

Antimicrobial Activities of Endophytic *Streptomyces* sp Bdusmp R25 from the Root of the Medicinal Plant *Solanum trilobatum*

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ABSTRACT

Medicinal plants contain endophytic *Streptomyces* that may have bioactive compounds that are still underexplored. We isolated and screened the antimicrobial endophytic *Streptomyces* from the surface sterilized root of *Solanum trilobatum* from Southern India, Kolli hills. A total of 25 isolates were evaluated for potential antimicrobial activity, and one isolate was chosen for further study. *Streptomyces* sp BDUSMP R25 is a potential isolate based on 16S rRNA sequencing. The strain grew well at 28 °C and pH 7. The isolate was found to utilize xylose as its sole carbon source. Moreover, the strain produced small droplets of yellow color extracellular exudates pigments. Based on antimicrobial activity against bacterial pathogens, this isolate has the potential to be further investigated for natural compound isolation.

Keywords: *Solanum trilobatum*, endophytic *Streptomyces*, antibacterial activity, polyphasic taxonomy.

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Introduction

While most clinical drugs, such as antibiotics, originate from bacterial natural products, escalating antimicrobial resistance stresses the need for novel antibacterial compounds. Natural compounds have been discovered by extensively researching several readily cultured actinomycetes from the soil. However, rediscovery of known compounds requires the study of underexplored environments. A source of understudied actinomycetes is the endophytic actinomycetes of medicinal plants. Exploring plant tissues inhabiting endophytic *Streptomyces* can lead to the discovery of unique metabolites with potential applications in medicine (Sivalingam et al. 2024).

Endophytic bacteria that live inside healthy plant tissues have a symbiotic or mutualistic relationship with the host plant (Mo et al. 2024). In addition, plant endophytes are reported not to cause any disease (Santoyo et al. 2016). Studies of endophytic bacteria from medicinal plants have expanded in recent years due to their potential

antibacterial (Gos et al. 2017), antifungal (Wardecki et al. 2015), and growth-promoting properties (Verma et al. 2011). In part, this is due to a unique niche and a particular adaptation to the conditions of a plant environment, which can result in the evolution of novel species. *Solanum trilobatum* is one of the herbal plants in Tamil Nadu, India used in the ayurvedic treatment of various respiratory diseases (Govindan et al. 1999).

Researchers have documented that *S. trilobatum* has antibacterial (Latha and Kannabiran 2006), anti-inflammatory (Pandurangan et al. 2011), antioxidant (Sini and Devi 2004) and mosquitocidal properties (Premalatha et al. 2013). The diversity of endophytic actinomycetes and their biotechnological applications has been studied previously (Golinska et al. 2015). Previous studies have shown that isolation of endophytic *Streptomyces* spp. from the medicinal plant roots (Akshatha et al. 2014; Wardecki et al. 2015). Of note, roots are reported to harbor more endophytic actinomycetes than leaves and stems of the host plant (Qin et al. 2009). *Streptomyces* are gram

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positive filamentous bacteria and have a high G+C content in their genomes (Flårdh and Buttner 2009; McCormick and Flårdh 2012).

It is noteworthy that the genus *Streptomyces* contains over 900 validly published species to date (<https://www.bacterio.net/genus/streptomyces>). The genus *Streptomyces* is one of the most dominant phyla of bacteria associated with plants (Taechowisan et al. 2003; Bascom-Slack et al. 2009; Gohain et al. 2015). Further, *Streptomyces* are the most historically significant sources of natural bioactive drug leads, including antibiotics, cytotoxics, antifungals, and antihelminthics (Berdy 2005). The objective of this study was to identify and characterize the antimicrobial properties of an endophytic *Streptomyces* from the root of *S. trilobatum*.

Materials and methods

Collection of plant samples

Healthy medicinal plants of *S. trilobatum* were collected from Kolli Hills, Tamil Nadu, India (10°12' - 11°7' N, 76°-77°17' E). Plant parts (stem, leaf and root) were excised with pliers and placed in plastic bags and transported to the laboratory. Plant samples (Figure 1) were transported in an ice cooler box and stored in a cold room at 4 °C and processed for endophytic actinomycetes isolation within 24 h of collection.

Isolation of endophytic actinomycetes

To remove any soil particles, all root segments were washed in running tap water. Surface sterilization of root tissues was performed as described previously (Verma et al. 2009). Briefly, tap water washed roots were dried well and then rinsed in 0.1% Tween 20 for 30 sec. Following that, the roots were immersed in 70% (v/v) ethanol for 5 min and in 2% sodium hypochlorite (NaClO) for 10 min. After each treatment, roots were rinsed three times in sterile double distilled water. Surface sterilized root samples were dried and aseptically cut into small pieces. Afterwards, the root tissues were transferred to the humic acid-vitamin B Agar (HV) medium supplemented with antibiotics such as cycloheximide (25 µg/ml), nystatin (25 µg/ml) and nalidixic acid (20 µg/ml) to inhibit fungal and nonfilamentous bacterial growth, respectively. The HV agar plates with impregnated root tissues were incubated at 28 °C for three to four weeks. Surface sterilization procedures were validated using previously described methods (Goudjal et al. 2013). A surface sterilized solution was added to 5 ml of sterile water and mixed

thoroughly. Following that, 0.3 ml of the sample was inoculated onto humic acid-vitamin B agar (HV). For three to four weeks, the plate was incubated at 28 °C and microbial growth was observed. There were no colonies on the plate, indicating that Surface sterilization was complete. We observed actinomycetes colonies on root tissues grown on HV agar medium with features such as earthy odour, a folded or dry appearance, and the presence of mycelia and spores. After repeated streaking on ISP4 medium, pure cultures were obtained (HI MEDIA Labs, India).

Morphological, cultural and physio-biochemical properties and Chemotaxonomy

Cultural characteristics were performed on the International *Streptomyces* Project (ISP) ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7 agar media after 14 days of incubation at 28 °C according to the International *Streptomyces* Project (ISP) method (Shirling and Gottlieb 1966). Analysis of color patterns of aerial and substrate mycelia was performed as previously described (KL 1964). General morphology of aerial and substrate mycelium was observed and recorded under high power magnification using a light microscope (Jasco). All images were obtained with an Olympus bh-2 upright phase contrast microscope. The spore morphology of *Streptomyces* sp BDUSMP R25 was observed by scanning electron microscope (SEM model, JEOL-JSM 6390, Japan) of two weeks old cultures on ISP4 agar media at 28 °C. The isomer type of the 2, 6 diaminopimelic (DAP) acid in the peptidoglycan layer in the cell hydrolysates was determined by thin layer chromatography (TLC) as previously described (Lee et al. 2005). The solvent of methanol/water/10 M HCl/pyridine (80: 26: 2.5: 20, by vol.) was used (Becker et al. 1965). Ninhydrin staining (0.1% w/v ninhydrin in Acetone) was used to observe the chromatogram. Growth at different temperatures of 25°C, 28°C, 32°C, 37°C, 40°C, and 45°C was examined on ISP2 agar media for 1-2 weeks (Xu et al. 2009). Effect of pH determined at 4, 6, 7, 8, 9 and 10, cultured in ISP2 at 28°C for 7 days and agitated in shake flasks at 220 rpm (Xu et al. 2009). Tolerance to NaCl was determined by adding 0, 1, 3, 5, 7,9,11, and 13% (w/v) NaCl to the ISP 2 agar media slants and incubating at 28 °C for 1 week (Bian et al. 2012). Utilization of carbon and nitrogen sources was monitored in minimal medium containing M9 salts (6g Na₂HPO₄, 3g K₂HPO₄, 0.5g NaCl, 1g NH₄Cl per liter, filter – sterilized), supplemented with 1ml 1M

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MgSO₄, 1ml 0.1M CaCl₂, (Savic et al. 2007) and 1% carbon source (w/v) and 1% nitrogen source (w/v) (Miller 1992) for 7 days at 30°C, respectively. pH was adjusted to 7 prior to seeding. Analyses of starch hydrolysis, gelatin hydrolysis, citrate utilization, H₂S production, catalase, nitrate reduction, and urease production were performed using standard methods.

Phylogenetic analysis

Genomic DNA was isolated as previously described (Hopwood et al. 1985). 16S rRNA was amplified using universal primer pairs of 27F 5' - AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-GGTTACCTTGTACGACTT-3. The purified 16S rRNA PCR product was sequenced using ABI 310 automatic DNA sequencer (Applied Biosystems) using the 27f, 533f and 1492r primers by Eurofins Genomics India Pvt Ltd., India. BLASTn (Altschul et al. 1997) at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to analyse the 16s rRNA sequences. The sequences obtained were aligned against selected sequences that are publicly available in GenBank using the CLUSTAL X program (Thompson et al. 1997). The phylogenetic tree was inferred using the neighbour-joining method and maximum parsimony algorithms contained in the PHYLIP package (Saitou and Nei 1987). According to the Jukes and Cantor model, an evolutionary distance matrix was generated (Jukes 1969). Bootstrap analyses based on 1,000 replicates with MEGA 6.0 were conducted (Jukes 1969) to determine the confidence values of branches of the phylogenetic tree software package (Felsenstein 1985; Tamura et al. 2013). The 16S rRNA gene sequence was deposited in GenBank, NCBI database under the accession number KF918268.

Test bacterial strains

Bacterial pathogens including *Vibrio cholerae* (MTCC 3906); *Proteus mirabilis* (MTCC 425); *Klebsiella pneumoniae* (MTCC 3384); *Staphylococcus aureus* (MTCC 3160); *Enterococcus faecalis* (MTCC 439); *Escherichia coli* (MTCC 1687); and *Salmonella typhi* (MTCC 3231) were obtained from the Microbial Type Culture Collection (MTCC), IMTECH, India. Luria broth or Luria agar was used to culture the bacterial strains at 37 °C.

Disc diffusion method

Disc diffusion method was adopted on Muller Hinton agar plates to evaluate the bactericidal

activity of cell free supernatant against test bacterial pathogens. Antimicrobial activities of an isolate were determined using its cell free supernatant (CFS) by recording the Zone of inhibition (ZoI). Sterile antibiotic discs (6 mm diameter) were purchased from HIMEDIA Laboratories, India. To test the antimicrobial activity, 60 µl CFS of was loaded onto the disc and air dried. LB agar medium was spread, plated with about 10⁶ CFU/ml of bacterial cultures, placed with the sample loaded disc and incubated at 37 °C for 18 hrs. ZoI was measured after incubation using a vernier calliper. Sterile water was used as a negative control. The experiments were performed in triplicate.

Results and discussion

Isolation of endophytic actinomycetes from the root of *S. trilobatum*

We isolated 25 strains of endophytic actinomycetes from surface sterilized root parts of *S. trilobatum*. It was found that the incubation time was crucial in determining the isolation of endophytic actinomycetes. Generally, cultures grow well after two weeks of incubation and reach their maximal growth after two to three weeks (Figure 1B). As a result, it is suggested that incubation time played an important role in the isolation of endophytic actinomycetes from medicinal plants. The results are shown in Figure 1B. Spores and aerial mycelium were well developed in all actinomycete colonies. It was observed that colonies exhibited white, yellow, brown, whitish gray and blackish white aerial mycelium. Moreover, the colonies showed fine droplets of extracellular exudate on their surfaces. There was no growth of microbial growth on the HV agar medium when surfaces sterilized wash solutions were spread on the medium. Hence, the isolate was undeniably an endophyte rather than an epiphyte. Endophytic microorganisms have evolved with their respective plant and are known to produce novel bioactive compounds (Li et al. 2008). In agreement with previous studies (Verma et al. 2009; Gohain et al. 2015; Musa et al. 2020), roots are favorable niches for endophytic actinomycetes.

Morphological, physio-chemical and chemotaxonomic characteristics

The life cycle of *Streptomyces* provides three important features for microscopic study, including vegetative mycelium, aerial mycelium bearing chains of spores, and spores themselves

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(Williams et al. 1983; Anderson and Wellington 2001). Mycelia clumps of *Streptomyces* sp *R25* displayed a mixture of dispersed and aggregated features (Figure 2A). A straight to rectiflexible spiral spore chain with elliptoid shape and nonmotile spore was observed for *Streptomyces* sp *BDUSMP* R25 at 28 °C for two weeks (Figure 2B). The results of cultural, physiological, and chemical characteristics are presented in Table 1. *Streptomyces* sp *BDUSMP* R25 produced light yellow color diffusible pigments in all ISP media tested except ISP5 and ISP7. *BDUSMP* R25 is characterized as yellowish white with a smooth surface. The cultural characteristics of the strain are shown in figure 2C.

It was observed that substrate mycelium developed well on almost all media used and aerial mycelium poorly formed on ISP 5 and ISP7 media. Aerial mycelium varied in color from yellow to gray, while substrate mycelium varied in color from reddish to yellow (Table 1). Many other researchers have studied *Streptomyces* species spore surface morphology, with evidently distinctive spore surface configurations in some species (Bian et al. 2012; Slama et al. 2014; Mo et al. 2024). The catalase production test was positive for *Streptomyces* sp *BDUSMP* R25. A number of physiological properties have been shown to influence *Streptomyces* growth rates (Shimizu et al. 2000).

Isolates grew at the following temperatures: 25 °C, 28 °C (optimal) and 32 °C; however, it could not grow above 40 °C. It was found that the isolates grew well at neutral pH 7.0. Previous studies have shown growth of *Streptomyces* sp with pH values ranging from 5.5 to 9.5 (Sun et al. 2007). Thus, the isolates can be cultured at pH 7 in order to maximize growth and yield high mycelia biomass. Growth of the strain was good in the presence of 0 - 5 % (w/v) NaCl. It has been reported that carbon sources of certain sugars play a major role in the growth of *Streptomyces* mycelia and morphology (Jonsbu et al. 2002). One notable characteristic of *Streptomyces* sp *R25* is that it uses Xylose as its sole carbon source. Several studies have shown that nitrogen source greatly influences *Streptomyces* morphological types (Choi et al. 2000).

Streptomyces sp *BDUSMP* R25 utilized arginine, while phenylalanine was not utilized. The analysis of Diaminopimelic acid isomers (A2pm) in the cell wall of aerobic actinomycetes could be considered a distinct amino acid (chemotype -I) in the *Streptomyces* taxonomy (Lechevalier and Lechevalier

1970). A previous report demonstrated the use of chemotaxonomy analysis of DAP to identify the novel species in polyphasic taxonomy (Xu et al. 2009; Deng et al. 2023). Studies have demonstrated that *Streptomyces* contain 2, 6 Diaminopimelic acids in their cell walls (Jiang et al. 2007; Xu et al. 2009; Long et al. 2024). Similar to previous studies, the cell wall hydrolysates of this strain contained 2, 6 diaminopimelic acid (DAP), a characteristic of *Streptomyces* (Figure 3).

Phylogenetic analysis

Molecular techniques involving 16S rRNA sequencing have revolutionized bacterial systematics (Lane et al. 1985). Traditionally, 16S rRNA genes sequencing has been used to identify novel *Streptomyces* species (Bian et al. 2012; Slama et al. 2014). Phylogenetic analysis of the 16S rRNA gene sequence (1393 base pairs) demonstrated that strain *BDUSMP* R25 belongs to the *Streptomyces* genus (figure 4). A rooted phylogenetic tree of *BDUSMP* R25 showed that the strain was closely related to *Streptomyces parvulus*. Based on the neighbour-joining method, distinct branches are formed from the rooted tree (figure 4).

Antibacterial activity

The results of antimicrobial activity against recorded bacterial pathogens are shown in table 2. The results indicate that *K.pneumoniae* is the most susceptible, whereas *S.aureus* is the least susceptible. However, no antimicrobial activity was observed against *Enterococcus faecalis*. It clearly demonstrates that *Streptomyces* sp *BDUSMP*R25 could be a potential natural drug discovery candidate. *Streptomyces* isolated from medicinal plants have been reported to possess antimicrobial properties (Ezra et al. 2004; Gos et al. 2017). Exploiting novel endophytic actinomycetes from ethanopharmacologically valuable plants can lead to exploration of their potential in unprecedented detail through the use of natural drug discovery. As a result, potential endophytic strain isolated from this study may lead to the discovery of antibacterial agents that will combat emerging bacterial infections in the future. Further, it has been reported that culture medium and incubation time play a crucial role in the production of antibacterial secondary metabolites (Vilches et al. 1990; Holmalahti et al. 1998; Arul Jose et al. 2011). The present study found promising growth inhibitory activity against test bacteria when cultures are grown for more than seven days.

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Conclusion

In this study, *Streptomyces* sp. BDUSMP R25 was isolated from the surface sterilized root of *S. trilobatum*. This strain displayed broad spectrum antimicrobial properties. Based on polyphasic taxonomic characterization involving morphological, physio-biochemical, chemotaxonomy and molecular analyses, the strain belongs to the *Streptomyces* genus and is closely related to *Streptomyces parvalus*. A bioassay guided the isolation of chemicals and the structure elucidation of natural drugs that were to be studied further. Additionally, genome mining of strain chromosomal DNA can uncover hidden novel drug leads that can be further exploited for pharmacokinetic studies and isolation of novel natural compounds.

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Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

- Akshatha V, Nalini M, D'souza C, Prakash H (2014) Streptomycete endophytes from anti-diabetic medicinal plants of the Western Ghats inhibit alpha-amylase and promote glucose uptake. *Letters in applied microbiology* 58:433-439
- Altschul SF et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research* 25:3389-3402
- Anderson AS, Wellington E (2001) The taxonomy of *Streptomyces* and related genera. *International journal of systematic and evolutionary microbiology* 51:797-814
- Arul Jose P, Satheja Santhi V, Jebakumar SR (2011) Phylogenetic-affiliation, antimicrobial potential and PKS gene sequence analysis of moderately halophilic *Streptomyces* sp. inhabiting an Indian saltpan. *Journal of Basic Microbiology* 51:348-356
- Bascom-Slack CA et al. (2009) Multiple, novel biologically active endophytic actinomycetes isolated from upper Amazonian rainforests. *Microbial ecology* 58:374-383
- Becker B, Lechevalier M, Lechevalier H (1965) Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Applied Microbiology* 13:236-243
- Berdy J (2005) Bioactive microbial metabolites. *The Journal of antibiotics* 58:1-26
- Bian G-K et al. (2012) *Streptomyces* phytohabitans sp. nov., a novel endophytic actinomycete isolated from medicinal plant *Curcuma phaeocaulis*. *Antonie van Leeuwenhoek* 102:289-296
- Choi DB, Park EY, Okabe M (2000) Dependence of apparent viscosity on mycelial morphology of *Streptomyces fradiae* culture in various nitrogen sources. *Biotechnology progress* 16:525-532
- Deng A, Luo Y, Wu C, Chen D, Mo P, Li B (2023) *Streptomyces cynarae* sp. nov., a novel actinomycete isolated from the leaves of *Cynara scolymus* L. *Antonie van Leeuwenhoek* 116:1277-1284 doi: 10.1007/s10482-023-01885-2
- Ezra D et al. (2004) Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp.(MSU-2110) endophytic on *Monstera* sp. *Microbiology* 150:785-793
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *evolution* 39:783-791
- Flärdh K, Buttner MJ (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. *Nature Reviews Microbiology* 7:36-49 doi: 10.1038/nrmicro1968
- Gohain A et al. (2015) Antimicrobial biosynthetic potential and genetic diversity of endophytic actinomycetes associated with medicinal plants. *FEMS Microbiology Letters* 362 doi: 10.1093/femsle/fnv158
- Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M (2015) Endophytic actinobacteria of medicinal plants: diversity and bioactivity. *Antonie Van Leeuwenhoek* 108:267-289
- Gos FMWR et al. (2017) Antibacterial Activity of Endophytic Actinomycetes Isolated from the Medicinal Plant *Vochysia divergens* (Pantanal, Brazil). *Frontiers in Microbiology* Volume 8 - 2017 doi: 10.3389/fmicb.2017.01642

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- Goudjal Y, Toumatia O, Sabaou N, Barakate M, Mathieu F, Zitouni A (2013) Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. *World Journal of Microbiology and Biotechnology* 29:1821-1829 doi: 10.1007/s11274-013-1344-y
- Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R (1999) A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. *Journal of ethnopharmacology* 66:205-210
- Holmalahti, Raatikainen, Laatsch, Spohr, Lyngberg, Nielsen (1998) Production of dihydroabikoviromycin by *Streptomyces anulatus*: production parameters and chemical characterization of genotoxicity. *Journal of applied microbiology* 85:61-68
- Hopwood D et al. (1985) *A Laboratory Manual, the John Innes Foundation*. Norwich, United Kingdom:356
- Jiang Y, Tang S-K, Wiese J, Xu L-H, Imhoff JF, Jiang C-L (2007) *Streptomyces hainanensis* sp. nov., a novel member of the genus *Streptomyces*. *International journal of systematic and evolutionary microbiology* 57:2694-2698
- Jonsbu E, McIntyre M, Nielsen J (2002) The influence of carbon sources and morphology on nystatin production by *Streptomyces noursei*. *Journal of Biotechnology* 95:133-144
- Jukes C (1969) Evolution of protein molecules. In Munro (ed.), *Mammalian Protein Metabolism*. Academic Press London. 21–132. .
- KL K (1964) *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Print Office.
- Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences* 82:6955-6959
- Latha PS, Kannabiran K (2006) Antimicrobial activity and phytochemicals of *Solanum trilobatum* Linn. *African Journal of Biotechnology* 5
- Lechevalier MP, Lechevalier H (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. In. *Microbiology Society*, pp 435-443
- Lee JY, Lee JY, Jung HW, Hwang BK (2005) *Streptomyces koyangensis* sp. nov., a novel actinomycete that produces 4-phenyl-3-butenoic acid. *International journal of systematic and evolutionary microbiology* 55:257-262
- Li J et al. (2008) Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. *Letters in applied microbiology* 47:574-580
- Long P-L, Liu J-X, Xiao Y, Mo P, Gao J (2024) *Streptomyces albidocamelliae* sp. nov., an endophytic actinomycete isolated from the leaves of *Camellia oleifera*. *The Journal of Antibiotics* 77:786-793 doi: 10.1038/s41429-024-00776-6
- McCormick JR, Flårdh K (2012) Signals and regulators that govern *Streptomyces* development. *FEMS Microbiology Reviews* 36:206-231 doi: 10.1111/j.1574-6976.2011.00317.x
- Miller (1992) Cold Spring Harbor Laboratory NY.
- Mo P et al. (2024) *Streptomyces cathayae* sp. nov., an endophytic actinobacterium from the root tissue of *Cathaya argyrophylla*. *Antonie van Leeuwenhoek* 117:31
- Musa Z et al. (2020) Diversity and antimicrobial potential of cultivable endophytic actinobacteria associated with the medicinal plant *Thymus roseus*. *Frontiers in microbiology* 11:191
- Pandurangan A, Khosa R, Hemalatha S (2011) Anti-inflammatory activity of an alkaloid from *Solanum trilobatum* on acute and chronic inflammation models. *Natural product research* 25:1132-1141
- Premalatha S, Elumalai K, Jeyasankar A (2013) Mosquitocidal properties of *Solanum trilobatum* L.(Solanaceae) leaf extracts against three important human vector mosquitoes (Diptera: Culicidae). *Asian Pacific journal of tropical medicine* 6:854-858

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- Qin S et al. (2009) *Glycomyces scopariae* sp. nov. and *Glycomyces mayteni* sp. nov., isolated from medicinal plants in China. *International journal of systematic and evolutionary microbiology* 59:1023-1027
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution* 4:406-425
- Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. *Microbiological Research* 183:92-99 doi: <https://doi.org/10.1016/j.micres.2015.11.008>
- Savic M, Bratic I, Vasiljevic B (2007) *Streptomyces durmitorensis* sp. nov., a producer of an FK506-like immunosuppressant. *International journal of systematic and evolutionary microbiology* 57:2119-2124
- Shimizu M et al. (2000) Studies on endophytic actinomycetes (I) *Streptomyces* sp. isolated from rhododendron and its antifungal activity. *Journal of General Plant Pathology* 66:360-366
- Shirling ET, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. *International Journal of Systematic and Evolutionary Microbiology* 16:313-340
- Sini H, Devi K (2004) Antioxidant activities of the chloroform extract of *Solanum trilobatum*. *Pharmaceutical biology* 42:462-466
- Sivalingam P, Easwaran M, Ganapathy D, Basha SF, Poté J (2024) Endophytic *Streptomyces*: an underexplored source with potential for novel natural drug discovery and development. *Archives of Microbiology* 206:442 doi: 10.1007/s00203-024-04169-z
- Slama N et al. (2014) *Streptomyces tunisiensis* sp. nov., a novel *Streptomyces* species with antibacterial activity. *Antonie van Leeuwenhoek* 105:377-387
- Sun W, Huang Y, Zhang Y-Q, Liu Z-H (2007) *Streptomyces emeiensis* sp. nov., a novel streptomycete from soil in China. *International journal of systematic and evolutionary microbiology* 57:1635-1639
- Taechowisan T, Peberdy JF, Lumyong S (2003) Isolation of endophytic actinomycetes from selected plants and their antifungal activity. *World Journal of Microbiology and Biotechnology* 19:381-385
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution* 30:2725-2729
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research* 25:4876-4882
- Verma VC, Gond SK, Kumar A, Mishra A, Kharwar RN, Gange AC (2009) Endophytic actinomycetes from *Azadirachta indica* A. Juss.: isolation, diversity, and antimicrobial activity. *Microbial ecology* 57:749-756
- Verma VC, Singh SK, Prakash S (2011) Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *Journal of Basic Microbiology* 51:550-556 doi: <https://doi.org/10.1002/jobm.201000155>
- Vilches C, Méndez C, Hardisson C, Salas JA (1990) Biosynthesis of oleandomycin by *Streptomyces antibioticus*: influence of nutritional conditions and development of resistance. *Microbiology* 136:1447-1454
- Wardecki T et al. (2015) Endophytic *Streptomyces* in the traditional medicinal plant *Arnica montana* L.: secondary metabolites and biological activity. *Antonie van Leeuwenhoek* 108:391-402 doi: 10.1007/s10482-015-0492-5
- Williams S, Goodfellow M, Alderson G, Wellington E, Sneath P, Sackin M (1983) Numerical classification of *Streptomyces* and related genera. *Microbiology* 129:1743-1813
- Xu J, Wang Y, Xie S-J, Xu J, Xiao J, Ruan J-S (2009) *Streptomyces xiamenensis* sp. nov., isolated from mangrove sediment. *International Journal of Systematic and Evolutionary Microbiology* 59:472-476

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Figure legends

Table 1. Cultural, physiological and biochemical characteristics features of the isolated *Streptomyces* sp BDUSMP R25 from *S. trilobatum*

Table 2. Antibacterial activity of cell free supernatants derived from *Streptomyces* sp BDUSMP R25

Figure 1. A) *S. trilobatum* tissues; B) isolation of endophytic actinomycetes on HV agar; C) Pure culture of *Streptomyces* sp BDUSMP R25 grown on ISP4

Figure 2. A) Light microscopy analysis of mycelium; B) SEM analysis of spore morphology; C) cultural characteristics on ISP media

Figure 3. Thin layer chromatography analysis of Diaminopimelic acid isomers (A2pm). M: 2,6 diaminopimelic acid 0.1M; R25 : Isolate cell wall hydrolysates diaminopimelic acid.

Figure 4. The phylogenetic tree of *Streptomyces* sp BDUSMP R25 based on partial nucleotide sequences of 16S Rrna was constructed using the neighbour-joining method with aid of MEGA 6.0 program. The Bootstrap values above 50 %, presented as percentages of 1000 replications, are shown at the branch points. Bar 2 for 16S rRNA substitutions per nucleotide position respectively.

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Table 1. Cultural, physiological and biochemical characteristics features of the isolated *Streptomyces* sp R25 from *S. trilobatum*

Characteristics	<i>Streptomyces</i> sp R25					
Features	ISP2	ISP3	ISP4	ISP5	ISP6	ISP7
Growth	Good	Good	Good	Moderate	Good	Moderate
Sporulation	Good	Good	Good	Moderate	Good	Moderate
Colour of aerial mycelium	Y/W	G	W/G	G	Y/W	G
Colour of substrate mycelium	R	Y	Y	G	Y	Light Yellow
Diffusible Pigment	Y	Y	Y	-	Y	-
Temperature (°C)				25-37		
Growth pH				6-8		
NaCl (%)				0-5		
<i>Biochemical</i>						
Gram staining				Positive		
Citrate utilization				Positive		
Methyl Red				Negative		
Voges-Proskauer				Negative		
H ₂ S production				Negative		
Nitrate reduction				Positive		
Catalase				Positive		
Urea hydrolysis				Positive		
Gelatin hydrolysis				Negative		
Starch hydrolysis				Negative		
<i>Utilization of carbon</i>						
Arabinose				+		
Dextrose				+		
Fructose				+		
Inositol				+		
Lactose				-		
Mannitol				+		
Maltose				-		
Sucrose				+		
Xylose				+		
<i>Growth on sole nitrogen source</i>						
Alanine				+		
Arginine				+		
Asparagine				-		
Cysteine				-		
Methionine				+		
Phenylalanine				-		

Y/W: Yellowish White; W/G: Whitish Gray; G: Gray; R: Red; Y: Yellow; +: positive; -: negative

Antimicrobial Activities of Endophytic *Streptomyces* sp Bdusmp R25 from the Root of the Medicinal Plant *Solanum trilobatum*

Table 2. Antibacterial activity of cell free supernatants derived from *Streptomyces* sp R25

Test bacterial strains	Mean Zone of inhibition (mm)
<i>Escherichia coli</i> (MTCC 1687)	16.33 ± 0.57
<i>Vibrio cholerae</i> (MTCC 3906)	12.66 ± 0.57
<i>Proteus mirabilis</i> (MTCC 425)	14 ± 1.00
<i>Klebsiella pneumoniae</i> (MTCC3384)	10.33 ± 0.57
<i>Staphylococcus aureus</i> (MTCC 3160)	13 ± 0.00
<i>Salmonella typhi</i> (MTCC3231)	15.66 ± 1.00

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Figure 1. A) *S. trilobatum* tissues; B) isolation of endophytic actinomycetes on HV agar; C) Pure culture of *Streptomyces* sp BDUSMP R25 grown on ISP4

Figure 1A

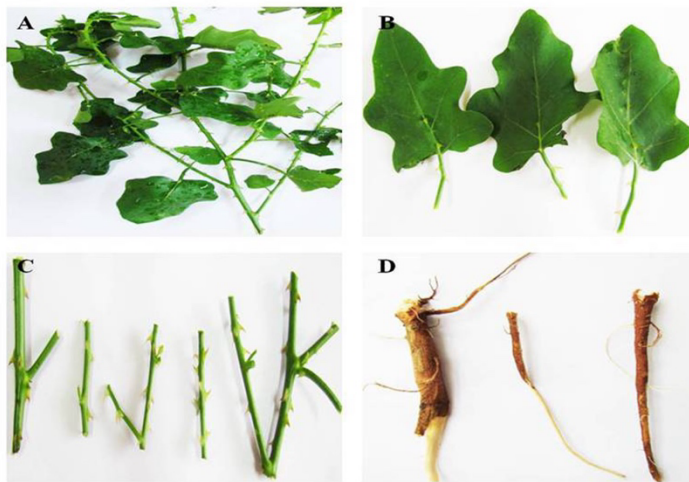


Figure 1B

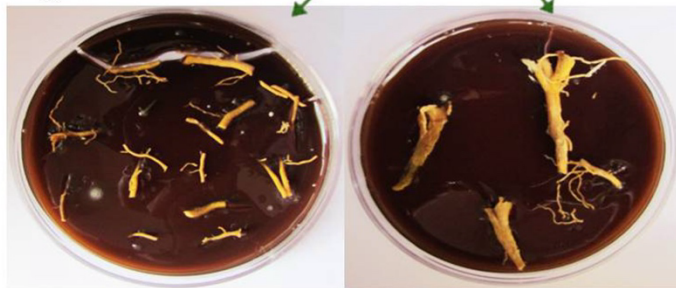


Figure 1C



Antimicrobial Activities of Endophytic Streptomyces sp Bdu SMP R25 from the Root of the Medicinal Plant Solanum trilobatum

Figure 2. A) Light microscopy analysis of mycelium; B) SEM analysis of spore morphology; C) cultural characteristics on ISP media

Figure 2

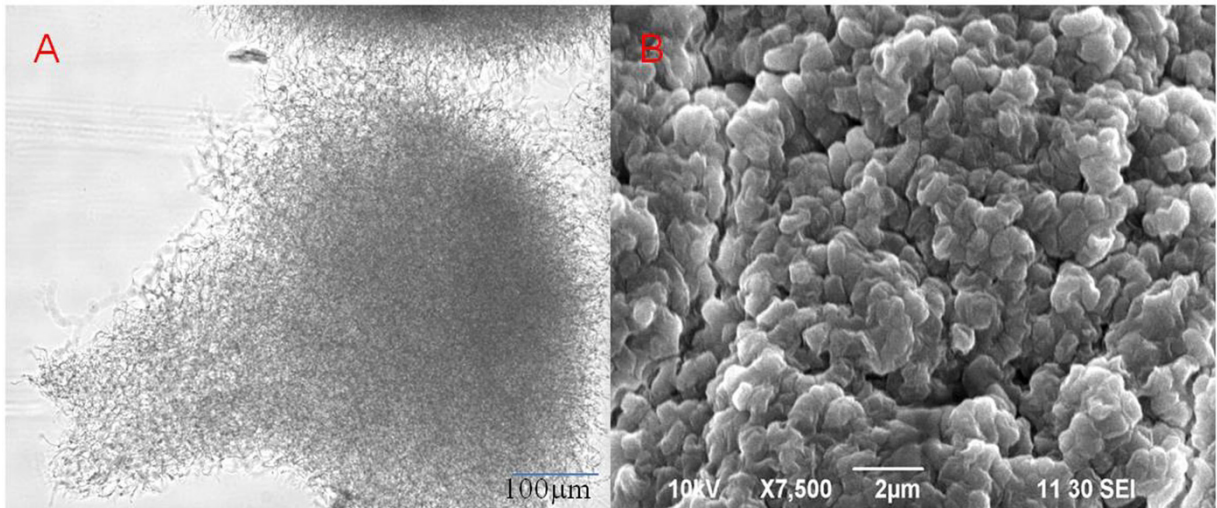
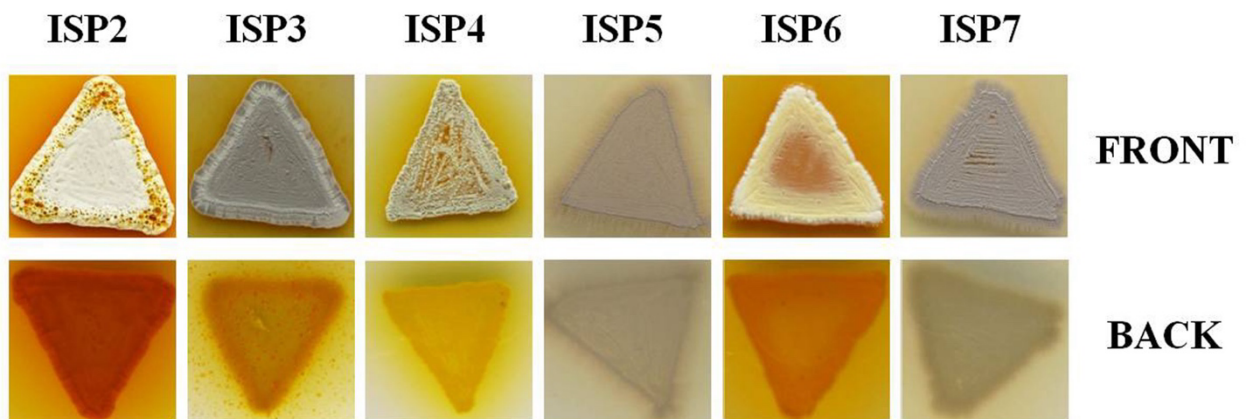
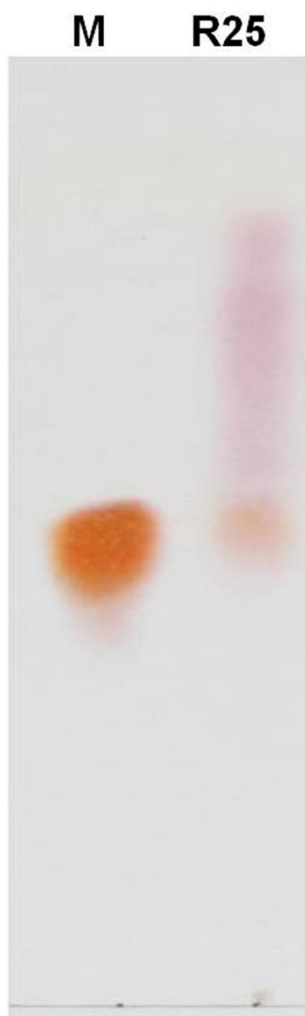


Figure 2 C



Antimicrobial Activities of Endophytic *Streptomyces* sp Bdu SMP R25 from the Root of the Medicinal Plant *Solanum trilobatum*

Figure 3. Thin layer chromatography analysis of Diaminopimelic acid isomers (A2pm). M: 2,6 diaminopimelic acid 0.1M; R25 : Isolate cell wall hydrolysates diaminopimelic acid.



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