ISSN: 2634 - 8861

Journal of Infectious Diseases & Case Reports

SCIENTIFIC Research and Community

Research Article

Open Access

Isolation and Biochemical Characterization of *Streptomyces* sp. MS14 Producing Antibiotics from Soil of Chhattisgarh

Neha Singh[1,2]*, Nikita Sherwani[1], Vibhuti Rai[2]

[1]Department of Microbiology, Pt Jawahar Lal Nehru Memorial Medical College Raipur Chhattisgarh, 492001, India

[2]Department of Microbiology, School of Life Sciences, Pt. Ravishankar Shukla University Raipur, Chhattisgarh, 492001, India

ABSTRACT

The rising incidence of multidrug-resistant (MDR) pathogens underscores the urgent need for novel antibiotic sources. Soil-dwelling actinomycetes, particularly members of the genus Streptomyces, are well-established producers of diverse secondary metabolites with antimicrobial properties. In this study, a novel actinomycete isolate, designated MS14, was recovered from soil samples collected from forest-rich regions of Durg, Chhattisgarh, India. The isolate demonstrated significant antibacterial activity, producing inhibition zones of 19±0.5 mm against *Escherichia coli* (ATCC 25922), 22±0.5 mm against *Staphylococcus aureus* (ATCC 25923), and 17±0.5 mm against *Pseudomonas aeruginosa* (ATCC 27853) respectively. Comprehensive characterization of the isolate MS14, including morphological, biochemical, physiological, and chemotaxonomic analyses, supported its classification. Further identification using PIB-Win software yielded a high confidence match (similarity index: 0.99) with *Streptomyces atroolivaceus*. These findings highlight the untapped microbial diversity of the Durg District's Forest soil and its potential as a valuable reservoir for antibiotic-producing actinomycetes.

***Corresponding author**

Neha Singh, Department of Microbiology, Pt Jawahar Lal Nehru Memorial Medical College Raipur Chhattisgarh, 492001, India and Department of Microbiology, School of Life Sciences, Pt. Ravishankar Shukla University Raipur, Chhattisgarh, 492001, India.

Received: July 01, 2025; **Accepted:** July 07, 2025; **Published:** August 20, 2025

Keywords: Streptomyces, Antibiotics, Actinomycetes, Soil Microbiota, Chhattisgarh, Antimicrobial Activity, Secondary Metabolites, Biochemical Characterization

Introduction

The discovery of antibiotics from soil-dwelling actinomycetes, particularly *Streptomyces,* marked a major breakthrough in medical microbiology. The emergence of multidrug-resistant (MDR) pathogens has intensified the need for new and effective antimicrobial agents, prompting renewed interest in the exploration of natural sources, particularly soil-dwelling actinomycetes. Among these, the genus *Streptomyces* is one of the most prolific producers of bioactive secondary metabolites, including antibiotics, antifungals, antivirals, immunosuppressants, and antitumor agents [1]. More than 70% of currently known antibiotics, including well-known examples such as streptomycin, tetracycline, and erythromycin, have been derived from *Streptomyces* spp [2,3]. Soil remains a highly diverse and largely untapped reservoir of microbial biodiversity. India, with its diverse ecological niches and varied soil types, offers a promising ground for the isolation of novel actinomycetes [4-6]. The state of Chhattisgarh, located in central India, comprises mineral-rich, forested, and undisturbed ecosystems which may harbor unexplored microbial flora. Despite this ecological richness, systematic exploration of soil microbes from this region remains limited. The isolation of indigenous actinomycetes such as *Streptomyces* sp. MS14 from such niches could lead to the discovery of new bioactive compounds with broad-spectrum antimicrobial potential. The genus *Streptomyces* is characterized by its Gram-positive, filamentous morphology and high G+C content in its DNA [7]. These bacteria undergo complex life cycles and produce an array of secondary metabolites, primarily during the stationary phase of growth [8,9] (Kieser et al., 2000). Their metabolic versatility is a result of their large genomes, which harbor a multitude of biosynthetic gene clusters [10]. Isolating such strains from soil samples and subjecting them to biochemical and antimicrobial screening can provide critical insights into their metabolic potential and therapeutic relevance. Biochemical characterization remains an essential tool for preliminary identification and differentiation of *Streptomyces* species [11]. These tests also aid in differentiating novel isolates from known species. Furthermore, antimicrobial assays against standard Gram-positive and Gram-negative bacterial strains allow researchers to evaluate the spectrum and potency of the antibiotics produced [12]. In this study, we focus on the isolation and biochemical characterization of a novel antimicrobial producing strain, *Streptomyces* sp. MS14, obtained from soil samples collected in the Chhattisgarh region. The primary objective is to screen for antimicrobial activity against selected bacterial pathogens and to assess its potential as a source of broad-spectrum antibiotics. This investigation contributes to the ongoing search for novel antibiotic-producing actinomycetes from underexplored ecological niches and provides a foundation for future molecular and pharmacological studies.

Materials and Methods

- **Sample Collection**

  Soil samples were collected from forest areas and agricultural fields in Durg districts of Chhattisgarh, Latitude & Longitude:

21.190449° N, 81.284920° E (approximately 21° 11′ 25.6″ N, 81° 17′ 5.7″ E); Elevation: around 295–297 meters (≈ 968–974 feet) above mean sea level. Approximately 200–300 grams of soil were collected from a depth of 5–10 cm using a sterile spatula and transferred into sterile polyethylene bags and labelled properly. Samples were air-dried and pre-treated with calcium carbonate to reduce non-actinomycete contamination and stored at 4°C until further processing [13].

- **Isolation of Streptomyces**
  Soil samples were subjected to serial dilution for the selective isolation of Streptomyces spp. One gram of each air-dried soil sample was suspended in 9 mL of sterile distilled water in a test tube and vortexed thoroughly to ensure uniform dispersion. From this suspension, a tenfold serial dilution was prepared up to 10^{-6} using sterile distilled water. From each dilution, 0.1 mL aliquots were aseptically spread onto Starch Casein Agar (SCA) plates supplemented with cycloheximide (50 µg/mL) and nalidixic acid (20 µg/mL) to inhibit fungal and fast-growing bacterial contaminants, respectively. The inoculated plates were incubated at 28 ± 2°C for 7–14 days [14-16].

- **Morphological, physiological and Biochemical Characterization**
  The isolate Streptomyces sp. MS14 was characterized based on colony morphology and microscopic features. The biochemical characterization of Streptomyces sp. MS14 was carried out following the methodology described by [17,18]. A series of conventional biochemical tests were performed to assess enzymatic and metabolic traits relevant to the identification of the isolate. All morphological and biochemical assays were performed in triplicates, and results were recorded after incubation at 28 ± 2°C for 7-14 days. The observed biochemical profile was compared with standard actinomycete identification keys for confirmation.

- **Antibacterial Activity Screening**
  Antimicrobial activity of isolates was screened using the agar well diffusion method against test organisms: E. coli (ATCC 25922), S. aureus (ATCC 25923), and P. aeruginosa (ATCC 27853) [19].

Results

- **Morphological, Physiological and Biochemical Characterization**
  Isolate designated as MS14 showed dominant antibacterial activity. The colonies on starch casein agar were chalky white to grey, with a dry, powdery surface and earthy odor, typical of Streptomyces species after 7-10 days of incubation. Aerial and substrate mycelia were well-developed, with no diffusible pigment observed initially but dark brown after 10-14 days (Figure 1). Sporulation was noted after 7 to 8 days of incubation at 28 ± 2°C. Gram staining revealed Gram-positive, filamentous mycelial structures, well-branched network of hyphae forming compact spore chains under the light microscopy. Physiological and biochemical activities are given in (Table 1; Figure 1). The isolate Streptomyces sp. MS14 exhibited the ability to hydrolyse a broad range of substrates, including starch, gelatine, adenine, xanthine, pectin, esculin, Tween 20, urea, and guanine, indicating robust enzymatic activity. Biochemical profiling revealed positive reactions for catalase, oxidase, nitrate reduction, hydrogen sulphide (H_2S) production, and milk coagulation. Cell wall analysis confirmed the presence of diaminopimelic acid (DAP), characteristic of actinomycetes. The isolate demonstrated tolerance to a wide range of salt concentrations, showing growth in the presence of 1–7% NaCl, as well as resistance to 0.001% potassium tellurite and 0.1% phenol. The strain was capable of utilizing various carbon and nitrogen sources, including D-glucose, D-fructose, D-galactose, D-lactose, D-maltose, D-mannitol, D-sorbitol, cellobiose, L-arabinose, L-rhamnose, L-phenylalanine, L-arginine, L-asparagine, L-serine, L-cysteine, L-histidine, and DL-α-amino-n-butyric acid. Optimal growth was observed over a temperature range of 15–37°C and a pH range of 6.0–8.0. Antibiotic susceptibility testing revealed that the isolate was sensitive to twelve antibiotics, resistant to eight, and exhibited intermediate susceptibility to two antibiotics. Based on morphological, biochemical, physiological, and chemotaxonomic characteristics, and confirmed through PIB-Win software analysis with a confidence score of 0.99, the isolate MS14 was identified as Streptomyces atroolivaceus.

Table 1: Sensitivity and Resistance Pattern of Streptomyces sp. MS14 to Various Antibiotics

Antibiotic (µg/disc)	No of Streptomyces isolates
	MS14
Gentamycin (G^{30}),	I
Chloromphenicol (C^{10})	S
Linezolid (LZ^{30})	S
Ciprofloxacin (CF^{5})	S
Amoxicillin (AM^{10}),	R
Methicillin(M^{10}),	R
Nalidixic acid (NA^{30})	R
Novobiocin (Nv^{5})	S
Tetracycline (T^{10})	S
Oleondamycin (OL^{15})	S
Streptomycin (S^{25})	S
Vancomycin (VA^{5})	S
Kanamycin (K^{30}),	S
Polymixim B (PB^{100})	S
Neomycin (N^{30}),	S
Rifamycin (R^{5}),	R
Erythromycin (E^{15}),	S
Cephalosporin (CR^{30}),	R
Penicillin G (P^{10})	R
Amphotericin B (AP^{30})	R
Fluconazole (F^{10})	R
Miconazole (MIC^{3})	I

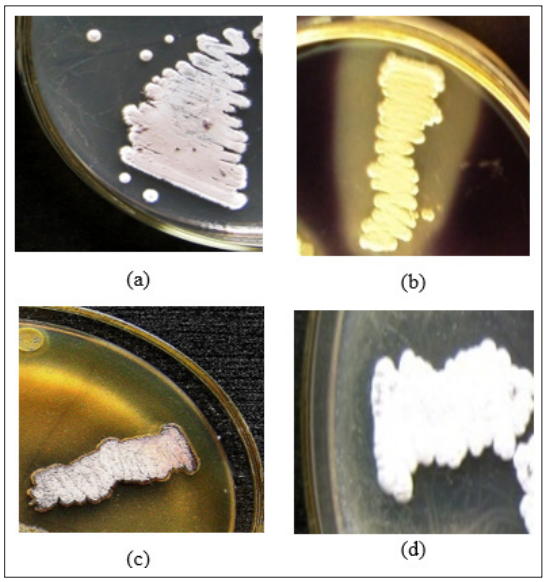

Figure 1: (a) Colony morphology of MS14: chalky white to grey, with a dry, powdery surface and earthy odor, aerial and substrate mycelia were well-developed, with no diffusible pigment (b) Starch hydrolysis test: A clear zone around the colony indicated positive amylolytic activity due to starch degradation after incubation at 28 ± 2°C for 5–7 days (c) Pectinolytic activity; confirming its capability to degrade pectin as a carbon source. (d) Adenine degradation; exhibited the ability to hydrolyze adenine, indicating the presence of purine-degrading enzymatic activity.

- **Antibacterial Activity**
  The isolate *Streptomyces* sp. MS14 demonstrated broad-spectrum antibacterial activity against tested bacterial pathogens. 100 ul supernatant was loaded in each well. The crude supernatant obtained from MS14 exhibited significant inhibitory effects, as evidenced by the formation of clear zones of inhibition. The measured diameters of inhibition zones were 19±0.5 mm, 22±0.5 mm, and 17±0.5 mm against the respective target bacterial strains namely E. coli (ATCC 25922), *S. aureus* (ATCC 25923), and *P. aeruginosa* (ATCC 27853) indicating the potent bioactive potential of the secondary metabolites produced by the isolate (Figure 2).

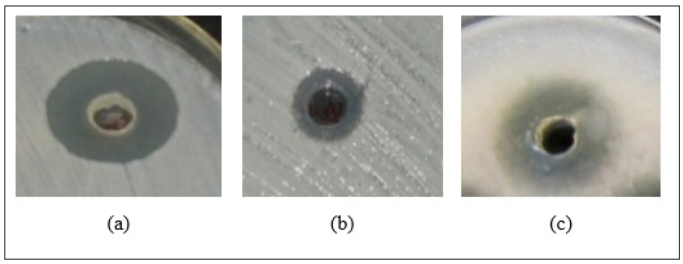

Figure 2: Antimicrobial activity of MS41, exhibiting inhibition zones 19 mm, 22 mm, and 17 mm against the E. coli (ATCC 25922), S. aureus (ATCC 25923), and P. aeruginosa (ATCC 27853). respectively.

Discussion

Soil remains a prolific source of antibiotic-producing microorganisms, especially *Streptomyces*. Our isolate MS14, obtained from the relatively unexplored region of Chhattisgarh, demonstrated strong antimicrobial activity. The biochemical characteristics aligned with typical *Streptomyces* spp. The results of this study demonstrate that Streptomyces sp. MS14 exhibits significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, indicating that the secondary metabolites produced by this strain may possess broad-spectrum potential. This finding is particularly significant in the current era of increasing antimicrobial resistance, where novel bioactive compounds are urgently needed to address the limitations of existing antibiotics. The genus *Streptomyces* is well known for its remarkable capacity to produce a diverse array of secondary metabolites, including over two-thirds of clinically useful antibiotics such as streptomycin, tetracycline, and erythromycin. The ability of MS14 to inhibit organisms across a wide taxonomic range suggests that it may produce compounds with either multiple targets or mechanisms of action capable of bypassing traditional resistance mechanisms. In Gram-negative bacteria, the presence of an outer membrane often limits the permeability of antibiotics, while Gram-positive bacteria are generally more susceptible due to the absence of this barrier [20]. Therefore, the activity of MS14 against both groups implies that its metabolites may either target universally conserved pathways or possess unique properties that facilitate membrane penetration.

Previous studies have identified several Streptomyces strains with broad-spectrum antimicrobial properties. For instance, *Streptomyces albidoflavus* has been reported to produce antibacterial compounds effective against both *Staphylococcus aureus* and *Escherichia coli*. Similarly, Streptomyces hygroscopicus and Streptomyces griseus are known to produce metabolites like hygromycin and streptomycin, respectively, that have broad-spectrum activity [21]. These findings support the potential of MS14 as a novel source of bioactive compounds. Furthermore, the ecological origin of MS14, likely from soil, may contribute to its bioactive potential. Soil-dwelling Streptomyces species are frequently exposed to diverse microbial populations, which drives the evolution of a rich arsenal of antimicrobial compounds as a means of survival and competition [22]. Such natural selective pressures often result in the production of structurally diverse and pharmacologically potent metabolites. The antimicrobial activity observed in this study may be attributed to the presence of polyketides, non-ribosomal peptides, or other classes of secondary metabolites. Polyketides, for example, are a large class of natural products with diverse biological activities, many of which are synthesized by Streptomyces species [23]. Further chemical characterization and purification of the active components from MS14 would help elucidate their structure and mechanism of action. Moreover, the use of modern tools such as genome mining and metabolomics could aid in identifying novel biosynthetic gene clusters responsible for these bioactivities [24]. Given the promising bioactivity of MS14, its metabolites hold potential not only for therapeutic applications but also for agricultural and industrial uses. However, to fully evaluate its clinical utility, further studies are needed, including the determination of minimum inhibitory concentrations (MICs), cytotoxicity assays, and in vivo efficacy models. In conclusion, the broad-spectrum antimicrobial activity exhibited by Streptomyces sp. MS14 underscores its potential as a valuable source of novel antibiotics. This finding contributes to the growing body of evidence supporting the role of soil-derived actinomycetes in the discovery of new antimicrobial agents, particularly in the face of the global threat posed by multidrug-resistant pathogens.

Conclusion

A novel *Streptomyces species*, designated as strain MS14, was isolated and characterized from soil samples collected in Chhattisgarh. Biochemical profiling and antimicrobial assays revealed that this isolate possesses significant potential for the production of novel antibiotic compounds. Molecular identification using PIB-Win software identified the strain as *Streptomyces atroolivaceus* with a high confidence score of 0.99. The findings underscore the importance of exploring biodiversity-rich and under-explored regions such as Chhattisgarh for the discovery of novel actinomycetes with antimicrobial properties, which may contribute to combating the growing threat of antimicrobial resistance.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

1. Berdy J. (2005) Bioactive microbial metabolites. J Antibiot (Tokyo) 58:1-26.
2. Bhange K, Singh N (2023) Rising Antibiotic Resistance: Growing Concern. J Bacteriol Mycol Open Access 11: 110-112.
3. Hopwood DA (2007) Streptomyces in Nature and Medicine: The Antibiotic Makers. Oxford: Oxford University Press https://academic.oup.com/book/50903.
4. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical Streptomyces Genetics. Norwich, UK: John Innes Foundation https://streptomyces.org.uk/PracticalStreptomycesGenetics.pdf.
5. Challis GL, Hopwood DA (2003) Synergy and Contingency as Driving Forces for the Evolution of Multiple Secondary Metabolite Production by Streptomyces Species. Proc Natl Acad Sci USA 100: 14555-14561.
6. Williams ST, Goodfellow M, Alderson G (1983) Genus Streptomyces Waksman and Henrici 1943. In: Bergey's Manual of Systematic Bacteriology. Baltimore: Williams & Wilkins 4: 2452-2492.
7. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, et al. (2016) Taxonomy, Physiology, and Natural Products of Actinobacteria. Microbiol Mol Biol Rev 80: 1-43.
8. Manivasagan P, Venkatesan J, Sivakumar K, Kim SK (2014) Marine Actinobacterial Metabolites: Current Status and Future Perspectives. Microbiol Res 169: 262-278.
9. El-Naggar NEA, El-Shweihy NM (2015) Antibacterial Activities of Bioactive Metabolites Produced by a Novel Streptomyces Strain Isolated From Egyptian Soil. J Appl Pharm Sci 5: 1-8.
10. Baltz RH (2007) Antimicrobials from Actinomycetes: Back to the Future. Microbe 2: 125-131.
11. Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How Many Antibiotics are Produced by the Genus Streptomyces? Arch Microbiol 176: 386-390.
12. Singh N, Rai V, Tripathi CKM (2012) Oxytetracycline Production by Immobilized Cells of a New Isolate of Streptomyces Rimosus MTCC 10792. J Pharm Res 5: 2477-2480.
13. Singh N, Rai V (2013) In Vitro Antimycotic Activity of a New Isolate Streptomyces Fradiae MTCC 11051 Against Multi-Drug-Resistant Pathogenic Fungi. J Pharm Res 7: 331-336.
14. Singh N, Rai V, Tripathi CKM (2013) Purification and Chemical Characterization of Antimicrobial Compounds from a New Soil Isolate Streptomyces Rimosus MTCC 10792. Appl Biochem Microbiol 49: 467-475.
15. Singh N, Rai V (2012) Improved Antimicrobial Compound Production by A New Isolate Streptomyces Hygroscopic us MTCC 4003 Using Plackett-Burman Design and Response Surface Methodology. Bioinformation 8: 1021-1025.
16. Singh N, Patil S, Shahnawaz M, Rai V, Patil A, et al. (2024) Green Extraction of Puromycin-Based Antibiotics from Streptomyces Albofaciens (MS38) for Sustainable Biopharmaceutical Applications. Front Chem 11: 1326310-1326328.
17. Singh N, Das D, Sherwani N, Sinha A (2025) Streptomyces: Sources of Novel Discoveries in Antibiotic Research to Combat Antimicrobial Resistance. Virol Immunol J 9: 000350-000356.
18. Singh N, Rai V (2011) Isolation and Characterization of Streptomyces sp. from drug District of Chhattisgarh for Antimicrobial Activity. Curr Trends Biotechnology Pharm 5: 1043-1053.
19. Nikaido H (2003) Molecular Basis of Bacterial Outer Membrane Permeability Revisited. Microbiol Mol Biol Rev 67: 593-656.
20. El-Naggar NE, El-Shweihy NM, El-Ewasy SM (2006) Broad-Spectrum Antimicrobial Activities of Streptomyces Albidoflavus. Res J Pharm Biol Chem Sci 1: 68-78.
21. Shen B (2003) Polyketide Biosynthesis Beyond the Type I, II and III Polyketide Synthase Paradigms. Curr Opin Chem Biol 7: 285-294.
22. Ziemert N, Alanjary M, Weber T (2016) The Evolution of Genome Mining in Microbes – A Review. Nat Prod Rep 33: 988-1005.
23. Singh N, Bhange K (2024) The Growing Threat of Antimicrobial Resistance in India: Challenges and Solutions. Virol Immunol J 8: 000350-000352.
24. Neha Singh, Vibhuti Rai, Kamlesh Jain, Nirmal Verma, Sharma OP (2023) Antimicrobial Resistance and Drug Discovery. Scholars Research Library https://www.scholarsresearchlibrary.com/articles/antimicrobial-resistance-and-drug-discovery-94879.html.