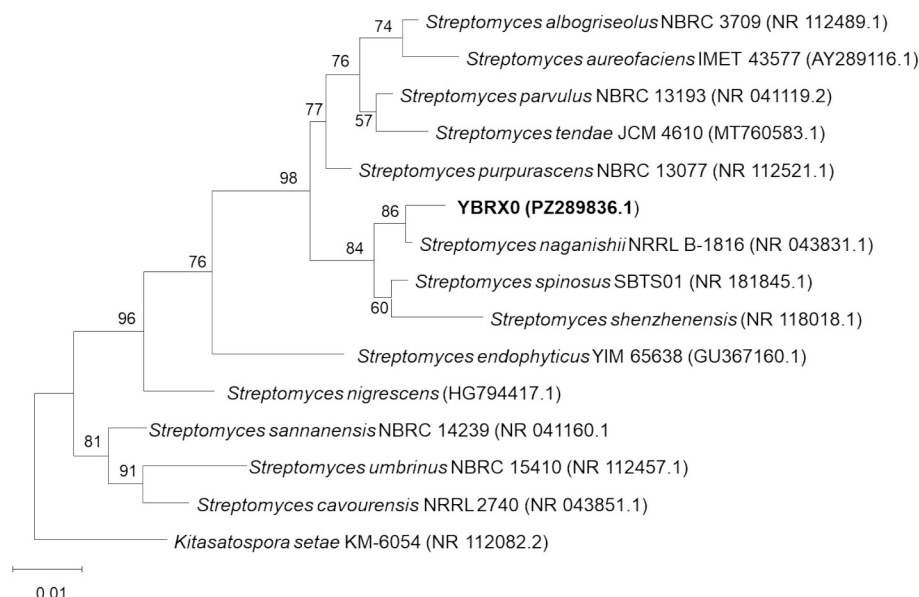
The isolate *Streptomyces* sp. YBRX0 exhibited robust growth across nearly all tested ISP media. This observation contrasts with the findings of Singh et al. (2018)⁴⁰ who reported only moderate-to-weak growth of their *Streptomyces* strains on similar media. However, our results align with the vigorous growth patterns documented by Daigham and Mahfouz (2021).⁴¹ Such discrepancies in growth performance likely stem from strain-specific metabolic profiles, as nutrient availability and media composition are known to profoundly influence actinomycete development.

Distinctive coloration of both substrate and aerial mycelia was also observed in strain YBRX0. While previous studies have frequently reported *Streptomyces* pigments ranging from pink and red to black and yellow-green^{40–42} The present isolate exhibited a distinct color palette comprising white, beige, orange, and brown. These variations in mycelial pigmentation and the secretion of soluble pigments are likely reflective of the strain's specific metabolic state and its capacity for secondary metabolite synthesis under the investigated conditions.⁴³

Table 3Antibacterial activity of the ethyl acetate extract from *Streptomyces* strain YBRX0.

Tested strains	Zone of inhibition (D±SD, mm) [#]
Gram-negative bacteria	
<i>E.coli</i> ATCC 25922	19.97 ± 0.55 ^{ab}
<i>S. typhimurium</i> ATCC 14028	18.83 ± 0.35 ^a
<i>P. aeruginosa</i> ATCC 9027	20.90 ± 0.89 ^b
<i>E. faecalis</i> ATCC 29212	23.13 ± 0.67 ^c
Gram-positive bacteria	
<i>B.cereus</i> ATCC 1306	23.23 ± 0.64 ^c
<i>MRSA</i> ATCC 33519	28.73 ± 0.59 ^c
<i>R.equi</i> ATCC 6939	24.97 ± 0.81 ^d

[#] : Mean ± SD (n = 3). Different letters (a–e) denote significant differences (Fisher's LSD, P < 0.05).

**Fig. 3.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of *Streptomyces* sp. YBRX0.

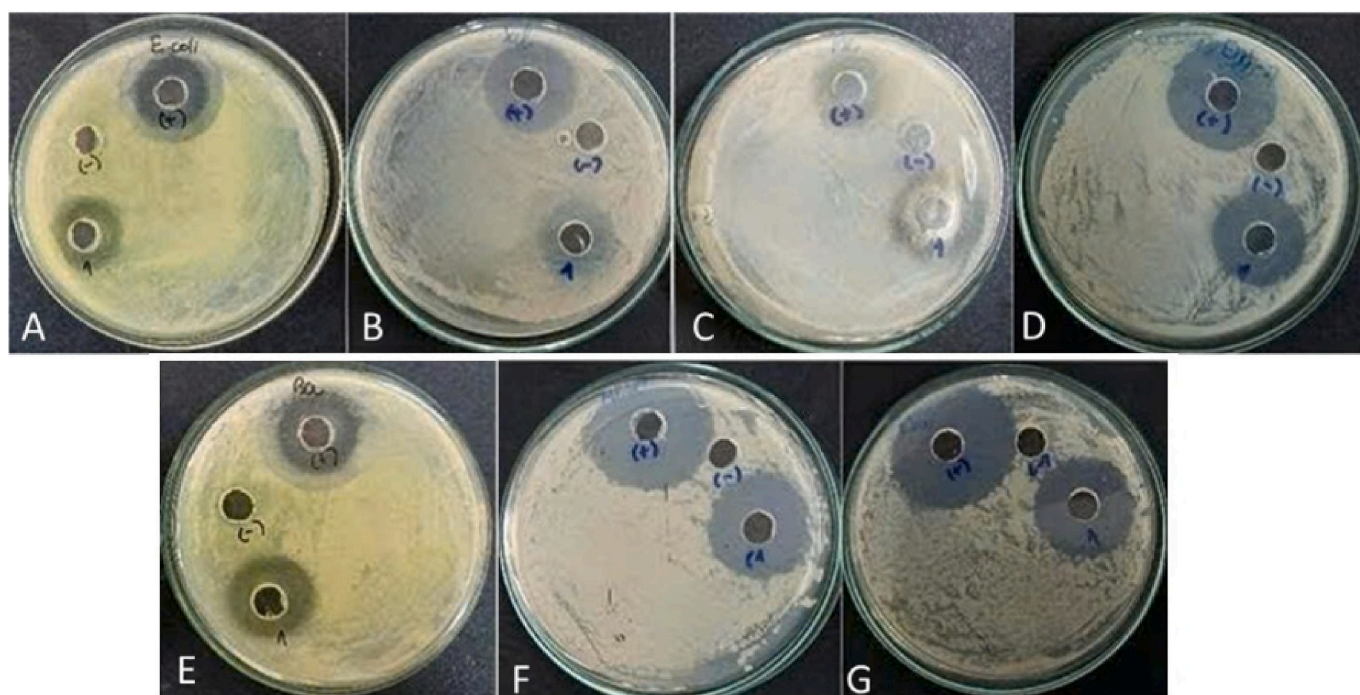


Fig. 4. Antibacterial activity of *Streptomyces* strain YBRX0 against: (A) *E. coli* ATCC 25922, (B) *S. typhimurium* ATCC 14028, (C) *P. aeruginosa* ATCC 9027, (D) *E. faecalis* ATCC 29212, (E) *B. cereus* ATCC 1306, (F) MRSA ATCC 33519, (G) *R. equi* ATCC 6939. (+), positive control (Gentamicin or Ampicillin); (−), negative control (DMSO); (1), ethyl acetate extract of *Streptomyces* strain YBRX0.

Table 4

MIC values of *Streptomyces* strain YBRX0 extract against tested pathogenic bacteria.

Tested strains	Extract (μg/mL)	Gentamicin (μg/mL)	Ampicillin (μg/mL)
Gram-negative bacteria			
<i>E. coli</i> ATCC 25922	14	10	
<i>S. typhimurium</i> ATCC 14028	16	10	
<i>P. aeruginosa</i> ATCC 9027	12	10	
<i>E. faecalis</i> ATCC 29212	10	10	
Gram-positive bacteria			
<i>B. cereus</i> ATCC 1306	8		10
MRSA ATCC 33519	8		10
<i>R. equi</i> ATCC 6939	10		10

Note: The MIC values were obtained through the broth microdilution method.

SEM examination at 2000× revealed a densely branched mycelial network. Individual hyphae were cylindrical with smooth outer walls, measuring 0.6–1.0 μm in diameter. Spore chains were visible at hyphal tips, displaying morphological hallmarks consistent with those previously described for the genus *Streptomyces*.^{44–46}

Molecular characterization yielded an amplicon of approximately 1.5 kb corresponding to the near-full-length 16S rRNA gene, a fragment size consistent with those reported in analogous taxonomic studies employing the same primer pair.^{47,48} Integrated assessment of phenotypic, physiological, and sequence data unambiguously places the isolate within the genus *Streptomyces*.

4.3. Bioactivity of *Streptomyces* strain YBRX0

The actinomycete strain YBRX0 exhibited antibacterial activity against both Gram-positive and Gram-negative strains of bacteria, demonstrating a broad spectrum of action. The observed inhibitory activity suggests that strain YBRX0 produces metabolites that target diverse bacterial cell types. Gram-positive test pathogens were generally more susceptible, yielding larger inhibition zones than their Gram-negative counterparts. This differential response is consistent with well-established structural differences between the two cell envelope architectures, and has been similarly documented for endophyte-derived extracts across the literature.^{49–51} Gram-positive cell walls, composed predominantly of peptidoglycan without an outer membrane, offer limited resistance to the penetration of hydrophobic metabolites. Gram-negative organisms, by contrast, present multiple layers of

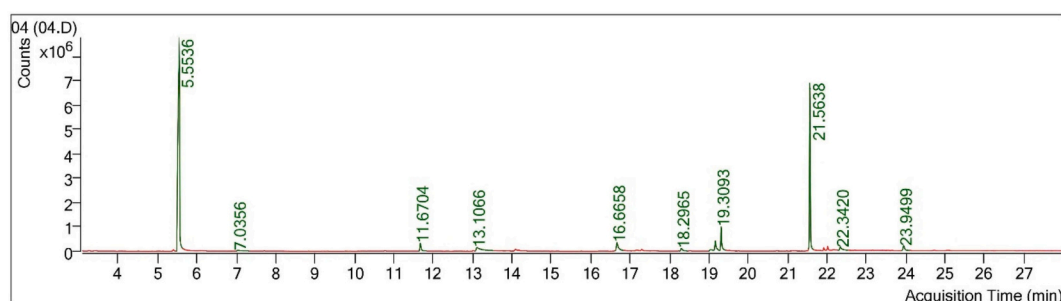


Fig. 5. GC-MS chromatogram of the secondary metabolites produced by *Streptomyces* strain YBRX0.

Table 5
Metabolite profiling of *Streptomyces* strain YBRX0 using GC–MS analysis.

Component RT	Compound Name	CAS#	Formula	Area	Match Score
5.5536	Ethanol, 2-butoxy-	111-76-2	C ₆ H ₁₄ O ₂	25,616,680	98.1
7.0356	1,2-Dimethoxy-2-methylpropan-1-amine	1,000,445-17-2	C ₆ H ₁₅ NO ₂	266,110	79.1
11.6704	Butyl 2-ethylhexanoate	68,443-63-0	C ₁₂ H ₂₄ O ₂	731,892	98.6
13.1066	Benzeneacetamide	103-81-1	C ₈ H ₉ NO	1,323,608	83.4
16.6658	Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-	30,316-36-0	C ₁₃ H ₁₈	1,135,009	89.3
18.2965	Cyclo(L-prolyl-L-valine)	2854-40-2	C ₁₀ H ₁₆ N ₂ O ₂	360,222	96.1
19.0461	2,2-Dimethyl-4-oxo-1,2,3,4-tetrahydroquinazoline	77,726-78-4	C ₁₀ H ₁₂ N ₂ O	269,600	81.9
19.1663	Pyrrolo[1,2- <i>a</i>]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	5654-86-4	C ₁₁ H ₁₈ N ₂ O ₂	1,261,929	91.0
19.3093	Pyrrolo[1,2- <i>a</i>]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	5654-86-4	C ₁₁ H ₁₈ N ₂ O ₂	2,126,211	94.7
21.5638	Myristohydroxamic acid	17,698-03-2	C ₁₄ H ₂₉ NO ₂	9,409,519	71.7
22.3420	Pyrrolo[1,2- <i>a</i>]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	14,705-60-3	C ₁₄ H ₁₆ N ₂ O ₂	519,466	87.8
23.9499	Squalane	111-01-3	C ₃₀ H ₆₂	608,334	92.1

intrinsic defense: their outer membrane, anchored by lipopolysaccharide, reduces passive diffusion of antimicrobial compounds, while active efflux pumps and enzymatic modification of target sites collectively diminish susceptibility to many naturally derived agents, including those of actinomycete origin.⁵²

Although the MIC values of the crude extract (8–16 µg/mL) were broadly similar in magnitude to those of the reference antibiotics Gentamicin and Ampicillin (10 µg/mL), this comparison warrants careful interpretation. Unlike the purified reference antibiotics, the EtOAc extract is an unfractionated mixture containing at least twelve distinct metabolites, as revealed by GC–MS profiling (Table 5), of which only a subset is presumed to contribute to the observed antibacterial effect. The apparent inhibitory potency of the crude extract is therefore distributed across multiple constituents present at comparatively low individual concentrations, suggesting that the extract may contain active constituents with higher intrinsic antibacterial potency following isolation and purification. This interpretation is consistent with established classification criteria for natural product extracts, under which crude extracts displaying MIC values below 100 µg/mL are generally considered to exhibit significant antibacterial activity.⁵³ By this benchmark, the activity profile of strain YBRX0, particularly against *B. cereus* and MRSA (MIC = 8 µg/mL), compares favorably with previously reported crude extracts from endophytic actinomycetes. The comparable activity observed against MRSA, a clinically significant methicillin-resistant pathogen, using an unfractionated extract further supports the potential of strain YBRX0 as a promising source of antibacterial metabolites and provides a strong rationale for future bioassay-guided fractionation. Within the broader drug discovery pipeline, these results should be regarded as an early-stage screening outcome rather than a terminal efficacy benchmark, since crude extracts generally underestimate the intrinsic activity of their individual bioactive constituents, which can only be accurately evaluated after purification.

Strain YBRX0 demonstrated moderate activity as an antioxidant, exhibiting an IC₅₀ value of approximately 197 µg/mL. These findings are consistent with the broader literature demonstrating antioxidant activity among endophytic *Streptomyces* strains from medicinal and coastal plant species, though the reported potencies vary considerably across studies.^{56,57} Notably, isolates derived from host plants with inherently high polyphenolic content, such as those from mangrove ecosystems, have, in several cases, yielded extracts with substantially greater radical-scavenging efficiency than observed here. This disparity may reflect the degree to which host-plant metabolic environments shape endophyte secondary metabolism. The moderate activity recorded for YBRX0 thus warrants further fractionation efforts to determine whether specific compound classes within the crude extract contribute disproportionately to the observed scavenging capacity. Broader screening efforts targeting actinomycete endophytes from South Asian medicinal flora have similarly revealed widespread, though variable, antioxidant output across isolates.⁵⁴ That YBRX0 falls toward the lower end of the reported activity spectrum is consistent with the recognized heterogeneity in secondary metabolite profiles among endophytic

strains, which is attributable to variability in biosynthetic gene content, regulatory networks, and the chemical nature of the host-plant environment from which each isolate was recovered.

4.4. Metabolite profiling of *Streptomyces* strain YBRX0

Among the tentatively identified constituents, Pyrrolo[1,2-*a*]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) was the dominant compound detected by GC–MS. Pyrrolopyrazines are well-recognized secondary metabolites produced by diverse *Streptomyces* species.^{55,56} A growing body of literature documents their pharmacological versatility: antifungal effects against *Pyricularia oryzae* and *Candida albicans* have been reported, alongside significant antibacterial and anti-biofilm potency, including activity against MRSA.^{57–61} Furthermore, it has demonstrated antioxidant potential and selective cytotoxicity against cancer cells.^{55,62} The antioxidant activity of Pyrrolo[1,2-*a*]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) has also been documented in previous studies. This compound has been isolated from *Streptomyces* strains inhabiting mangroves and medicinal plant tissues, demonstrating robust free radical scavenging capabilities in DPPH and ABTS assays, alongside low toxicity in cell models.^{55,63,64} However, GC–MS-based identification remains tentative pending confirmation by NMR and high-resolution mass spectrometry. The observed bioactivities are, moreover, likely the result of a complex mixture of metabolites acting in concert, rather than attributable to a single constituent. Further investigations involving compound isolation, purification, and individual bioactivity assays are therefore required before definitive mechanistic conclusions can be drawn. Future studies should additionally examine how environmental and cultivation conditions modulate the metabolite output of this strain.

5. Conclusion

The endophytic actinomycete strain *Streptomyces* YBRX0, isolated from the roots of *Croton tonkinensis* Gagnep. (Khô sâm cho lá) in Viet Cuong commune, Tran Yen district, Yen Bai province, Vietnam, exhibited broad-spectrum antibacterial activity and moderate antioxidant capacity. Notably, this antibacterial potency was achieved by an unpurified crude extract at concentrations comparable to purified reference antibiotics, highlighting the strain's potential as a source of structurally novel antimicrobial leads rather than a direct antibiotic substitute at this stage of investigation. GC–MS analysis revealed that several compounds were tentatively identified, including Pyrrolo[1,2-*a*]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-, as major constituents of the extract. Published evidence for structurally related pyrrolopyrazine compounds points to a broad pharmacological profile encompassing antimicrobial, antifungal, free-radical-scavenging, anti-biofilm, and cytotoxic activities, lending biological plausibility to the effects reported here. However, as GC–MS identification provides only tentative compound assignments, definitive attribution of the observed bioactivities to specific constituents will require targeted isolation,

chromatographic purification, and rigorous structural characterization by NMR and high-resolution mass spectrometry. Until such work is completed, the data presented should be interpreted as evidence that *Streptomyces* sp. YBRX0 harbors a metabolically active secondary metabolome, warranting systematic investigation, particularly in the ongoing search for structurally novel agents against drug-resistant pathogens.

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CRediT authorship contribution statement

Ha Trang Phan: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Hung Vuong Pham:** Visualization, Methodology, Investigation. **Xuan Truong Nguyen:** Methodology, Investigation, Data curation. **Thuy Hang Dam:** Methodology, Investigation, Formal analysis, Data curation. **Huu Nghi Do:** Methodology, Investigation. **Trung Hai Huynh:** Supervision, Methodology, Conceptualization. **Quoc Phong Truong:** Supervision, Methodology, Conceptualization. **Truong Giang Nguyen:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgeb.2026.100786>.

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