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Potential Anti-Tuberculosis Metabolites from Streptomyces rochei HS2D4 Isolated from the Indian Himalayan Region

Ranjani Singaraj
Sathyabama Institute of Science and Technology
Radhakrishnan Manikkam
mrkactinos@gmail.com
Sathyabama Institute of Science and Technology
Suresh Arumugam
Meenakshi Medical College Hospital & Research Institute, Meenakshi
Kishore kumar Annamalai
Sathyabama Institute of Science and Technology
Anita Pandey
Graphic Era University

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Abstract

Actinobacteria are significant producers of bioactive compounds, and always been playground in isolation of various lead molecules. Extreme ecosystems drive the evolution of novel secondary metabolic pathways in Actinobacteria, increasing the potential for discovering new biologically active compounds.
The present study aimed to isolate and characterize Actinobacteria from underexplored Indian terrestrial soil samples, focusing on strains with antimycobacterial activity. A total of 130 actinobacterial isolates were screened, among which One potent strain, HS2D4, was identified using morphological and molecular methods. Phylogenetic analysis based on 16S rRNA sequencing placed HS2D4 as closely related (97% similarity) to *Streptomyces rochei*. Metabolite profiling was done by Gas chromatography-mass spectrometry (GC-MS), which identified 31 different bioactive compounds. In silico molecular docking of the 31 compounds assessed the interactions of key compounds with important *Mycobacterium tuberculosis* targets like DNA gyrase and InhA.
Strain HS2D4 exhibited antimycobacterial activity against *M. smegmatis* and *M. tuberculosis* H37Rv. It was shown that the crude extracts contained Key compounds including 3,5-Di-tert-butylphenol, 2,4-Di-tert-butylphenol, and TBHQ through GC-MS and Insilco molecular docking demonstrated strong binding affinities to therapeutic targets, against *Mycobacterium tuberculosis*, highlighting their potential as drug leads.
Streptomyces sp. HS2D4 isolated from Indian Himalaya Region is promising source for isolation of anti-tuberculosis agents.

Introduction

Globally, Tuberculosis (TB) is attributed to be the leading cause of death. In 2024, the World Health Organization (WHO) stated nearly 10.8 million TB cases and 1.25 million deaths, marking TB as the most fatal infectious disease post-COVID-19 (WHO Report, 2024). Standard treatment for drug-sensitive TB involves a 6-month regimen, with cure rates between 80% and 90%. However, non-adherence of the patient and the long-term therapy contribute to the development of drug-resistance among the *Mycobacterium tuberculosis* (*M.tb*) strains, also treatment of multidrug-resistant TB (MDR-TB) traditionally requires up to 24 months of chemotherapy, making the management of the disease difficult [2].

The search for antibiotics and other bioactive metabolites from microorganisms has produced a large number of lead molecules over the last fifty years, many of which have gone on to become successful medications for a range of illnesses. Actinobacteria are well-known among microorganisms for their metabolic diversity and capacity to generate a broad variety of secondary metabolites, especially among soil-dwelling taxa like Streptomyces and Micromonospora [8]. Notably, about two-thirds of the naturally derived antibiotics now utilized in clinical settings are produced by Streptomyces species. Actinobacteria, particularly Streptomyces, are indeed prolific producers of antibiotics. Recent research supports the notion that Streptomyces remains a significant source of new antibiotics; it is estimated to produce around 80% of the antibiotics derived from Actinobacteria. These bacteria have been found to produce a wide range of bioactive compounds, including antibiotics, antitumor compounds, and antiparasitic compounds [2]. Between 2015 and 2020, researchers discovered 135 new species of Streptomyces, with 108 from terrestrial environments and 27 from marine sources. These new species have been found to produce 279 new secondary metabolites with diverse biological activities [10]. Anti-TB treatment began with the discovery of streptomycin from Streptomyces griseus, which opened the door for the creation of additional anti-TB medications, including rifampicin and kanamycin, which are also derived from terrestrial actinobacteria [2].

Actinobacteria have been producing anti-tuberculosis (anti-TB) drugs in large quantities for a number of decades Nevertheless, the discovery of new antimycobacterial metabolites has noticeably decreased as a result of the recurrent isolation of recognized chemicals brought about by the thorough screening of actinobacteria from typical habitats. A promising method for finding novel bioactive chemicals with anti-TB potential has been found by investigating actinobacteria from understudied or unusual environments in order to get around restriction. This study is to investigate the potential for the production of new antimycobacterial compounds by actinobacteria from certain selected undisturbed environments in India.

Materials and Methods

Isolation of culturable actinobacteria

Soil samples were collected from three geographically and ecologically distinct parts of India as described in Table I.

Table I. Geographic Locations and Details of Soil Samples Collected for Actinobacterial Isolation

Sample type	Location	State	Latitude	Longitude	No of samples
Soil	Baramulla	Kashmir	34.3711° N,	74.37694° E	2
Soil	Maibam Lokpa	Manipur	24.703° N,	93.817° E	1
Soil	Charoipandongba		24.665541° N,	93.720612° E	1
Soil	Angaan Ching		24.455618° N,	93.935410° E	1
Insect nest	Kanchipuram	Tamil Nadu	12°36'58 ° N	79°45'11° E	1

Soil samples were collected and processed according to Selim et.al. (2021). Culturable actinobacteria from the collected samples were isolated by the dilution and plating method. Two sets of Starch Casein Agar (SCA), Actinobacteria Isolation Agar (AIA), and Yeast Extract Malt Extract (YEME) Agar plates were prepared. A hundred µL aliquot of diluted soil sample from 10^{-3} to 10^{-5} dilutions was plated on all the media and spread using a sterile L-rod. Plating was done in triplicate, and all the plates were incubated at 28°C for 4 weeks. Colonies with actinobacterial morphology were recovered upon incubation using YEME agar plates at 28°C for 7–10 days. Morphologically distinct cultures were selected [11].

Determination of morphological characteristics of actinobacteria

All the actinobacterial isolates were grown on ISP2 media and incubated for 10 days at 28°C. After incubation, cultural features such as growth rate, colony consistency, texture, aerial mass color, reverse side pigment, and soluble pigment production were recorded. The slide culture method was used to perform micromorphological observations. [33].

In vitro evaluation of actinobacteria for anti-mycobacterial activity using M. smegmatis as a surrogate

The antimycobacterial activity of actinobacterial cultures was evaluated using the agar plug method according to Radhakrishnan et al. (2014). The antimycobacterial activity was measured by determining the diameter of the zone of inhibition around the agar plugs. In addition to the isolated cultures, 35 actinobacterial cultures isolated from the Indian Himalaya region were also screened for anti-*M. smegmatis* activity.

Preparation of extract libraries for potential actinobacterial cultures by liquid and solid-state fermentation

Bioactive metabolites from selected actinobacterial cultures (n = 14) that showed good anti-smegmatis activity in the agar plug assay was produced by adopting agar surface and submerged fermentation using YEME medium. Actinobacterial cultures were grown for 10 days at 28°C on YEME agar plates for agar surface fermentation and inoculated in liquid broth media for submerged fermentation. Bioactive metabolites from the YEME agar plates as well as from YEME broth was extracted using equal volume of ethyl acetate by adopting solid – liquid extraction and liquid-liquid extraction, respectively [4] Each 10 mg of all the crude extracts were dissolved in 10% sterile DMSO to get 10mg/ml stock concentration and filtered through 0.45 µm syringe filter to remove any debris and used for further activity testing.

Evaluation of actinobacterial extracts for antimycobacterial activity

Drugs and extracts

Rifampicin (10 mg/mL) and isoniazid (20 mg/mL), standard drugs, were purchased from Sigma-Aldrich (USA). actinobacterial crude extracts (50 mg/mL) were dissolved in 10% dimethyl sulfoxide (DMSO) [7].

Mycobacterial strains

M. smegmatis and *M. tuberculosis* H37Rv strains were obtained from the National Institute for Research in Tuberculosis (NIRT) Department of Bacteriology. The strains were cultured on Lowenstein-Jensen (LJ) medium (Himedia, SL001) at 37 ± 2°C. Cultures were grown to a density of approximately 10^8 CFU/mL and stored in 1 mL aliquots with 20% glycerol at -80°C. the cultures were reconstituted in Middlebrook 7H9 broth supplemented with 0.05% Tween 80, 0.2% glycerol, and 10% albumin-dextrose-catalase (ADC). The cultures were incubated at 37°C, with *M. smegmatis* requiring 3 days of pre-incubation and *M. tuberculosis* requiring 8–10 days of revival before use in assays [8].

Microplate Alamar Blue Assay (MABA)

The antimycobacterial potential of the extracts was assessed against *M. smegmatis* and *M. tuberculosis* H37Rv using the Microplate Alamar Blue Assay (MABA). The assay was performed in 96-well microtiter plates, with each well containing Middlebrook 7H9 broth supplemented with glycerol and albumin-dextrose-catalase. The test extracts were added at a concentration of 10 mg/mL, and bacterial suspensions were adjusted to an optical density (OD□□□) of 0.5. Controls included solvent (10% DMSO), growth control, and rifampicin as a drug control. The optical density was measured at 600 nm to evaluate the antimycobacterial activity of the extracts [26].

Selection and characterization of the potential actinobacterial strain

Based on the above experiments and screening, actinobacterial strain HS2D4, isolated from the Indian Himalaya Region, was selected as the potential strain for further studies.

Cultural and morphological characteristics

Cultural characteristics were studied by inoculating strain HS2D4 on different media such as such as Actinomycete agar (AIA), Starch casein agar (SCA), Nutrient Agar and into different ISP (International Streptomyces Project) media such as Tryptone agar (ISP1), Yeast extract- malt extract agar (ISP2), oatmeal agar (ISP3), Inorganic salts-starch agar (ISP4), glycerol-asparagine agar (ISP5), Peptone yeast extract iron agar (ISP6) and tyrosine agar (ISP7) (Shirling and Gottlieb, 1996). All the culture plates were incubated for 10 days at 28°C. Cultural properties such as the rate of growth, consistency, aerial mass colour, presence of reverse side pigment, and the soluble pigment production was observed [20]. The culture was observed under microscope to witness the hyphal and colony morphology.

Molecular identification through 16s rRNA sequencing

Genomic DNA was isolated from the fresh culture of HS2D4 by Cetyltrimethylammonium Bromide (CTAB) method (Gunawardana et al., 2024). Using the universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′), the 16S rRNA gene from actinobacterial isolates was amplified [6]. Following purification using the Qiagen Gel Extraction Kit, PCR products were validated using agarose gel electrophoresis, the purified amplicons were sequenced.

The BLASTn technique was used to compare the actinobacterial isolates 16S rRNA gene sequences with those found in the NCBI GenBank database in order to ascertain their phylogenetic affiliation [25]. Sequences that were closely linked were retrieved for tree construction and alignment. ClustalW, which is a feature of the MEGA X software, was used to do multiple sequence alignment [17]. To assess the robustness of the tree topology, phylogenetic trees were built with 1,000 bootstrap replications utilizing the neighbor-joining (NJ) approach [32]. The resulting phylogenetic relationships shed light on the isolates' taxonomic placements and evolutionary distances.

Production and evaluation of antimycobacterial metabolites from the actinobacterium HS2D4

Optimization of media by One-Factor-at-a-Time (OFAT) method

The traditional One-Factor-at-a-Time (OFAT) method, which modifies one variable while maintaining all other factors constant, was used to optimize the fermentation conditions for the actinobacterial strain HS2D4. To ascertain their ideal values, subsequent optimization entailed adjusting the amounts of important medium ingredients, such as pH, mineral salts, and carbon and nitrogen sources, separately [30]. Cell-free supernatant from ideally grown cultures was measured for activity to determine the efficacy of each condition. Following adequate development, 100 µL of the supernatant was taken out for each OFAT experiment was tested for anti *M. smegmatis* activity by well diffusion method [23]

Antimycobacterial metabolite production using optimized media

Antimycobacterial metabolites from the strain HS2D4 was produced using optimized medium components consist of Sucrose (4gm/l), yeast extract(10gm/l), calcium chloride (0.1gm/l) at pH 7. After 120 hours of incubation, the fermentation broth was collected and centrifuged at 10,000 rpm for 20 minutes to collect the cell-free supernatant. Extracellular metabolites from the cell-free supernatant were extracted by liquid-liquid extraction using an equal volume of ethyl acetate. The ethyl acetate portion was collected and concentrated using a rotary evaporator and concentrator plus and then quantified. Minimal inhibitory concentration of ethyl acetate extract was tested against *M. smegmatis* and *M. tuberculosis* H37Rv using MABA at 200–50 µg/ml concentrations [26].

Metabolite profiling of antimycobacterial extract by GC MS analysis

The actinobacterial strain HS2D4 showed strong antimycobacterial potential, and its secondary metabolites were profiled using Gas Chromatography–Mass Spectrometry (GC–MS). A Shimadzu GC-MS-QP2010 equipped with an RTX-5MS fused silica capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) was used for the analysis. The carrier gas was helium, which flowed at a steady rate of 1.0 mL/min. After two minutes at 60°C, the oven temperature was ramped up to 300°C at a rate of 10°C per minute, with a final hold of 10 minutes. Ionization was accomplished by electron impact at 70 eV after a 1 µL sample was injected in split mode (10:1) [23]

In silico analysis selected compounds from HS2D4 against M. tuberculosis targets

Ligand Optimization and Drug-Likeness Screening Based on Lipinski’s Rule

Based on the results of GC-MS profiling, the molecular structure of 31 compounds was obtained from PubChem in 2D SDF format. The MMFF94 force field in OpenBabel, which is renowned for its efficient geometry optimization, including electrostatic and hydrogen-bonding characteristics, was used to transform them into optimal 3D structures. For docking tool compatibility, the energy-minimized ligands were stored in PDB format. Compounds that complied with Lipinski's Rule of Five were then chosen for docking after their physicochemical characteristics—molecular weight, hydrogen bond donors/acceptors, and log P—were evaluated [1].

In Silico Optimization and Binding Site Mapping of Essential Tuberculosis Enzymes for Ligand Interaction Studies

Due to their crucial roles in *M. tuberculosis* survival and virulence, three essential bacterial enzymes—DNA gyrase (PDB ID: 3M4I), InhA (PDB ID: 2H7M), and decaprenyl phosphoryl-β-D-ribose 2′-epimerase (DprE1)—were chosen as molecular targets (Batt et al., 2020; Sharma et al., 2021). The RCSB Protein Data Bank provided the three-dimensional crystal structures of these enzymes. Using Biovia Discovery Studio Visualizer 2021, protein preparation was carried out by removing water molecules and co-crystallized ligands, adding hydrogen atoms, and modeling any missing loops or residues. To guarantee stability and accuracy in docking simulations, the structures were additionally exposed to energy minimization using the CHARMM force field. To guarantee biologically appropriate docking areas, the active sites were determined using crystallographic ligand coordinates and prior research [30].

Molecular Interaction Analysis of Actinobacterial Ligands with Tuberculosis Targets

Molecular docking was performed in ArgusLab 4.0.1 (ArgusDock), using rigid proteins and fully flexible ligands within a 3D grid focused on the predefined active site. For each ligand, up to 150 poses were generated and scored with AScore, and the lowest binding free energy conformation was selected. Top poses were then analyzed in Biovia Discovery Studio Visualizer 2021 to characterize hydrogen bonds, hydrophobic contacts, π–π stacking, nearby residues within 5 Å, and overall binding geometry

Results

Isolation and characterization of actinobacteria

Soil samples were taken from three different parts of India, yielding a total of 95 actinobacterial isolates. Good growth was demonstrated by the isolates, which formed powdery colonies with different colored aerial mycelia. Moreover, a few of the isolates had characteristic reverse-side coloring. Both aerial (reproductive) and substrate (vegetative) mycelium were found in all cultures by bright-field microscopic analysis. The genus Streptomyces seems to be the most prevalent group, and the majority of the isolates shared physical characteristics with its members.

In vitro evaluation of actinobacteria for anti-mycobacterial activity using M. smegmatis as a surrogate

All 130 actinobacterial isolates were checked for antimycobacterial activity using the agar plug diffusion assay against *M. smegmatis* as part of the first screening process. Of these, 23 isolates showed distinct zones of inhibition, suggesting that they may have antimycobacterial properties. The diameter of the zone of inhibition varied between 10 and 25 mm. In total, about 18% of the isolates that were evaluated showed activity against *M. smegmatis*. 14 isolates were chosen for secondary screening based on the size of the inhibitory zone, which measured between 20 and 30 mm.

Preparation of extract libraries for potential actinobacterial cultures by liquid and solid-state fermentation and antimycobacterial activity against M. smegmatis and M. tuberculosis

Using both liquid-state and solid-state fermentation techniques, crude extracts were isolated from 14 actinobacterial strains. The Microplate Alamar Blue Assay (MABA) was used to evaluate the antimycobacterial activity against *M. smegmatis* and *M. tuberculosis* H37Rv. Three concentrations in triplicate were used for screening: 50 µg, 100 µg, and 200 µg per well. The most potent strain, HS2D4, was selected based on the percentage of inhibition, which was isolated from the Indian Himalaya Region. It showed more than 85% inhibition against *M. smegmatis* and *M. tuberculosis* H37Rv at all concentrations (Figure I). Remarkably, this strain's liquid and solid extracts both demonstrated strong inhibitory action, indicating that the bioactive metabolites were generated in both fermentation scenarios

Figure I. Anti-mycobacterial activity of HS2D4 extracts isolated both from solid state and liquid state fermentation. a: antimycobacterial activity against *M. smegmatis*; b: *anti tb activity against M.tb H37Rv*

Molecular characterization of actinobacterial strain HS2D4

The PCR amplification of 16s rRNA gene of actinomycete strain HS2D4 yielded around 1400 base pair sequence. The BLAST analysis showed 99.51% similarity to Streptomyces rochei strain NRRL B- 1559. Phylogenetic relationship of the strain HS2D4 and related taxa are given in Figure II. The 16s rRNA gene sequence of Strain HS2D4 is submitted to GenBank under the accession number SUB15585234 HS24

Figure II: Phylogenetic relationship of the strain HS2D4 and related taxa, based on 16S rDNA analysis. Substitution per 100 nucleotide positions

Optimization of the medium through OFAT for bioactive molecule production of Actinobacterial strain HS2D4

Antimycobacterial metabolites from the strain HS2D4 was produced using optimized medium components consist of Mann

itol (4gm/l), yeast extract(10gm/l), calcium chloride (0.1gm/l) at pH. This imply that actinobacteria have differential ability to synthesize bioactive metabolites under different nutritional conditions. (fig III). Minimum Inhibitory Concentration was checked for the crude extract isolated from the optimized media by MABA at different concentrations and it was observed that at 50 µg/ml the growth of *M. tuberculosis* and *M. smegmatis* was inhibited

Figure. III: Effect of different components of media on antimycobacterial property of HS2D4 against *M. smegmatis*: a-Carbon, b-Nitrogen, c- Mineral, d- pH

Identification of Bioactive Compounds by GC-MS Analysis

Significant binding affinities toward DNA gyrase and InhA, two important *M. tuberculosis* target enzymes, were demonstrated by 31 compounds found by GC-MS, indicating possible inhibitory effects. To assess the interaction patterns of particular ligands with these proteins' active sites, molecular docking analysis was used. 3,5-Di-tert-butylphenol showed the best binding interaction with DNA gyrase (PDB ID: 3M4I) among the compounds on the shortlist, with a binding energy of -11.1122 kcal/mol. In addition to many hydrophobic contacts that together maintained its orientation within the active site cleft, this molecule established a strong hydrogen bond with the catalytically crucial residue HIS539 (bond distance: 2.032 Å). These interactions point to the compound's potential as a lead contender for additional development by indicating a strong and specific binding conformation. Positive docking scores and interactions with InhA (enoyl-ACP reductase) were also shown by other drugs, suggesting the possibility of dual-targeting. Table II provides a summary of the extensive docking profiles and interaction maps for the most active chemicals.

Table II: Interaction Summary of Lead Molecules with DNA Gyrase and InhA

Compound	Target	H-Bond Residues	Binding Energy (kcal/mol)
2,4-Di-tert-butylphenol	DNA gyrase **E. coli**	50TYR	-9.73692 kcal/mol
2,4-Di-tert-butylphenol	InhA (Enoyl-[acyl-carrier-protein] reductase [NADH)	96GLY; 118LYS; 126SER; 64ASP; GLN66; 63LEU; 67ASN	-11.4394 kcal/mol
2,4 -Di-tert-butylphenol	DNA gyrase MTB 3M4I	539HIS	-11.1122 kcal/mol
tert-Butylhydroquinone (TBHQ)	InhA	66GLN; 65VAL; 14GLY; 39THR	-10.181 kcal/mol
tert-Butylhydroquinone (TBHQ)	DNA gyrase	219THR; 268VAL; 267GLN; 91ARG; 97SER	-9.742 kcal/mol

Table III: Interaction Summary of Lead Molecules with DNA Gyrase and InhA

Compound	Target	H-Bond Residues
2,4-Di-tert-butylphenol	DNA gyrase ***E. coli***	50TYR
2,4-Di-tert-butylphenol	InhA (Enoyl-[acyl-carrier-protein] reductase [NADH)	96GLY; 118LYS; 126SER; 64ASP; GLN66; 63
2,4 -Di-tert-butylphenol	DNA gyrase MTB 3M4I	539HIS
tert-Butylhydroquinone (TBHQ)	InhA	66GLN; 65VAL; 14GLY; 39THR
tert-Butylhydroquinone (TBHQ)	DNA gyrase	219THR; 268VAL; 267GLN; 91ARG; 97SER

Molecular Docking Insights into InhA and DNA Gyrase Inhibition

2,4-Di-tert-butylphenol showed strong affinity for InhA (ΔG − 11.44 kcal/mol), forming multiple hydrogen bonds with key active-site residues, indicating high inhibitory potential on this essential enzyme in the mycolic acid pathway. Tert-butylhydroquinone (TBHQ) also bound efficiently to InhA (ΔG − 10.18 kcal/mol) and interacted favorably with DNA gyrase (ΔG − 9.74 kcal/mol), supported by hydrogen bonds and hydrophobic contacts with catalytic residues, suggesting dual inhibition of DNA replication and cell wall biosynthesis

Binding Affinity and Multi-Target Potential of GC-MS-Derived Compounds

2,4-Di-tert-butylphenol showed moderate affinity for DNA gyrase (ΔG − 9.74 kcal/mol) and formed a key hydrogen bond with TYR50, supporting its role as a potential multi-target inhibitor. Elemol and Cyclo(L-prolyl-L-valine) displayed weaker binding (ΔG − 7.5 to − 8.5 kcal/mol), driven mainly by hydrophobic interactions and lacking strong, directed hydrogen bonds, suggesting lower selectivity and stability. No meaningful hydrogen bonding was observed for most ligands with DprE1, whereas 3,5-Di-tert-butylphenol, 2,4-Di-tert-butylphenol, and TBHQ bound strongly to DNA gyrase and InhA, highlighting them as promising lead candidates.

Computational Assessment of Actinobacterial Metabolites Targeting M. tuberculosis

There is strong evidence from the in silico molecular docking investigation that several metabolites from the potent actinobacterial strain HS2D4 have a high binding affinity for important *M. tuberculosis* targets. Specifically, tert-Butylhydroquinone (TBHQ), 2,4-Di-tert-butylphenol, and 3,5-Di-tert-butylphenol showed strong interactions with catalytically necessary residues in DNA gyrase and InhA active sites, indicating their potential as lead compounds for anti-TB medication development (Fig IV).

Figure IV: Hydrophobic interactions of **a**: Interaction between 2,4-Di-tert-butylphenol and DNA gyrase E. coli; **b**: Interaction between 2,4-Di-tert-butylphenol and InhA; **c**: Interaction between 2,4-Di-tert-butylphenol and DNA gyrase MTB; **d**: Interaction between TBHQ and DNA gyrase; **e**: Interaction between TBHQ and InhA

Discussion

Actinobacteria from ecologically distinct regions such as the Indian Himalaya show marked morphological diversity and adaptation to harsh conditions, with strong capacity to produce antimicrobial metabolites and industrial enzymes. Several studies report broad-spectrum antibacterial activities from such isolates, and notably, Hussain et al. demonstrated that 10 of 26 actinobacterial strains produced metabolites active against *Mycobacterium tuberculosis* H37Rv. In the present work, 23 of 130 isolates from lesser-known ecosystems inhibited *M. smegmatis*, and the Himalayan strain HS2D4 showed 85% inhibition at 50 µg against both *M. smegmatis* and *M. tuberculosis*.

Nutrient composition strongly influenced secondary metabolite production by HS2D4, with mannitol, yeast extract, and CaCl supporting maximal antimycobacterial activity and neutral pH 7 giving the largest inhibition zones, in agreement with previous optimization reports for other *Streptomyces* strains. These components were therefore used to formulate an optimized medium for larger-scale metabolite production. GC-MS profiling of HS2D4 extract revealed 31 compounds spanning phenolics, fatty acid derivatives, aromatic compounds, diketopiperazines, and other classes, consistent with reports that Himalayan actinomycetes yield chemically diverse antimycobacterial metabolites.

From these, 2,4-di-tert-butylphenol, 3,5-di-tert-butylphenol, TBHQ, and Elemol were prioritized based on known antimicrobial, antioxidant, and anti-inflammatory properties, and were docked against DNA gyrase and InhA, two validated *M. tuberculosis* drug targets. Docking showed particularly strong and dual inhibitory potential for 2,4-di-tert-butylphenol and TBHQ, which interacted favorably with residues in both enzymes, suggesting simultaneous disruption of DNA replication and cell wall biosynthesis. Overall, the data support Himalayan actinobacteria, especially HS2D4, as a promising source of new antimycobacterial leads, warranting further bioassays, SAR work, and optimization of these metabolites for drug development.

Conclusion

The study aims to summarize the biodiversity and biosynthesis of novel secondary metabolite compounds of the phylum Actinobacteria and the diverse range of secondary metabolites produced. Screening and exploring the bioactivity of actinobacteria isolated from extreme environments and ecosystems led us to identify an actinobacterial strain HS2D4 with potent antimycobacterial activity against *M. smegmatis* and *M. tuberculosis* sp. Therefore, it is evident that Actinobacteria adapted to survive in extreme environments represent an important source of a wide range of bioactive compounds.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Ethics approval

This is an observational study. The Research Ethics Committee has confirmed that no ethical approval is required.

Consent to publish

Not Applicable

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ranjani Singaraj, Radhakrishnan Manikkam and Kishore Kumar Annamalai, Anita Pandey, Arumugam Suresh. The first draft of the manuscript was written by Ranjani Singaraj and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

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Data Availability

“The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. The 16S rRNA gene sequence of strain HS2D4 has been deposited in GenBank under accession number SUB15585234.”

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Figures

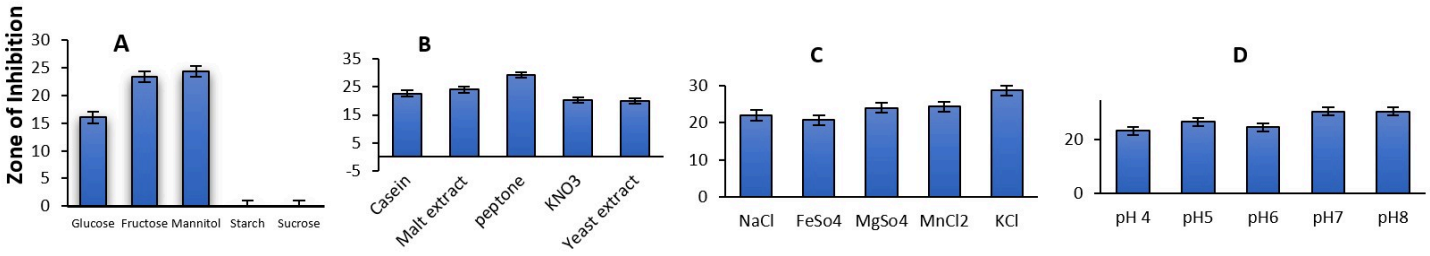


Figure 1

Effect of different components of media on antimycobacterial property of HS2D4 against *M. smegmatis:* A-Carbon, B- Nitrogen, C-Mineral, D- pH

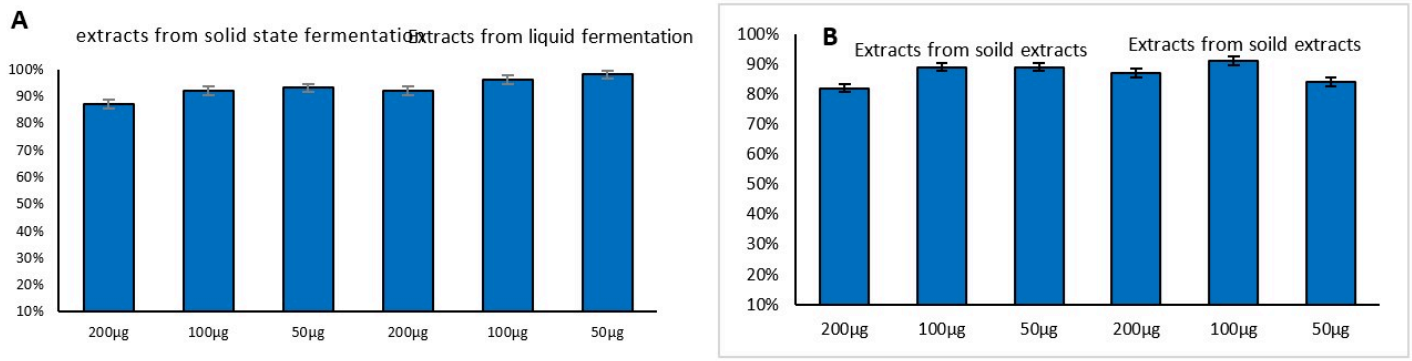


Figure 2

Anti-mycobacterial activity of HS2D4 extracts isolated both from solid state and liquid state fermentation. A: antimycobacterial activity against *M.smegmatis*; B*: anti tb activity against M.tb H37Rv*



Figure 3

Growth of strain HS2D4 on ISP2 agar medium

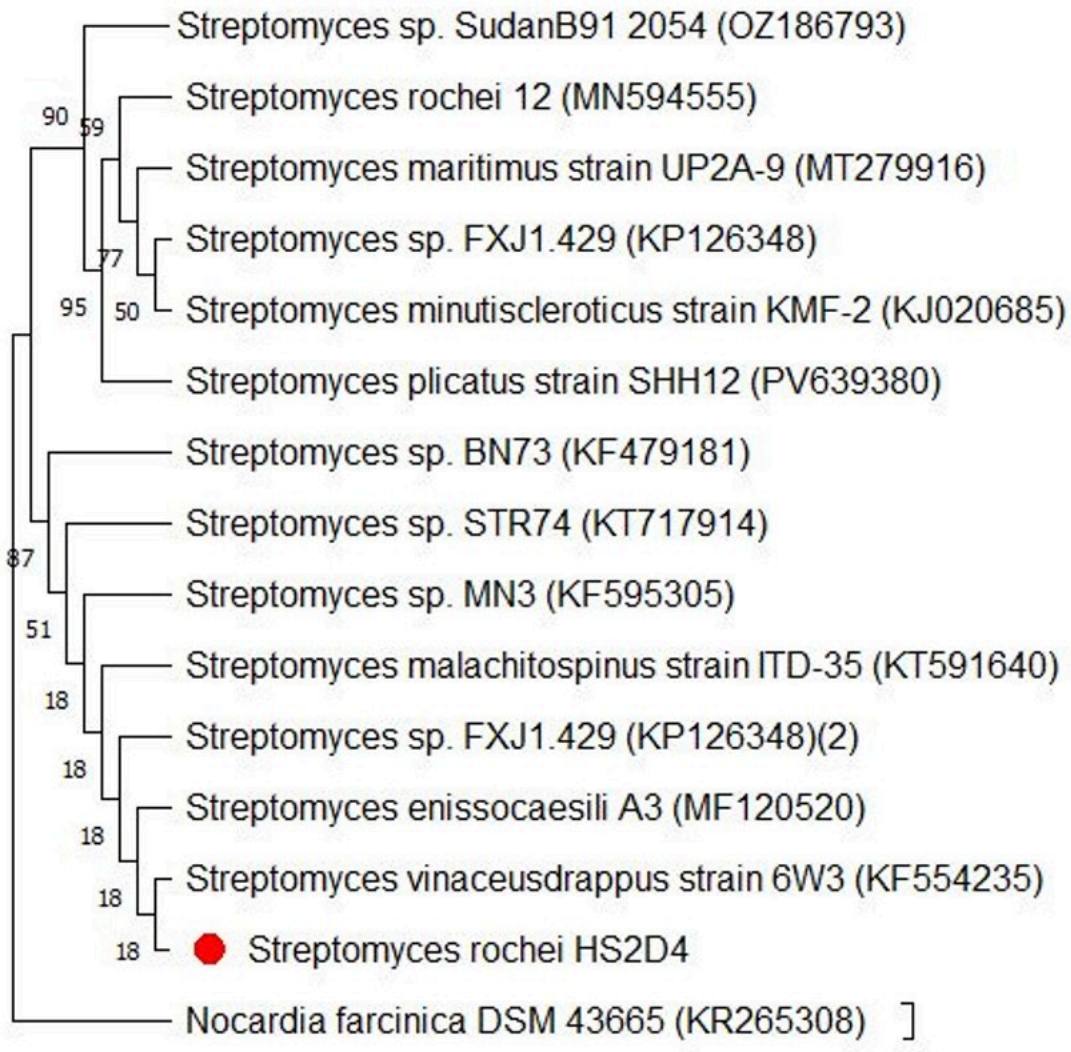


Figure 4

Phylogenetic relationship of the strain HS2D4 and related taxa, based on 16S rDNA analysis. Substitution per 100 nucleotide positions.

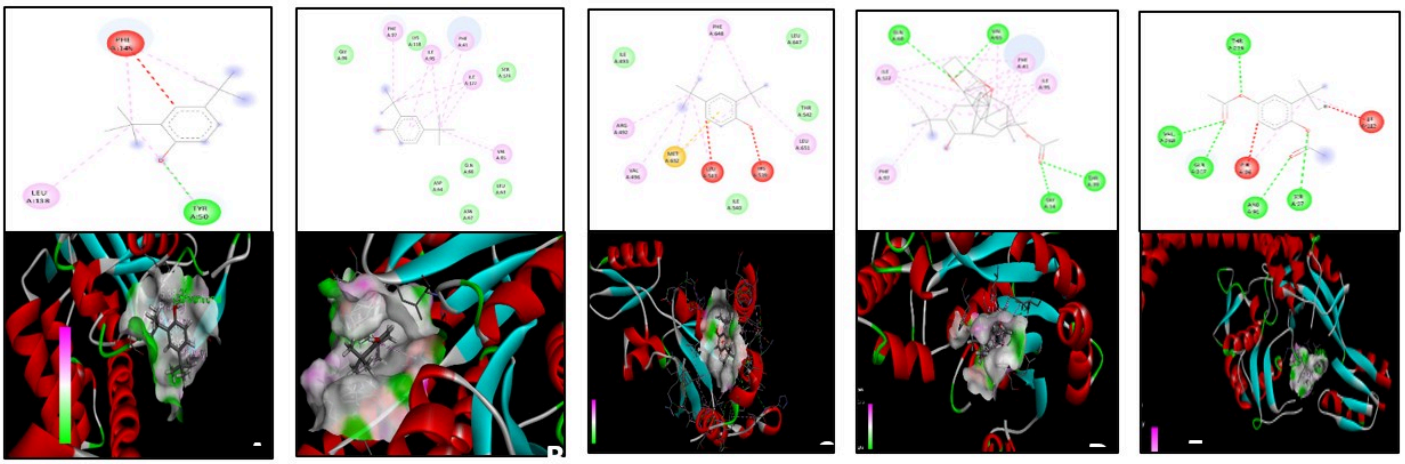


Figure 5

Hydrophobic interactions of **A**: Interaction between 2,4-Di-tert-butylphenol and DNA gyrase E. coli ; **B**: Interaction between 2,4-Di-tert-butylphenol and InhA; **C**: Interaction between 2,4-Di-tert-butylphenol and DNA gyrase MTB; **D**: Interaction between TBHQ and DNA

gyrase; **E**: Interaction between TBHQ and InhA