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Polyphasic characterization, bioactive potential and metabolite profiling of *Streptomyces violaceoruber* R6 isolated from *Ocimum tenuiflorum*

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Abstract

The emergence of antimicrobial resistance necessitates the exploration of novel bioactive compounds. We conducted this study to explore the biological capabilities of R6, an endophytic actinomycete isolated from *Ocimum tenuiflorum*. The strain exhibited significant antibacterial activity, particularly against ESBL-producing *Escherichia coli* (20 mm) and *Klebsiella pneumoniae* (25 mm). Moderate antifungal activity was also observed against *Aspergillus fumigatus* (9–10 mm). Cytotoxicity screening using the MTT assay revealed 75% cell mortality at 100 mg/mL against Baby Hamster Kidney fibroblast (BHK-21) cell lines. Thin-layer chromatography (TLC) and HPLC-UV/Vis analysis indicated the presence of polyketides, indoles, and amines, suggesting the synthesis of biologically active compounds. The detection of type I polyketide synthase (PKS-I) gene cluster further confirmed its potential for synthesizing novel secondary metabolites. Genetic identification through analysis of 16 S rRNA sequencing and phylogenetics indicated it to be *Streptomyces violaceoruber*. To the best of our understanding, this is the first report of the isolation of *Streptomyces violaceoruber* from the medicinal plant, *Ocimum tenuiflorum*. These findings support the prospect of medicinal plant endophytes as a potential source of antimicrobial and cytotoxic compounds, encouraging further research on their pharmaceutical applications.

Keywords Antimicrobials, Endophytes, ESBL, *Ocimum*, *Streptomyces*

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Background

Ocimum tenuiflorum, widely recognized as Tulsi or Holy Basil, is a highly valued medicinal plant in both traditional and modern medicine. It is widely used in Ayurveda and is recognized for its antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties [1]. Research has shown that it is effective against a variety of human pathogens, highlighting its significance as a candidate for drug discovery [2]. The medicinal properties of *O. tenuiflorum* are primarily associated to its bioactive secondary metabolites, which include phenolics, flavonoids, terpenoids, and essential oils [3].

In addition to these phytochemicals, recent studies have emphasized the role of endophytes residing within plant tissues in enhancing the plant's bioactivity and overall medicinal value [4]. Endophytes especially endophytic actinomycetes, have gained attention for their ability to produce bioactive compounds that contribute to plant health and defense [5]. Particularly the fact that recent investigations have revealed that endophytic *Streptomyces* strains isolated from medicinal plants can produce compounds identical to or analogous with those originally identified in their plant hosts. For instance, *Streptomyces* sp. YINM00001, derived from *Peperomia dindygulensis*, was found to harbor biosynthetic pathways for cycloheximide, dinactin, and anthracimycin with known antimicrobial or anticancer activities mirroring the plant's reported bioactive profile [6]. In case of *O. tenuiflorum*, studies have identified endophytes that exhibited significant pharmacological potential [7], against drug-resistant pathogens [8] such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and diverse fungal pathogens [2, 9].

Streptomyces violaceoruber is a filamentous, spore-forming actinomycete recognized for its complex aerial mycelium and prolific secondary metabolism. It has drawn attention due to its diverse biosynthetic range, including volatile organic compounds with antimicrobial potential and enzymes such as alkaline proteases with biomedical relevance [10, 11]. Over the decades, this species has been recognized for producing a suite of bioactive metabolites including kendomycin [10], granaticin, protoactinorhodin, methylenomycin A, and phospholipase A₂ demonstrating its continued relevance in pharmaceutical and biotechnological research [12–14]. While this species has been studied in various ecological niches for its metabolite production, there have been no reports to date of endophytic *S. violaceoruber* inhabiting medicinal plants.

Studies regarding the production of novel polyketide antibiotics have revealed their synthesis through biosynthetic gene clusters (BGCs) in endophytic *Streptomyces* spp [10, 11]. Such antibiotics are synthesized through

the polyketide synthase enzymes and some of the well known examples include antibiotics such as streptomycin, erythromycin and tetracycline [10]. Polyketide synthases (PKSs) are key enzymes essential for polyketide biosynthesis, functioning similarly to fatty acid synthases. However, they constitute only a part of a complex metabolic pathway that involves multiple genes responsible for precursor synthesis and product modification [15]. Type I polyketide synthases (PKS-I) function as complex, multifunctional enzymes composed of many domains. These enzymes are typically large and modular, with each module facilitating a single cycle of chain elongation during polyketide synthesis. Advancements in molecular techniques have led to the successful identification of numerous undiscovered PKS genes. For example, genome analysis of *Streptomyces* spp. has revealed that it may contain multiple PKS genes having yet unidentified functions. This suggests that *Streptomyces* possess the capability to synthesize previously undiscovered polyketides. PKS gene screening is widely employed as a tool for detecting polyketides that may be novel, as their presence serves as a strong indicator of its potential to produce new polyketide compounds [16].

Our study indicated that despite its established role as a soil and environmental isolate, *S. violaceoruber* could reside as an endophyte within medicinal plants. Our current study reports for the first time its isolation from *O. tenuiflorum* (Tulsi), enhancing our understanding of the species' ecological range. Unlike previously described soil or marine isolates, which typically produced specific metabolites such as granaticin or kendomycin, R6 demonstrated a broader metabolite potential, including polyketides, indoles, and amines. It also exhibited strong antibacterial activity against ESBL-producing *E. coli* and *K. pneumoniae* as well as moderate antifungal activity against *A. fumigatus*, distinguishing it from soil-derived strains. This unique ecological origin and enhanced bioactive profile suggests that R6 may possess biosynthetic gene clusters that may produce structurally novel secondary metabolites, making it a compelling candidate for antimicrobial and cytotoxic product discovery.

Methods

Isolation of endophytic actinomycetes from *O. tenuiflorum*

O. tenuiflorum was collected from botanical garden (31.57° N, 74.30° E) situated in the main campus of University of Veterinary and Animal Sciences (UVAS) Lahore. The plant were uprooted with care so that the roots and leaves remain intact and it was immediately transported to the laboratory for processing.

The plant was rinsed with tap water to wash away any attached soil residues. Surface sterilization was performed following the method explained by Qin et al. [17]. Briefly, the plant parts (roots, stem and leaves) were air

dried and cut into small segments of 0.5 cm using a sterile scalpel. The plant segments were dipped in ethanol (70%) for 5 min, followed by treatment with sodium hypochlorite (0.9%) for 20 min. Subsequently, the segments were washed with distilled water three times to ensure complete removal of disinfectants. To prevent fungal contamination and facilitate tissue agitation, the samples were treated with NaHCO₃ (10%) for 10 min. Finally, the segments were thoroughly rinsed with autoclaved distilled water, picked up using sterile forceps, and plated onto actinomycete isolation agar (Difco Laboratories, USA). These segments were also crushed separately in a sterile pestle and mortar. A small amount of autoclaved distilled water (100 µl) was used to prepare the slurry. This slurry was spread (100 µl) on the actinomycetes isolation agar plates. The inoculated plates were incubated at 28 °C for one to three weeks (7–21 days). To verify surface sterilization, the plant segments were dragged across the agar plates before incubation. This sterilization protocol was optimized following Qin et al. [17] until no microbial contamination was detected. The resulting actinomycete colonies were subcultured onto GYM (Glucose Yeast Extract Malt Extract) agar [18].

Preliminary screening for bioactivity

The isolated endophytes were checked for their bioactivity with agar plug method as outlined by Fatima et al. [19]. According to this method, agar plugs were cut out from freshly grown strains on GYM agar and placed on Mueller-Hinton agar (MHA) plates inoculated with the test organisms (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*). It revealed an endophyte, R6 to be bioactive against both Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*).

Morphology, biochemical and physiological characterization

The morphological features of strain R6 were documented after incubation on GYM agar for up to 7 days. Microscopic examination of aerial and substrate mycelium was conducted using slides prepared according to the method outlined by Kieser et al. [20] and the substrate and aerial mycelium were examined using Gram staining (Cappuccino and Sherman 2005). The physiological properties were assessed using various sugars as carbon sources and melanin production, following the guidelines of the International *Streptomyces* Project (ISP). Additionally, hydrolysis of urea and hydrolysis of esculin or arbutin for biochemical characterization were performed as outlined [18].

16 S sequencing

The taxonomic classification of strain R6 was confirmed through 16 S rRNA sequencing facility provided by a commercial sequencing service (1st Base Inc, Singapore). For this purpose, total genomic DNA was obtained by following the method of Tanvir et al. [21]. The data was analysed using the NCBI website using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) and a phylogenetic tree was generated using the Neighbor-Joining (BLAST: Basic Local Alignment Search Tool) method as described by Saitou and Nei [22] in MEGA 7 [23] to analyze those sequences that were exhibiting 99% homology. A closely related genus, *Streptacidiphilus jiangxiensis* was used as an outgroup for phylogenetic analysis. The sequence was submitted to GenBank, where it was assigned the accession number MN704645.

Small Scale cultivation of *S. violaceoruber* R6

The selected strain R6 was cultivated in two separate Erlenmeyer flasks of GYM broth (300 ml) with pH adjusted to 7.8 before autoclaving at 121 °C for 20 min. Prior to inoculation, a pre-culture of R6 was prepared in GYM broth, and 10% of this pre-culture was transferred to the autoclaved medium. The inoculated flasks were incubated at 28 °C under continuous agitation on a linear shaker set to 180 rpm for 3–5 days. After incubation, the cultures were subjected to sonication for 10 min to facilitate cell disruption. Ethyl acetate (1:1) was added to the disrupted culture, and the upper organic layer was carefully separated [24]. This organic phase was then evaporated under vacuum using a rotary evaporator (Heidolph 4000, GmbH & Co, KG, Germany). The extracted metabolites were dissolved in absolute methanol for further analysis and stored in a glass vial at 4 °C.

Determination of antibacterial and antifungal activity

The antimicrobial activity of the R6 extract was assessed with agar well diffusion method, as mentioned by Balouiri et al. [25]. Both bacterial and fungal pathogens were used as test organisms, including *Escherichia coli*, *Campylobacter jejuni*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Acinetobacter baumannii* (University Diagnostic Lab (UDL), UVAS, Lahore), and five toxigenic strains of *Aspergillus fumigatus*. (Quality Operations Lab (QOL), UVAS, Lahore). The assay was performed by dispensing a volume of 60 µl of crude extract (5 mg/ml) into wells created using a sterile cork borer and allowed to diffuse. For bacterial pathogens, MHA was used, while Sabouraud dextrose agar (SDA) (Difco Laboratories) was utilized for fungal pathogens. The plates were incubated at 37 °C for 24 h, after which the zones of inhibition were measured in millimeters (mm).

Determination of anti-ESBL activity

The anti-ESBL activity of *S. violaceoruber* R6 extract was determined against eight ESBLs producing urinary isolates (UO1-UO8) of *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *E. coli* (Combined Military Hospital (CMH), Lahore) using agar plug method and agar well diffusion method as previously outlined. Incubation was carried out at 37 °C for 24 h and the zones were measured.

In vitro screening for cytotoxic activity using MTT assay

For determining the cytotoxicity of *S. violaceoruber* R6, the extract was dried, weighed and re-dissolved in 1% (v/v) DMSO in double-distilled water [26] to prepare the required concentrations (0.2, 0.4, 0.8, 1.6, 3.1, 6.2, 12.5, 25, 50, 100 mg/ml). The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) assay was done following the method described by Supino [27]. For this purpose, cell culture media, Glasgow Modified Essential Medium (GMEM) [28] was dissolved in double-distilled water, followed by supplementation with fetal bovine serum (10%) (Thermo Fisher Scientific, USA) and sodium bicarbonate (4.17 mM) [29]. To prevent bacterial contamination, penicillin (100 U/ml), streptomycin (50 mg), gentamycin (50 mg), and amphotericin B (25 mg) were incorporated [30]. The media was filtered using a capsule filter (Merck, USA) and its sterility was verified by culturing on nutrient agar and blood agar [31]. Then trypsin (10 gm) [32] was introduced, and the cell count of Baby Hamster Kidney fibroblast (BHK-21 Clone 13) cell line (Sigma Aldrich, USA) was adjusted to 300,000 cells/ml. A diluted cell suspension (0.1 ml) was distributed into the wells of a 96-well microplate and incubated at 37 °C under 5% CO₂ incubator for 72 h. Once a confluent monolayer was established, the media was removed, and a 100 mg/ml working solution of endophytic actinomycetes concentrated broth was added to the first well, mixed thoroughly, and transferred to the second well to perform a two-fold serial dilution. The plate was further incubated at 37 °C in a 5% CO₂ incubator for 72 h. The positive control was BHK-21 cells without the extract while negative control was 1% (v/v) DMSO. To assess cytopathic effects (CPEs), the plates were examined after 24, 48, and 72 h. Following the 72-hrs incubation, the concentrated broth was discarded, and 10 µl of MTT reagent (5 mg/ml) (Thermo Fisher Scientific, USA) was put in each well. The plate was then incubated for 4 to 24 h. After removing the medium, 200 µl of methanol was added. The optical density (OD) was observed at 595 nm with a microplate reader (BioRad, USA), and the percentage of cell viability was calculated using the following formula:

$$\text{Cell survival percentage (\%)} = \frac{\text{abs. of R6 extract} - \text{abs. of DMSO}}{\text{Abs. of control} - \text{abs. of DMSO}} \times 100$$

Abs = Absorbance.

Molecular confirmation of polyketide synthase type I (PKS-I) gene

Molecular confirmation was done to determine the presence of biosynthetic genes that result in type I polyketide synthase (PKS-I) production. For this confirmation, two sets of degenerate primers, MDPQQR F: 5'-RTRGAYCCNCAGCAICG – 3' and HGTGT R: 5'-VGTNCCNGTGCCRTGS – 3' to target the alpha-keto synthase gene of PKS-I, generating an expected amplicon of 750 bp [33]. PKS/K1 F: 5'-SAAGTCSAACATCCGBCA – 3' and PKS/M6 R: 5'-CGCAGGTTSCSGTACCAGTA-3' with expected amplicon size of 1200–1400 bp [34]. The reaction mixture (20 µl) was prepared using 10 µl Green Taq 2 × (Cat # K1081), 1 µl template DNA and 2 µl of each forward and reverse primers and 5 µl deionized (DNase and RNase free) water. The parameters were similar for both primer sets and amplifications were performed in a SENSOQUEST labcycler (GmbH, Germany) as explained by Graça et al. [33]. The thermal cycling conditions was of 30 cycles, with an initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min (for PKS-I primers), and extension at 72 °C for 5 min. A final extension at 72 °C for 5 min was performed. The amplicons of the required sizes were analyzed using a 1 kb DNA ladder (Thermo Scientific GeneRuler, USA) as a molecular marker via 1% agarose gel electrophoresis prepared in 1× TAE buffer.

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) of the R6 extract was carried out as mentioned previously by Kirchner et al. [35]. Briefly, a silica gel plate was cut (20 × 20 cm) (Merck, Germany) and the extract was applied using a capillary tube. The plate was subsequently developed using an optimized solvent system consisting of chloroform (CHCl₃) and methanol (MeOH). Visualization was performed under UV light at 254 nm and 365 nm, and the absorbing bands were marked accordingly. To analyze further, the plate was stained with an anisaldehyde/H₂SO₄ reagent and examined for different colored bands.

Preparative TLC and high performance liquid chromatography-UV/Vis (HPLC-UV/Vis)

Preparative TLC was carried out to partially purify the metabolites in the R6 extract as described by Eber et al. [36]. Silica gel TLC glass plates (Merck, Germany) were taken and a baseline was drawn 2 cm from the bottom edge and 2 ml of the crude extract was applied uniformly along this line. The chromatographic separation was

carried out using a chloroform/methanol (9:1) solvent system. After development, the plates were examined under UV light at 254 nm to visualize the bands. Those showing strong absorbance were carefully scraped from the plate using a sterile scalpel and ground into a fine powder. The powdered silica was then added to absolute methanol, filtered using a 0.45 µm syringe filter (Merck, Germany), and stored in a glass vial at 4 °C.

The partially purified R6 extract were examined for diversity of metabolites using HPLC-UV/Vis with a HPLC system integrated with the Clarity Chromatography Data System (Sykam, GmbH, Germany). The chromatographic system comprised of two pressure pumps and an injection port with a 20 µl loop, along with a UV/Vis detector. Analysis was carried out on an RP C18 column (Hypersil, Thermo Fisher Scientific, USA) using a methanol-to-water mobile phase (95:5) at a flow rate of 1 ml/min. Prior to injection, the extracts were prepared

by dissolving in 200 µl of methanol, and 20 µl of the prepared sample was introduced into the system. The total run time for each sample was 15 min, and UV absorbance was recorded at 254 nm.

Statistical analysis

All experiments were done in triplicate, and the results were shown as mean values. For the statistical analysis, one-way ANOVA was performed through SPSS version 28.0 (SPSS, USA).

Results

Isolation and identification of *S. violaceoruber* R6

O. tenuiflorum was collected from the botanical garden within the Institute of Pharmaceutical Sciences (IPS), UVAS. The stem, roots, and leaves underwent surface sterilization before being plated on actinomycete isolation agar. Following incubation, an actinomycetes colony (R6) was noted due to its bright red color and distinct morphology and it was subsequently purified on GYM agar. Based on Fig. 1a, the strain exhibited morphological features characteristic of *Streptomyces*, forming rough, embedded colonies with a bright red aerial mycelium and a deep red substrate mycelium, along with moderate sporulation. It also produced deep red pigments, which diffused into the surrounding medium. In Fig. 1b, Gram staining confirmed the presence of Gram-positive cells arranged in filamentous chains.

R6 demonstrated the ability to utilize various carbon sources, including glucose, mannose, sucrose, sorbitol, arabinose, fructose, and xylose. Additionally, biochemical tests confirmed its ability to produce melanin. Table 1 presents a detailed summary of the morphological and physiological characteristics of R6. Comparative analysis with actinomycetes as explained in Bergey's Manual of Determinative Bacteriology [37] indicated that it was from the genus *Streptomyces*.

To confirm its identity, 16 S rRNA gene sequencing was performed, yielding a 1,414 bp sequence. BLAST analysis revealed 99% similarity with various *Streptomyces* strains therefore a phylogenetic tree was generated to determine the evolutionary relationship of strain R6. As seen in Fig. 2, a close relationship was indicated between strain R6 and *S. violaceoruber* (GenBank Accession No. MN704645).

Antibacterial, anti-ESBL and antifungal activity of *S. violaceoruber* R6

The processes involved in sample preparation, extraction, and chromatographic analysis are critical in ensuring the accuracy and reliability of experimental results. Additionally, there is a growing preference for faster, more efficient, and environmentally sustainable techniques

S. violaceoruber

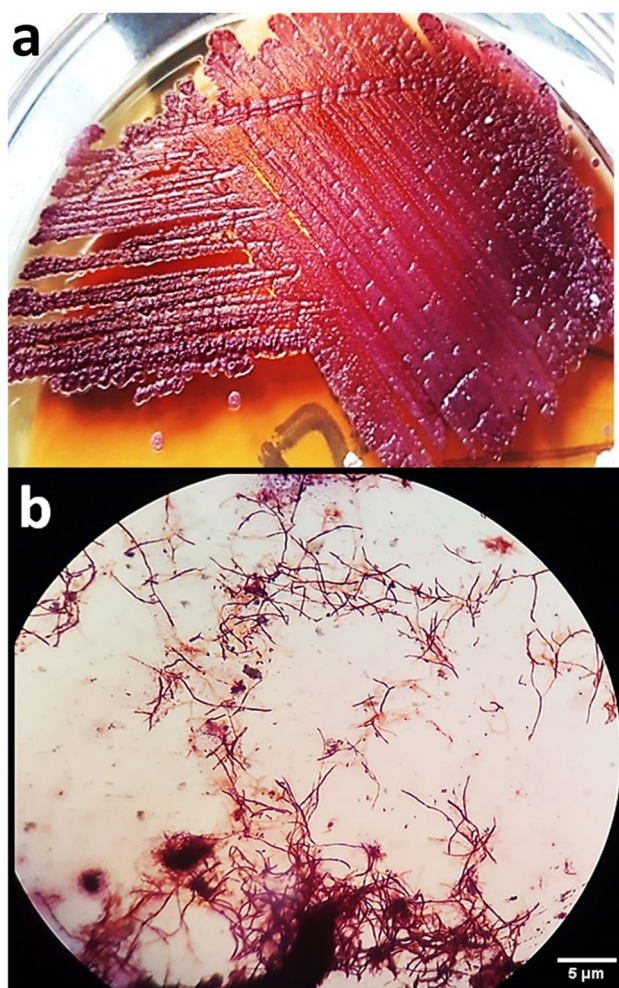


Fig. 1 a Substrate and aerial mycelium of *Streptomyces violaceoruber* R6 b Gram staining of *Streptomyces violaceoruber* R6

Table 1 Morphological and physiological characteristics of *Streptomyces violaceoruber* R6

Morphological characterization	Size (mm)	2 mm
	Shape	Circular
	Margin	Filamentous
	Texture	Rough
	Consistency	Leathery
	Sporulation	Moderate
	Growth Pattern	Well grown
	Substrate mycelium	Deep red
	Aerial mycelium	Bright red
Physiological characterization with sugar utilization as carbon source	Soluble Pigments	Red pigments
	Glu	+
	Man	+
	Suc	+
	Sor	+
	Ara	+
	Fru	+
	Xyl	+
Physiological characterization with melanin test, hydrolysis of esculin or arbutin and hydrolysis of urea	Mel	-
	HEA	+
	HUA	+

Glu Glucose, Man Mannose, Suc Sucrose, Sor Sorbitol, Ara Arabinose, Fru Fructose, Xyl Xylose, Mel Melanin, HEA Hydrolysis of esculin and arbutin, HUA Hydrolysis of urea and allantoin, (-) negative results; (+) positive results

[38]. The biological potential of *S. violaceoruber* R6 was assessed through.

various bioactivity assays. Initially, the antimicrobial activity was determined with agar plug method, as described by Fatima et al. [17], against Gram-negative as well as Gram-positive pathogens. It exhibited equal inhibitory effects against *Escherichia coli* and *Staphylococcus aureus*, producing 20 mm zones of inhibition in both cases. Further testing with the agar well diffusion assay, as per Balouiri et al. [23], demonstrated the highest activity against Gram-negative bacteria, including *Campylobacter jejuni* (13.6 mm), *Salmonella enteritidis* (12.3 mm), *Escherichia coli* (10.6 mm), and *Proteus mirabilis* (8.3 mm). Notably, *Acinetobacter baumannii* was not inhibited, whereas moderate activity was detected against *Staphylococcus aureus* (8.3 mm).

The anti-ESBL activity of *S. violaceoruber* R6 was assessed using the agar plug diffusion assay, where the strain inhibited all eight urinary isolates (UO1–UO8). The largest inhibition zone (25 mm) was observed against *Klebsiella pneumoniae*, while significant activity (20 mm) was recorded against ESBL-producing *E. coli* isolates. Table 2 presents the results of agar well diffusion method and further confirm these findings, with the highest inhibition observed against *Enterobacter aerogenes* (16 mm) and *K. pneumoniae* (15 mm). Moderate activity (13 mm) was detected against ESBL-producing *E. coli*.

The antifungal potential of *S. violaceoruber* R6 was evaluated against toxigenic isolates of *Aspergillus fumigatus*. The strain exhibited moderate antifungal activity, producing zones of inhibition ranging from 9 to 10 mm against three pathogenic strains (EF2, EF3, and EF5).

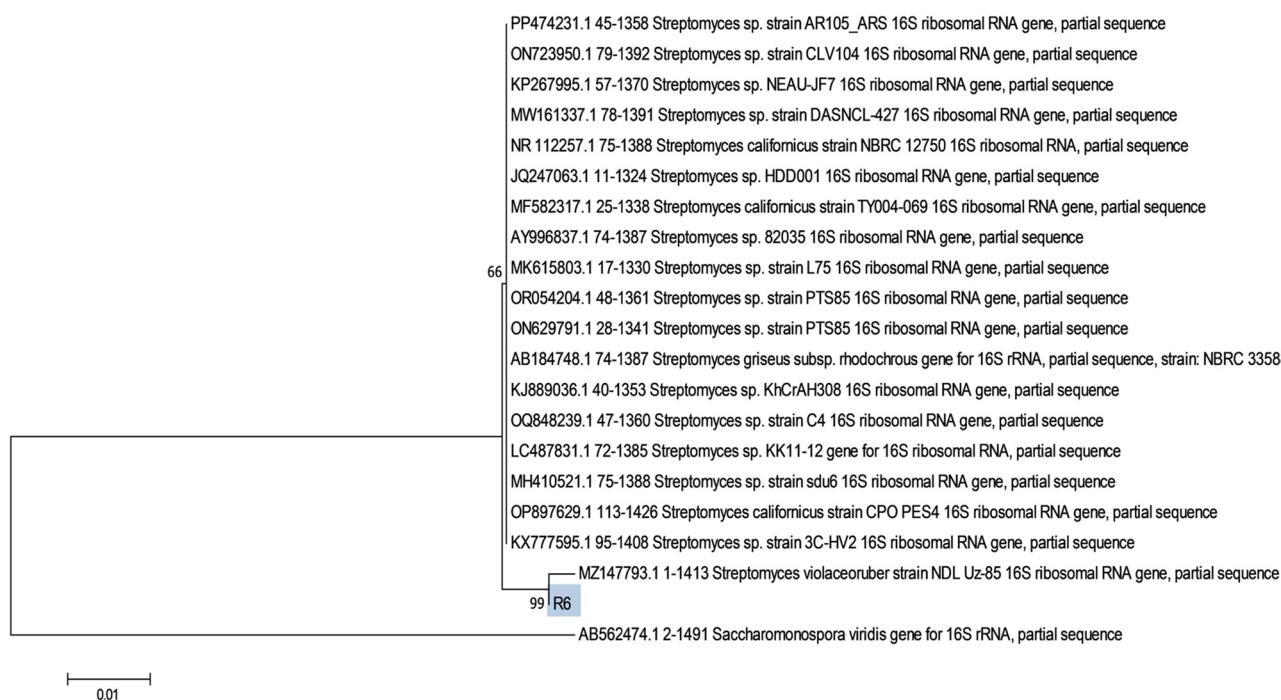
**Fig. 2** Phylogenetic tree of *Streptomyces violaceoruber* R6

Table 2 Antibacterial, anti-ESBL activity of *Streptomyces violaceoruber*R6

Zone of inhibition (mm)		ESBLs producing urinary isolates														
Strain	Clinical isolates															
	<i>E. coli</i>	<i>C. jejuni</i>	<i>P. mirabilis</i>	<i>S. enteritidis</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>UO-1</i>	<i>UO-2</i>	<i>UO-3</i>	<i>UO-4</i>	<i>UO-5</i>	<i>UO-6</i>	<i>UO-7</i>	<i>UO-8</i>		
R6	10.6	13.6	8.3	12.3	–	8.3	12	10	6	10	25	20	15	20		

E. coli Escherichia coli, *C. jejuni* Campylobacter jejuni, *P. mirabilis* Proteus mirabilis, *S. aureus* Staphylococcus aureus, *S. enteritidis* Salmonella enteritidis, *A. baumannii* Acinetobacter baumannii, *UO-1*= Klebsiella pneumoniae, *UO-2*= Enterobacter aerogenes, *UO-3*= Escherichia coli, *UO-4*= Escherichia coli, *UO-5*= Klebsiella pneumoniae, *UO-6*= Klebsiella pneumoniae, *UO-7*= Klebsiella pneumoniae, *UO-8*= Klebsiella pneumoniae, zones of inhibition were measured in mm(s)

Table 3 Antifungal activity of *Streptomyces violaceoruber*R6

Zone of inhibition (mm)		Aspergillus fumigatus isolates				
Strain		EF2	EF3	EF4	EF5	
EF1						
R6	-	10	9	-	10	

EF1-5 Toxigenic *Aspergillus fumigatus* isolates, (-) no inhibition; zones of inhibition were measured in mm(s)

However, no inhibition was observed against the remaining two *A. fumigatus* strains as seen in Table 3.

Cytotoxic activity of *S. violaceoruber* R6 against BHK-21 cell line

Previous research indicates that cytotoxicity assays can serve as predictive tools for anticancer activity, as they may correlate with cytotoxic effects on solid tumors. However, while these assays are valuable for screening potential anticancer compounds, they have limitations in differentiating between strong, moderate, and weak cytotoxicity. Despite this, they remain a widely adopted approach for the preliminary evaluation of cytotoxins [39]. The MTT assay is one of the most commonly employed methods for assessing cell viability and cytotoxicity, particularly in drug screening applications. This technique utilizes a colorimetric approach to evaluate cell viability [40].

In this study, *S. violaceoruber* R6 exhibited a notable cytotoxicity profile in the MTT assay with BHK-21 cell lines. The strain produced bioactive compounds that resulted in 25% cell survival at a 100 mg/ml concentration, which represented the highest level of cytotoxicity observed. A dose dependent reduction in cytotoxicity was detected, with 26% cell survival at 50 mg/ml, 28% cell survival at 25 mg/ml and 29% cell survival at 12.5 mg/ml. The results can be seen in Table 4 where the highest cell survival rate (90%) was recorded at 0.1 mg/mL, a level comparable to the positive control (BHK-21 cells, 100% cell survival) and the negative control (1% DMSO, 100% cell survival).

PKS type I gene amplification in *S. violaceoruber* R6

The screening of natural products has traditionally focused on the discovery of bioactive metabolites; however, recent advancements now enable the direct identification of genes involved in secondary metabolite biosynthesis [41]. These developments have facilitated the genetic manipulation of polyketide biosynthetic pathways, allowing for the production of novel bioactive compounds [42]. In this study, the polyketide synthase (PKS) biosynthetic gene cluster was specifically targeted due to its role in encoding one of the largest classes of bioactive secondary metabolites, which exhibit diverse biological activities and pharmacological properties. A well-known example of a PKS-encoded antibiotic is erythromycin, a

Table 4 Cytotoxicity of *Streptomyces violaceoruber* R6

Bioactivity	Sample	Cell survival percentage (%) under concentration studied (mg/ml ⁻¹)										P-value
		100	50	25	12.5	6.2	3.1	1.5	0.7	0.3	0.1	
MTT assay	BHK-21 cells with R6 extract	25	26	28	29	36	40	58	62	70	90	0.000
	BHK-21 cells without R6 extract	100	100	100	100	100	100	100	100	100	100	
	(Positive control)											
	DMSO 1% in double distilled water	100	100	100	100	100	100	100	100	100	100	
	(Negative control)											

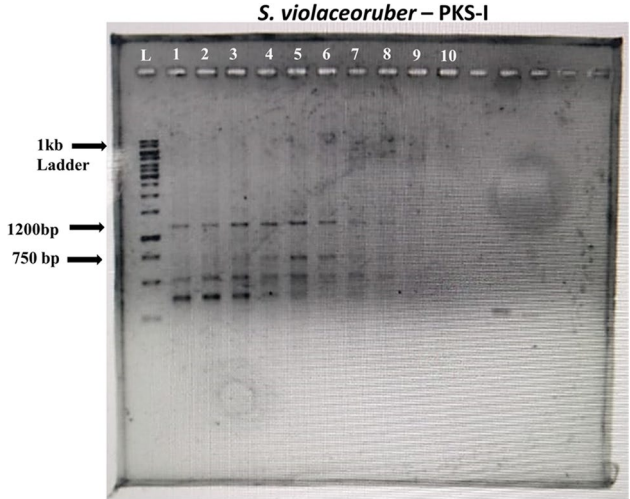


Fig. 3 Confirmation of PKS type I gene through PCR. An amplicon size of 750 and 1200 bp indicated a positive result. Left to right lane 1: 1 kb DNA Ladder, Lane 1–8: 1200 bp amplicons positive for PKS-I, Lane 4–6: 750 bp amplicons positive for PKS-I

widely used macrolide antibiotic [43]. Additionally, to the best of our understanding, the polyketide biosynthetic potential of endophytic *S. violaceoruber* isolated from *O. tenuiflorum* has not been previously characterized. This research aimed to explore the type I polyketide synthase (PKS-I) genes in strain R6. To achieve this, two sets of primers with high amino acid sequence identity specific to type I polyketide synthases (PKS-I) in actinomycetes were selected. In Fig. 3 it can be seen that the PCR amplification using primer set 1 yielded a 750 bp amplicon, confirming the presence of the PKS-I gene in *S. violaceoruber* R6. Similarly, amplification with primer set 2 produced a 1200 bp amplicon, further indicating that *S. violaceoruber* R6 harbors the PKS-I gene cluster, which is likely involved in the biosynthesis of novel secondary metabolites. An uncropped image of the PCR amplification is seen in supplementary Fig. 1.

Thin layer chromatography (TLC) and high performance liquid chromatography-UV/Vis (HPLC-UV/Vis) analysis

Thin-layer chromatography (TLC) is a widely used technique for separating and purifying chemical and biological compounds. Its simplicity, cost-effectiveness, ease of operation, and low solvent requirement make it suitable for various analytical applications. Additionally, TLC is considered an effective high-throughput separation method since its procedure is conducted in ambient conditions [44].

TLC analysis of the R6 extract exposed the presence of polar, medium-polar, and non-polar metabolites. As observed in Fig. 4a, b, on a normal-phase silica plate the extract displayed two polar and two medium-polar bands in the middle while a single non-polar band was

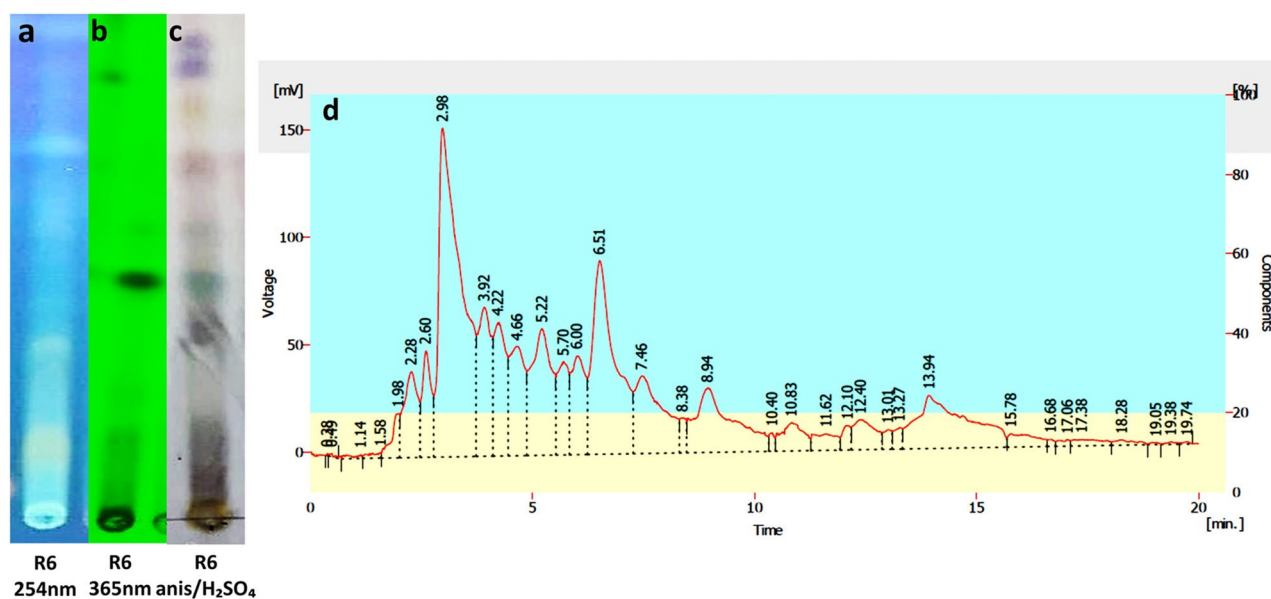


Fig. 4 **a** Observation of polar, medium-polar, and non-polar bands under UV 254 **b** Observation of polar, medium-polar, and non-polar bands under UV 365 **c** Observation of red, purple, yellow, and green bands after anisaldehyde/H₂SO₄ staining **d** HPLC-UV/Vis chromatogram of the R6 extract at UV 254 displaying peaks at different retention times (tr) of the partially purified compounds

detected near the top. The bands showed stronger absorbance at 254 nm compared to 365 nm under UV light. In Fig. 4c, after the anisaldehyde/H₂SO₄ staining the TLC plate displayed red (indole derivatives), purple (amines), yellow, and green bands, indicating the presence of phenols, steroids, and terpenes. Since the metabolites of *S. violaceoruber* R6 exhibited absorbance at 254 nm, further analysis was conducted after partially purifying the bands through preparative TLC and HPLC-UV/Vis was used to identify peaks of interest at specific retention times (tr). From Fig. 4d, the chromatographic analysis at 254 nm revealed a prominent peak at 2.98 min, with a peak area of 20.6%, suggesting a mixture of metabolites.

Discussion

Exploration of unique or underexplored ecological niches for the isolation of endophytes has proven to be a promising route for discovering new bioactive metabolites [45]. One such unexplored environment is the medicinal plants across the Indo-Pak region. Recent studies have revealed that the endophytic actinobacteria residing within them often carry broad-spectrum antimicrobial potential and diverse biosynthetic gene clusters [46]. In Pakistan, recent work has isolated endophytic actinobacteria from local medicinal plants such as *Cassia fistula*, *Phyllanthus emblica* and *Moringa oleifera*, showing both novel taxonomy and antimicrobial activities, especially from root tissues [47]. Among these medicinal plants, *O. tenuiflorum* (Tulsi) stands out because of its diverse medicinal use and the endophytic isolates, harbored by it may mirror the plant's traditional therapeutic properties.

This may offer potential to produce novel bioactive compounds not yet reported from this host, making targeted studies on Tulsi endophytes highly pertinent for new drug discovery [47]. In the present study, an endophytic actinobacterial strain, R6 isolated from *O. tenuiflorum* was noted for its bright red color and distinct morphology along with its broad spectrum activity. Polyphasic taxonomic characterization, including morphological, microscopic, biochemical, and physiological analysis, together with 16 S rRNA gene sequencing, identified the isolate as *S. violaceoruber*. Previous studies such as by Duangmal et al. [48] described the distinctive red color and pigment production as key characteristics of *S. violaceoruber*, which aligns with the colony morphology, pigmentation, and diffusible red pigment observed in our study. Abou-Dobara et al. [49] later identified this red pigment as protoactinorhodin, a bioactive naphthoquinone compound with antibacterial activity against *E. coli* and *K. pneumoniae*. Johnson [50] reported that *S. violaceoruber* Tü22 produces granaticin, a benzoisochromane quinolone antibiotic. More recently, Girão et al. [51] described the production of prodigiosins, decyl-prodigiosin (red-pigmented antibiotics), and actinorhodin or its analogs, with further studies from Xu et al. [52]. Notably, most studies have reported the isolation of *S. violaceoruber* from soil environments. However, Ma et al. [53] documented its isolation from an animal intestinal environment, and Girão et al. [51] reported its presence in green seaweed. To date, no study has documented the isolation of this species from medicinal plants, making

the discovery of *S. violaceoruber* R6 from *O. tenuiflorum* a novel finding.

Interestingly, Naragani et al. [54] reported similar findings to our study, where a soil isolate of *S. violaceoruber* exhibited notable antimicrobial activity, mainly against Gram-negative bacteria, *Escherichia coli* (18 mm), with comparatively lower inhibition against Gram-positive bacteria, *Staphylococcus aureus* (17 mm). However, a study by Hernández-Saldaña et al. [55] contradicted our findings, as *S. violaceoruber* JOCE01 did not inhibit *Salmonella* spp. or *Vibrio parahaemolyticus*. This study attributed the activity of *S. violaceoruber* JOCE01 to bacteriocin production, with optimized culture conditions playing a key role in its bioactivity. Sherman et al. [56] attributed the potential mechanism of bioactivity of *S. violaceoruber* Tü22 to the production of granaticin, a benzoisochromane quinone produced via PKS pathways. It is important to note that these potential bioactivity mechanisms studied by Naragani et al. [54] Hernández-Saldaña et al. [55] and Sherman et al. [56] focused on soil-derived *S. violaceoruber* strains, whereas our study examined an endophytic strain. In addition to antibiotics, a study by Abbasi et al. [57] found that hydrolytic enzymes such as chitinases, proteases along with pigment (melanin) production in *Streptomyces* isolates contribute to its possible mechanism of activity against microbes beyond bacteriocins. The observed differences in antimicrobial activity may be influenced by the plant-associated environment, as endophytes often produce secondary metabolites that mimic host-derived compounds to evade plant immune responses. In a parallel study, we compared the bioactivity of the crude extract from *O. tenuiflorum* roots with that of strain R6 and observed similar inhibition patterns, with the strongest activity exhibited against *E. coli* (Data not shown).

To the best of our knowledge, no prior studies have evaluated the anti-ESBL activity of *S. violaceoruber* against clinical ESBL-producing strains; thus, no direct comparisons could be made with the results of our study. However, we observed similarities in the bioactivity of R6 and *O. tenuiflorum* root extracts, with the strongest inhibition against ESBL-producing *K. pneumoniae* and *E. coli* (Data not shown). This supports the hypothesis that R6 may be mimicking host metabolites, as suggested by Agarwal et al. [58] and Risdian et al. [59] that *Streptomyces* possess diverse PKS types (Type I, Type II, and Type III) which allow them to synthesize polyketides forming the backbone of antibiotic activity in such strains. Our results partially align with those reported by Naragani et al. [54] where a soil-derived isolate of *S. violaceoruber* demonstrated activity against *Candida albicans* but showed no inhibition against *Aspergillus niger* and *Fusarium oxysporum*. In contrast, our endophytic *S. violaceoruber* R6 strain displayed moderate antifungal activity

against *A. fumigatus*, distinguishing it from soil-derived isolates.

In our study, R6 exhibited a notable cytotoxicity profile that coincided with a similar study conducted on a seaweed-derived isolate of *S. violaceoruber* reported 20% cell survival for colorectal carcinoma (HCT116) and breast carcinoma (T-47D) cell lines [51]. Additionally, Mhuan-tong et al. [41] described an endophytic *Streptomyces* sp. OS603R, isolated from *O. tenuiflorum*, with potent antitumor activity in cytotoxicity assays. More recently, Veilumuthu et al. [10] identified kendomycin, a macrocyclic polyketide antibiotic with anticancer properties, as a metabolite of *S. violaceoruber*, reinforcing the findings of this study. Furthermore, a forest soil-derived isolate of *S. violaceoruber* was reported to exhibit significant cytotoxic effects against human tumor cell lines, attributed to the production of Streptothiazolidine B [44, 60].

Previous studies on *S. violaceoruber* support these findings. Sherman et al. [56] reported that *S. violaceoruber* produces granaticin, an antibiotic encoded by PKS genes. More recently, Veilumuthu et al. [10] described the presence of a type I PKS gene cluster in *S. violaceoruber*, which encodes for kendomycin, a polyketide antibiotic. The study characterized kendomycin as a potent antibacterial and anticancer agent, further reinforcing the biological significance of PKS-encoded metabolites and corroborating our findings in the cytotoxicity assay as observed previously in Table 4.

Previous studies have shown that indole derivatives, which appear red with anisaldehyde/H₂SO₄ staining, are aromatic heterocyclic compounds that act as precursors for pharmaceutical agents and N-heterocycles. Additionally, Ehrlich's reagent reacts with indole-containing tryptophan residues in amino acids, producing a purple coloration in peptide bands [61]. In terms of metabolite production, recent genomic studies showed that *Streptomyces* strains such as *S. violaceoruber* harbor a wide array of biosynthetic gene clusters beyond those previously linked to granaticin or kendomycin. Mhuan-tong et al. reported an endophytic *Streptomyces* sp. OS603R isolated from *O. tenuiflorum* to contain numerous biosynthetic clusters, many of which are still uncharacterized, indicating the potential for discovering novel metabolites. Most recently, Wang et al. [44] also identified polyketides, chromanones, indoles, and amides in a soil-derived *S. violaceoruber* isolate, which exhibited antibacterial, anti-inflammatory, and cytotoxic properties. In this context, our findings align with those of Wang et al. [44], as we also detected the presence of a type I polyketide synthase (PKS-I) gene cluster, along with indications of indoles and amines, supporting the antibacterial and cytotoxic potential of the strain. Although, our findings are novel, as this particular *S. violaceoruber* strain was isolated from *O. tenuiflorum*, a well-known

medicinal plant, they also illustrate a major limitation: most studies report metabolite classes or preliminary activity screens but do not carry out full chemical characterization or detailed bioassays. The cytotoxicity testing also poses certain constraints as studies have reported percentage cell survival at a single concentration rather than complete dose–response curves making conclusions about potency limited. Also majority of the assays focus on a small panel of tumor cell lines without parallel testing on non-cancerous ones, which limits the understanding of selectivity and safety. The study by Veilumuthu et al. [10] highlights this need as kendomycin shows promising antitumor activity, but in vitro data alone cannot be extrapolated to in vivo efficacy without pharmacokinetic and toxicity evaluations.

The isolation of *S. violaceoruber* R6 from *O. tenuiflorum* highlights the value of exploring local medicinal plants for bioactive endophytes. Hence, our future research would be aimed towards genome–metabolome integration to connect the identified biosynthetic gene clusters with their metabolites and then their purification and structural characterization. We can also expand the cytotoxicity assays across diverse cancer and non-cancerous cell lines. These studies will clarify the activity of compounds such as polyketides, indoles, and amines derived from R6. The antifungal and antibacterial metabolites of R6 may also hold potential in agriculture as bio-control agents as an alternative to chemical pesticides.

Conclusions

In order to explore novel sources of bioactive metabolites, our study focused on isolating endophytic actinomycetes from *O. tenuiflorum*, a widely used medicinal plant. While endophytes have been extensively studied, their presence in medicinal plants, particularly in Pakistan, remains underexplored. Our findings showed that a strain identified as *S. violaceoruber* R6 is a significant producer of secondary metabolites, including polyketides, indoles, and amines, as evidenced by TLC and HPLC-UV/Vis analysis. The identification of PKS-I gene cluster further supports its potential for producing novel bioactive compounds. The strain demonstrated potent antibacterial effects against Gram-negative pathogens, including ESBL-producing strains of *E. coli* and *K. pneumoniae*, along with moderate antifungal activity against *A. fumigatus*. Additionally, its cytotoxic activity suggests the presence of antitumor compounds.

This study highlights *O. tenuiflorum* as an important ecological niche for bioactive endophytic actinomycetes, emphasizing the potential of medicinal plants as reservoirs of novel microbial strains. Given the pharmaceutical significance of polyketides and indole derivatives, further research should focus on the purification and structural characterization of the metabolites. Further

research could explore its cytotoxic potential against cancer and non-cancerous cell lines and specific breast and liver cancer cell lines, particularly those prevalent in Pakistan. The application of the antifungal and antibacterial metabolites of R6 can be expanded by checking them against agriculture pathogens as an alternative to chemical agents. The outcomes of this study encourage further exploration of medicinal plant endophytes for their pharmaceutical applications.

Limitation of the study

This study was constrained by the unavailability of funding for LC-MS analysis of the partially purified bands obtained from *S. violaceoruber* R6. Such analysis could provide valuable insights into the molecular weights of the bioactive compounds, enabling comparison with known actinomycete-derived metabolites in specialized compound databases to assess their potential novelty.

Abbreviations

Abs	Absorbance
ANOVA	Analysis of Variance
Ara	Arabinose
BGC	Biosynthetic Gene Cluster
BHK	Baby Hamster Kidney
BLAST	Basic Local Alignment Search Tool
bp	Base pair
cm	Centimetre
CMH	Combined Military Hospital
CPEs	Cytopathic Effects
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
EF	Toxigenic <i>Aspergillus fumigatus</i>
ESBL	Extended Spectrum Beta Lactamases
Fru	Fructose
Glu	Glucose
gm	Grams
GMEM	Glasgow Modified Essential Medium
GYM	Glucose Yeast Extract Malt Extract
HCT	Human Colorectal Carcinoma
HEA	Hydrolysis of Esculin and Arbutin
HEP	Hepatoblastoma
HM	High Mobility
HPLC-UV/Vis	High Performance Liquid Chromatography-Ultraviolet/Visible
hrs	Hours
HUA	Hydrolysis of Urea and Allantoin
IOM	Institute of Microbiology
IPS	Institute of Pharmaceutical Sciences
ISP	International Streptomyces Project
kb	Kilobases
LC-MS	Liquid Chromatography- Mass Spectrometry
Man	Mannose
MCF	Michigan Cancer Foundation
MEGA7	Molecular Evolutionary Genetics Analysis Version 7
Mel	Melanin
mg	Milligram
MHA	Mueller-Hinton agar
min	Minutes
ml	milliliter
mM	Millimolar
mm	Millimeters
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NCBI	National Center for Biotechnology Information
NJ	Neighbor-Joining

nm	Nanometers
OD	Optical Density
PCR	Polymerase Chain Reaction
PKS	Polyketide synthases
QOL	Quality Operations Lab
RNase	Ribonuclease
RP	Reverse Phase
rpm	Revolutions Per Minute
rRNA	Ribosomal Ribonucleic Acid
SDA	Sabouraud dextrose agar
Sor	Sorbitol
SPSS	Statistical Package for the Social Sciences
Suc	Sucrose
TAE	Tris Acetate EDTA
TLC	Thin Layer Chromatography
tR	Retention Time
U	Units
UDL	University Diagnostic Lab
UO	Urinary Isolates
UV	Ultraviolet
UVAS	University of Veterinary and Animal Sciences
VOC	Volatile Organic Compounds
Xyl	Xylose
µm	Micrometers
µl	Microliter

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Authors' contributions

R.T, A.A.A, M.N, A.A.S conceptualized the idea. A.I, E.A, H.S performed the experiments. R.T wrote the first draft. H.M and W.S helped with the data curation. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The 16 S sequence that supports the findings of this study have been deposited in the National Center for Biotechnology Information (NCBI) with the GenBank accession number MN704645 (<https://www.ncbi.nlm.nih.gov/nuccore/MN704645>).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Karthika M, Rasmi AR. Pharmacological potential of fungal endophytes associated with the genus *Ocimum* L. Int J Secondary Metab. 2023;10(1):1–0.
- Jalil MT, Zakaria NA, Ibrahim D. Effect of culture medium incorporated with *Ocimum sanctum* extract in enhancing anti-MRSA activity of

- endophytic fungus, *Lasiodiplodia Pseudotheobromae*. J Pure Appl Microbiol. 2021;15(3):1398–408.
- Musheer N, Jamil A, Choudhary A. Solo and combined applications of fungicides and bio-agents to reduce severity of *Fusarium oxysporum* and induce antioxidant metabolites in *Ocimum tenuiflorum* L. J Plant Pathol. 2023;105(1):237–51.
- Khan F, Tenguria RK. Endophytic biodiversity in *Ocimum sanctum*. J Adv Sci Res. 2021;12:340–2.
- Sahu PK, Singh S, Gupta AR, Gupta A, Singh UB, Manzar N, Bhowmik A, Singh HV, Saxena AK. Endophytic bacilli from medicinal-aromatic perennial holy Basil (*Ocimum tenuiflorum* L.) modulate plant growth promotion and induced systemic resistance against *Rhizoctonia Solani* in rice (*Oryza sativa* L). Biol Control. 2020;150:104353.
- Liu T, Ren Z, Chunyu WX, Li GD, Chen X, Zhang ZT-L, Sun HB, Wang M, Xie TP, Wang M, Chen J-Y, Zhou H, Ding Z-T, Yin M. Exploration of diverse secondary metabolites from *Streptomyces* sp. YINM00001, using genome mining and one strain many compounds approach. Front Microbiol. 2022;13:831174.
- Sheham A, Mohamed SS, El-Mehalawy AA, Abdou DA. Isolation. Identification and biological applications of endophytic fungi from *Ocimum Basilicum* and *Ammi Visanga*. Egypt J Microbiol. 2024;59:1.
- Pasrija P, Girdhar M, Kumar M, Arora S, Katyal A. Endophytes: an unexplored treasure to combat multidrug resistance. Phytomedicine Plus. 2022;2(2):100249.
- Ayswaria R, Alfred J, Gopalan G, Krishna R. Endophytic *Streptomyces* isolated from *Ocimum tenuiflorum* and *Catharanthus roseus* and its efficacy test to develop antibacterial fabric for wound caring. J Text Inst. 2023;114(4):530–41.
- Veilumuthu P, Nagarajan T, Magar S, Sundaresan S, Moses LJ, Theodore T, Christopher JG. Genomic insights into an endophytic *Streptomyces* sp. VITGV156 for antimicrobial compounds. Front Microbiol. 2024;15:1407289.
- Taneja T, Garg H, Sharma I, Singh R. Isolation and identification of endophytic fungi and evaluation of their interactions with plants from *Syzygium cumini* (Jamun) and *Ocimum tenuiflorum* (Tulsi). J Indian Bot Soc. 2023;234:103.
- Bode HB, Zecek A. Structure and biosynthesis of kendomycin, a carbocyclic ansa-compound from *Streptomyces*. J Chem Soc Perkin Trans. 2000;(3):323–8.
- Liu X. GMO and protein engineering. In: Whitehurst RJ, Oort MV, editors. Enzymes in food technology. Singapore: Wiley-Blackwell; 2010. <https://doi.org/10.1002/9781444309935.ch2>.
- Mander LN, Liu HW, Singh R, Kumar S, editors. Medicinal plants and antimicrobial therapies. Oxford: Elsevier Science; 2010.
- Melki SB, Gautier A, Mliki A. Genetic approach to target putative PKS genes in *Aspergillus* section *Nigri* species producing Ochratoxin A. Biol Life Sci Forum. 2023;24:15.
- Amin DH, Nageeb WM, Elkesh A, Makharita RR. Mining metagenomes reveals diverse antibiotic biosynthetic genes in uncultured microbial communities. Braz J Microbiol. 2023;54(2):983–95.
- Qin S, Xing K, Jiang JH, Xu LH, Li WJ. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol. 2011;89:457–73.
- Shirling ET, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Evol Microbiol. 1966;16(3):313–40.
- Fatima A, Aftab U, Shaaban KA, Thorson JS, Sajid I. Spore forming actinobacterial diversity of Cholistan desert pakistan: polyphasic taxonomy, antimicrobial potential and chemical profiling. BMC Microbiol. 2019;19:1–7.
- Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. Practical streptomyces genetics: John Innes Foundation. Colney: Norwich Research Park; 2000. pp. 44–61.
- Tanvir R, Sajid I, Hasnain S. Screening for type I polyketide synthases genes of endophytic streptomycetes isolated from *Parthenium hysterophorus* L. Mol Gen Microbiol Virol. 2013;28:32–9.
- Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
- Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–4.
- Tanvir R, Sajid I, Rehman Y, Hasnain S. Fatty acid based antimicrobials from *Streptomyces* sp. SORS-24, an endophyte isolated from *Sonchus oleraceus*. Lett Appl Microbiol. 2023;76(8):ovad080.
- Balouiri M, Sadiki M, Ibsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
- Morgan DML. Tetrazolium (MTT) assay for cellular viability and activity. In: Morgan DML, editor. Polyamine protocols. Methods in molecular biology, vol. 79. Totowa: Humana Press; 1998. pp. 179–84.

27. Supino R. MTT assays. In vitro toxicity testing protocols. Totowa: Humana Press; 1995. pp. 137–49.
28. Ahmad A, Davies J, Randall S, Skinner GR. Antiviral properties of extract of *Opuntia streptacantha*. Antiviral Res. 1996;30(2–3):75–85.
29. Burgener A, Butler M. Medium development. In: Ozturk S, Hu WS, editors. Cell culture technology for pharmaceutical and cell-based therapies. Boca Raton: CRC Press; 2006. pp. 41–79.
30. Zweygardt E, Josemans AI, Horn E. Serum-free media for the *in vitro* cultivation of *Cowdria ruminantium*. Ann NY Acad Sci. 1998;849(1):307–12.
31. Cappuccino JG, Sherman N. Microbiology: a laboratory manual. San Francisco: Pearson/Benjamin Cummings; 2005.
32. Tukay A, Bayindir F, Akyil MS. Cytotoxicity of soft and hard denture lining materials on MA-104, Vero and primary tooth cell culture. Mater Res Innov. 2010;14(3):220–3.
33. Graça AP, Calisto R, Lage OM. Planctomycetes as novel source of bioactive molecules. Front Microbiol. 2016;2:7:1241.
34. Passari AK, Mishra VK, Saikia R, Gupta VK, Singh BP. Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in vitro* antimicrobial biosynthetic potential. Front Microbiol. 2015;6:273.
35. Kirchner JG, Perry ES, Weissberger A. Thin-layer chromatography. USA: New York; 1967.
36. Ebere EC, Obinna IB, Wirnkor VA. Applications of column, paper, thin layer and ion exchange chromatography in purifying samples: mini review. SF J Pharm Anal Chem. 2019;2:1018.
37. Holt JG. Shorter bergey's manual of determinative bacteriology. USA: Lippincott Williams & Wilkins; 1994.
38. Krakowska-Sieprawska A, Kielbasa A, Rafińska K, Ligor M, Buszewski B. Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. Molecules. 2022;27(3):730.
39. Niksic H, Becic F, Koric E, Gusic I, Omeragic E, Muratovic S, Miladinovic B, Duric K. Cytotoxicity screening of *Thymus vulgaris* L. essential oil in Brine shrimp nauplii and cancer cell lines. Sci Rep. 2021;11(1):13178.
40. Bahuguna A, Khan I, Bajpai VK, Kang SC. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J Pharmacol. 2017;12(2):Online–Apr.
41. Mhuanthong W, Kwandee W, Boondireke S, Kongrit D. Draft genome sequence of *Streptomyces* sp. OS603R isolated from holy Basil roots with promising antimicrobial and antitumor potential. Microbiol Res Announc. 2022;12(10):e00332–23.
42. Nie L, Cao M, Ji Q, Gao Y, Wang S, Lyu Y, Feng Z. Regulated expression of an environmental DNA-derived type II polyketide gene cluster in *Streptomyces* hosts identified a new tetracenomycin derivative TCM Y. Curr Microbiol. 2022;79(11):336.
43. Geers AU, Buijs Y, Strube ML, Gram L, Bentzon-Tilia M. The natural product biosynthesis potential of the microbiomes of earth–bioprospecting for novel anti-microbial agents in the meta-omics era. Comput Struct Biotechnol J. 2022;20:343–52.
44. Wang T, Kang DW, Hu DB, Wang JA, Chen X, Cao YR, Wang Y, He JB. Streptothiazolidine B, a new cytotoxic alkaloid produced by *Streptomyces violaceoruber*. J Asian Nat Prod Res. 2023;25(6):595–602.
45. Younis T, Rahman L, Rahman S, Shinwari AK, Iqar I, Shinwari ZK. Endophytes. Potential source of bioactive compounds of pharmaceutical importance. Proc Pak Acad Sci B Life Environ Sci. 2022;59(4):1–13.
46. Hatami H, Bahrami Y, Kakaei E. Endophytic actinobacteria from *Mentha longifolia* and *Lonicera nummulariifolia*: a novel source against antibiotic resistance. BMC Microbiol. 2025;25(1):365.
47. Hassan A, Ponomareva LV, Thorson JS, Shaaban KA, Sajid I. Bioactivity and metabolomics profiling of endophytic actinobacteria isolated from roots of the medicinal plants dominant in South Asian region. Adv Life Sci. 2023;10(2):200–9.
48. Duangmal K, Ward AC, Goodfellow M. Selective isolation of members of the *Streptomyces violaceoruber* clade from soil. FEMS Microbiol Lett. 2005;245(2):321–7.
49. Abou-Dobara MI, El-Sayed A, Omar NF. *Streptomyces violaceoruber* ES: a producer of bioprospective metabolite for rapid and green synthesis of antibacterial silver nanoparticles. Egypt J Bot. 2017;57(7):31–43. th International Conf).
50. Johnson DA. Deoxysugars: occurrence, genetics, and mechanisms of biosynthesis. Compr Nat Prod Chem. 1999;3:311–65.
51. Girão M, Freitas S, Martins TP, Urbatzka R, Carvalho MF, Leão PN. Decylprodiginosin: a new member of the prodiginosin family isolated from a seaweed-associated *Streptomyces*. Front Pharmacol. 2024;15:1347485.
52. Xu BL, Wang YY, Dong CM. Study on marine actinomycetes and analysis of their secondary metabolites. Life Res. 2023;6(4):18.
53. Ma J, Lei H, Chen X, Bi X, Jiang Y, Han L, Huang X. New anti-inflammatory metabolites produced by *Streptomyces violaceoruber* isolated from *Equus Burchelli* feces. J Antibiot. 2017;70(10):991–4.
54. Naragani K, Munaganti RK, Sirigiri CK, Muvva V. Antimicrobial potential of *Streptomyces violaceoruber* VLK-4 isolated from South Coast of Andhra Pradesh, India. Int J Pharm Sci Rev Res. 2014;25:125–29.
55. Hernández-Saldaña OF, Barboza-Corona JE, Bideshi DK, Casados-Vázquez LE. New bacteriocin-like substances produced by *Streptomyces* species with activity against pathogens. Folia Microbiol. 2020;5:669–78.
56. Sherman DH, Malpartida F, Bibb MJ, Kieser HM, Bibb MJ, Hopwood DA. Structure and deduced function of the granaticin-producing polyketide synthase gene cluster of *Streptomyces violaceoruber* Tü22. EMBO J. 1989;8(9):2717–25.
57. Abbasi S, Spor A, Sadeghi A, et al. *Streptomyces* strains modulate dynamics of soil bacterial communities and their efficacy in disease suppression caused by *Phytophthora capsici*. Sci Rep. 2021;11:9317.
58. Agarwal S, Sharma G, Mathur V. Plant endophytes: a treasure house of antimicrobial compounds. In: Singh R, Kumar S, editors. Medicinal plants and antimicrobial therapies. Singapore: Springer Nature Singapore; 2024. pp. 107–306.
59. Risdian C, Mozef T, Wink J, et al. Biosynthesis of polyketides in *Streptomyces*. Microorganisms. 2019;7(5):124.
60. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front Pharmacol. 2023;14:1265178.
61. Lamb AC, Federico-Perez RA, Xue ZL. Product in Indole detection by ehrlich's reagent. Anal Biochem. 2015;484:21–3.

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