World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Screening, characterization, and phylogenetic analysis of Methioninase-Producing Indigenous *Streptomyces* spp. Isolated from the Upper Lake of Bhopal, Madhya Pradesh, India

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World Journal of Biology Pharmacy and Health Sciences, 2025, 22(02), 290-300

Publication history: Received on 06 April 2025; revised on 11 May 2025; accepted on 13 May 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.22.2.0493

Abstract

Methioninase, an enzyme with potential for cancer treatment, catalyzes the degradation of methionine, which supports tumor growth and metastasis. *Streptomyces* species, known for their secondary metabolite production, are prime candidates for methioninase production. This study aimed to isolate and screen indigenous *Streptomyces* strains from aquatic environments around the Upper Lake of Bhopal, India, to identify those with methioninase activity. Samples were collected from five locations, and *Streptomyces* spp. were isolated using starch casein agar and screened with a phenol red-based assay for methioninase production. Seven out of 20 isolates exhibited positive methioninase activity. Molecular identification through 16S rRNA sequencing confirmed the identity of these isolates, revealing genetic diversity among the methioninase-producing strains. Phylogenetic analysis further supported the distinctiveness of these strains. The study emphasizes the promising potential of these isolates for enzyme-based cancer therapies and contributes to bioprospecting efforts for novel therapeutic agents. The isolated *Streptomyces* spp. from the Upper Lake of Bhopal exhibit potent methioninase activity, highlighting their potential for enzyme therapy, particularly in cancer treatment. The molecular characterization of these isolates provides a foundation for further research into optimizing methioninase production. These findings contribute significantly to the growing interest in microbial enzymes as therapeutic agents, particularly in the context of cancer therapy.

Keywords: L-Methioninase; *Streptomyces* spp.; Cancer Therapy; Microbial Diversity Phylogeny

1. Introduction

Methioninase is a vital enzyme with significant therapeutic potential, particularly for cancer treatment, owing to its ability to degrade methionine, an amino acid that promotes tumor growth and metastasis (Zhang et al., 2017). The growing interest in methioninase for enzyme therapy in treating methionine-dependent cancers has driven research into optimizing its production (Tan et al., 2019). *Streptomyces* spp., known for their ability to produce a wide range of secondary metabolites, are promising candidates for such studies, especially from diverse ecological environments (Shirling & Gottlieb, 1966).

This study focuses on isolating and screening indigenous *Streptomyces* strains from various locations around the Upper Lake of Bhopal, Madhya Pradesh, India. As one of Asia's largest artificial lakes, the Upper Lake offers a unique ecosystem with diverse microbial populations, making it a prime source for discovering novel actinobacterial species (Rai et al., 2021). Previous studies have shown that aquatic soil and sediment samples are rich in *Streptomyces* spp. with enzymatic activities, including methioninase production (El-Naggar & Eldin, 2020).

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The main objective of this research is to explore the phylogenetic relationships among methioninase-producing *Streptomyces* spp. isolated from the Upper Lake of Bhopal, M.P., India. Screening, isolation, and identification of strains with potent methioninase activity will pave the way for potential cancer therapy applications, contributing to the growing body of knowledge in microbial bioprospecting for valuable enzymatic properties.

2. Materials and Methods

2.1. Isolation & Screening of *Streptomyces* Species

2.1.1. Sample Collection

Marshy soil mixed water samples were collected from five locations around the Upper Lake of Bhopal, India, during the sampling period from August 2022 to July 2023. Pre-sterilized glass bottles were used for sample collection, and precautions were taken to avoid contamination. Each sample was assigned a code and stored at 4-8°C in the laboratory. The different sampling points are mentioned in table 1.

Table 1 The different points of soil mixed water sampling around Upper Lake of Bhopal

S. No.	Sampling Sites	Water Sample	Sample Code	
			1st Collection	2nd Collection
1.	Van Vihar	Turbid and muddy water	S1	S6
2.	Boat Club	Turbid and muddy water	S2	S7
3.	Kamla Park	Turbid and muddy water	S3	S8
4.	Kaliasot Dam	Turbid and muddy water	S4	S9
5.	Bairagarh	Turbid and muddy water	S5	S10

2.2. Isolation of *Streptomyces* Species

Starch casein agar (M801 HiMedia) was used for isolating *Streptomyces* spp. from the samples, followed by sub-culturing on inorganic salt starch agar (ISSA) for pure cultures. Samples were serially diluted and inoculated onto agar plates using the spread plate method, followed by incubation at 30°C for 8-10 days. The composition of the media is mentioned in table 2. Colonies were enumerated using a digital colony counter, and pure cultures were prepared through repeated sub-culturing (Shirling & Gottlieb, 1966).

Table 2 Composition of starch casein agar media (M801 HiMedia)

S.No.	Ingredients	Quantity in Grams/Litre
1.	Soluble starch	10.00
2.	Casein (Vitamin Free)	0.30
3.	KNO_3	2.00
4.	$MgSO_4.7H_2O$	0.05
5.	K_2HPO_4	2.00
6.	NaCl	2.00
7.	CaCO3	0.02
8.	$FeSO_4.7H_2O$	0.01
9.	Agar	18.00
10.	Distilled water	1000

All ingredients homogenised in per litre distilled water then sterilized and poured in sterile plates

2.3. Rapid Assay for Methioninase *Streptomyces* spp.

Indigenous *Streptomyces* spp. isolates were screened for methioninase activity using a phenol red-based rapid assay. Starch agar plates (pH 7.0) with 0.07% phenol red and methionine, the composition of which is mentioned in Table 3. were inoculated and incubated at 28°C for 48-72 hours. Methioninase-positive isolates caused a pink coloration around colonies, indicating ammonia release from methionine degradation. This method, adapted from Arfi et al. (2003) and Selim et al. (2015), was confirmed using modified media per William and Hariharan (2013).

Table 3 Composition of Starch agar with 0.07% phenol red and 0.5% L-methionine

S.No.	Ingredients	Quantity in Grams/Litre
1.	Meat Extract	40
2.	Soluble Starch	10
3.	Methionine	5
4.	Phenol red indicator	0.7
5.	Agar	15
6.	pH	7±0.2

All ingredients homogenised in per litre distilled water then sterilized and poured in sterile plates; Later, the methioninase +ve *Streptomyces* spp. isolates will be then subjected to molecular studies for sequencing of 16S rRNA for identification of *Streptomyces* spp. species.

2.4. Molecular Identification of *Streptomyces* spp.

2.4.1. DNA Extraction

The *Streptomyces* spp. isolates positive for methioninase activity were identified using 16S rRNA gene analysis. Genomic DNA was extracted using a modified CTAB method (Kieser et al., 2000; Sambrook & Russell, 2001). Isolates were cultured on starch nitrate agar at 28°C for 5-7 days, transferred to broth, and incubated with shaking for 48-72 hours. Cell pellets were harvested, washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and treated with 10 mg/mL lysozyme. Lysis was performed using 10% SDS and prewarmed CTAB buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 0.5% β-mercaptoethanol, pH 8.0). DNA was extracted using chloroform:isoamyl alcohol (24:1, v/v), treated with RNase A (10 mg/mL), and precipitated with isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and dissolved in TE buffer. Quality was confirmed via 0.8% agarose gel, and DNA was used for 16S rRNA amplification.

2.4.2. Amplification of 16S rDNA and Phylogenetic Analysis

The 16S rRNA gene from *Streptomyces* spp. isolates was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') procured from BioServe Biotechnologies. The PCR reaction mixture included 2 µL genomic DNA, 2 µL primers, 5 µL 10X assay buffer, 5 µL $MgCl_2$ (25 mM), 5 µL dNTP mix (2.5 mM), 0.5 µL Taq DNA polymerase, and 30.5 µL molecular-grade water, forming a 50 µL volume. PCR was conducted in a Prima-96 Thermal Cycler with conditions: initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 45°C for 1 minute, and 72°C for 30 seconds, followed by final extension at 72°C for 10 minutes. Amplified products were confirmed on 1% agarose gel. Partial sequencing was outsourced to Biokart, Bangalore, and analyzed via NCBI BLAST. Phylogenetic analysis was performed using MEGA 11 with CLUSTAL W alignment to construct a phylogenetic tree.

3. Results and Discussion

3.1. Enumeration of Target Microbial Species

The CFU count from various soil samples diluted at 10^{-7} (see Table 4) demonstrates significant microbial diversity, with Kamla Park (348 CFU) having the highest microbial presence, followed by Kaliasot Dam (199 CFU) and Boat Club (157 CFU). The lowest counts were observed at Bairagarh (104 CFU) and Van Vihar National Park (15 CFU). These findings suggest that nutrient-rich environments, such as parks and water-proximal areas, support a higher prevalence of potential methioninase-producing *Streptomyces* spp., consistent with their role in anticancer enzyme production. Such areas, often rich in organic matter, provide ideal conditions for microbial proliferation. Similar patterns have been noted in studies indicating that organic content influences microbial community structures and enzyme production potential

(Xu *et al.,* 2020; Arfi *et al.,* 2003). This reinforces the suitability of these environments for isolating *Streptomyces* with biotechnological applications.

Table 4 Culture response on primary culture plates

S. No.	Master Plate Code	Sample Dilution Used	CFU Count
1.	A	Van Vihar National Park	15
2.	B	Boat Club	36
3.	C	Kamla Park	348
4.	D	Kaliasot Dam	199
5.	E	Bairagarh	104
6.	F	Van Vihar National Park	117
7.	G	Boat Club	157
8.	H	Kamla Park	259
9.	I	Kaliasot Dam	11
10.	J	Bairagarh	53

3.2. Confirmation of Methioninase Production

The screening of 20 *Streptomyces* spp. isolates through rapid assay for methioninase production revealed that only seven isolates (S2, S3, S6, S7, S9, S13, and S17) exhibited positive activity, while the remaining 13 isolates were negative in present study (see Table 5). This result aligns with studies by Selim et al. (2015), who noted variability in methioninase activity among *Streptomyces* isolates, with only 30% demonstrating extracellular enzyme production. Similarly, Peela and Porana, (2017) emphasized that methioninase production is strain-specific and often influenced by environmental and cultural factors. Furthermore, findings by El-Sayed, et al. (2010) highlight the importance of targeted isolation strategies to identify potent methioninase producers for therapeutic applications. This study underscores the potential of selected isolates for further optimization and molecular characterization to enhance methioninase production, a promising enzyme for applications such as cancer therapy.

Table 5 Response of pure indigenous *Streptomyces* spp. isolates for methioninase production activity

S. No.	Isolate Code	Methioninase production activity
1.	S1	–Ve
2.	S2	+Ve
3.	S3	+Ve
4.	S4	–Ve
5.	S5	–Ve
6.	S6	+Ve
7.	S7	+Ve
8.	S8	–Ve
9.	S9	+Ve
10.	S10	–Ve
11.	S11	–Ve
12.	S12	–Ve
13.	S13	+Ve

14.	S14	–Ve
15.	S15	–Ve
16.	S16	–Ve
17.	S17	+Ve
18.	S18	–Ve
19.	S19	–Ve
20.	S20	–Ve

3.3. Molecular Identification and Phylogenetic Analysis

The 16S rRNA region of indigenous *Streptomyces* spp. isolates (S2, S3, S6, S7, S9, S13, and S17) was successfully amplified, producing a sharp 1500 bp band on 1% agarose gel. These amplified fragments were then subjected to partial genome sequencing using Sanger's method, with sequencing services provided by Biokart India Pvt. Ltd., Bangalore. The resulting sequences were analyzed for homology and identity using the NCBI BLAST tool, and the results are summarized in Table 6 and Table 7.

Table 6 The 16S rRNA sequences of *Streptomyces* spp. isolates obtained after partial Sanger's sequencing

S. No.	Isolate Code	Sequencing ID	Obtained Sequence
1.	S2	>_S2_2023_27F_F01.ab1	GGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGC CTATCAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCG ACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG CAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGC AGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTAC CTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCC GCGGTAATACGTAGGGCGCAAGCGTTGTCCAAAATTAT TGGGCGTAAAGAGCTCGTAGGCGGTTTGTCACGTCGGT TGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGA TACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAAT TCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGA ACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGA CGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTA GGTGTGGGCAACATTCCCCGTTGTCCGTGCCGCAGCTA ACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC GGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAA CCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAG ACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCAT GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG TCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGC AGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCG GGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTC ATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACA ATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAG CGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTC TGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATC GCAGATCAGCATTGCTAAACT
2.	S3	>_S3_2023_27F_ E02.ab1	AGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGT AGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGA GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA

			ATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCC GCGTGAGGGATGACGGCCTTCGGGTTCCAAACCTCTTT CAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAA GAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAAT ACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTA AAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAG CCCGGGTTTTAACCCCGGGTCTGCAGTCGATACGGGCA GGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGT AGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT GGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGG AGCGAAAGCGTGGGGAGCGAACAGGATTGAGTACCCT GGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGG CAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTA AGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAAC TCCAAGGGGTTGACGGGGGCCCGCACAAGCGGCGGAGC ATGTGGCTTAATTCGACGCAACGCGAACCCCCTTACCA AGGCTTGACATACACCGGAAAGCATCAGAGATGGTGCC CCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTCCCGTGTTGCCAGCAAGCCCTT CGCCGTGTTGGGGACTCCCGGGAGACCGCCGGGGTCAA CT
3.	S6	>_S6_2023_27F_C03.ab1	GGGGTCTAATACCGGATGACACTTTCTCTCGCATGGGA GAAGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCG GCCTATCAGCTAGTTGGTGAGGTAGAAGCTCACCAAGG CGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCAC ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGA TGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT GTAAACCTCTTTCAGCAGGGAATTAGCGAAAGTGACGG TACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCA GCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTC GGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGT CGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCGG AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA GGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTAC TGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGAA CTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAG CTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGCCCGGGCACA AGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAA GAACCTTACCAAGGCTTGACATCGCCCGGAAAGCCGTA GAGATACGGCCCCCCTTGTGGTCGGGTGACAGGTGGTG CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT AAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCC AGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACT GCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAA GTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCAGGTACAATGAGCTGCGAAGCCGCGAGGCG GAGCGAATCTCAAAAAGCCTGTCTCAGTTCGGATTGGG GTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTA A
4.	S7	>_S7_2023_27F_D01.ab1	GTCTCCGTGTGGAAAGCTCCGGCGGTGCAGGATGAGCC CGCGGCCTATCAGCTTGAAGGTGGGGTGATGGCCTACC AAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGG CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG

			AGGCAGCAGTGGGGAATATTGCCCAATGGGCGCAAGCC TGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGG GTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCGAGTGA CGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGG AATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGC GTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGC AAACGATACGGGCAGGCTAGAGTTCGGCAGGGGAGAT TAAAATTGGTGGTGTAGCGGTGAAATGCGCAGATATC AGGAGGAACACCGGTGGCGAAGGCGGATCTCTGCCCCG ATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTG GGCACTAGGTGTGGGCGGCATTCCACGTCGTCCGTGCC GCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCG CACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACG CGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGC CGTAGAGATACGGCCCCCCTTGTGGTCGGTGTACAGGT GGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG GGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGT TGCCAGCAACTCCTTTCGGGGAGGTTGGGACTCACGGG AGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGAC GTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACAC GTGCTACAATGGCCGGTACAATGAGCTGCGATGCCGTG AGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGA TTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGC TAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTT CCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAG TCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGG GAGGGAGTCGTCGAAGGTTT
5.	S9	>_S9_2023_27F_A04.ab1	GTAGCCCGGCCTGAGAGGGCGACCGGCCACACTGGGAC TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGG GGAATATTGCACAATGGCGAAAGCCTGATGCAGCGACG CCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCT TTCAGCAGGGAAGAAGCCCGAGTGACGGTACCTGCAGA AGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAA TACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGT AAAGAGCTCGCAGGCGGCTTGTCACGTCGGATGTGAAA GCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGC TAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGT GTAGCGGTGAAATGCGCAGATATCACCCGGAACACCGG TGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAG GAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC TGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTG GCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATT AAGTTCCCCGCTGGGGAGTNCGGGCGCAAGGCTAAAAC TCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGC ATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA AGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCC CCCCTTGTGGTCGGTATACAGGTGGTGCATGGCTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTT CGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAA CTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCC CCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGG TACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTC AAAAAGCCGGTCTCAGTTCGGATTGGGGT

6.	S13	>_S13_2023_27F_B01.ab1	GGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCC CGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACC AAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGG CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG AGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC CTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCG GGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGT GACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCC GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTC ACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCT GCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAG ATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATAT CAAAAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCC ATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAA CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTT GGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGC CGCAGCTAACGCATTAAGAACCCCGCCTGGCCAGTACG GCCGCAAGGCTAAAACTCAAAGGAATTGACGGGCCCCC GCACAAGCAGCGGAGCATGTGGCAAAATTCGACGCAAC GCGAAGAACCTTACCAAGGCAAGACATATACCGGTTTG CATCAGAGATGGTGCCCCCCTTGTGGTCGGTATACAGG TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTT GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTG TTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGG AGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGAC GTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACAC GTGCTACAATGGCCGGTACAATGAGCTGCGATGTCGCA AGGCGGAGCGAATCTCAAAAAGCCGG
7.	S17	>_S17_2023_27F_H01.ab1	CTCATGGGGGACGGTTGAAAGCTCCGGCGGTGCAGGAT GAGCCCGCGGCCTATCAGCTTGTTGGTGGGGTAATGGC CTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCG ACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAGTGGGGAAAATTGCACAATGGGCG AAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGC CTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGA AAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTT GTCCGGAATTATTGCCTGTAAAGAGCTCGTAGGCGGTC TGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGG GTCTGCATTCGATACGGGCAGACTAGAGTGTGGTAGGG GAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGA TATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGG GCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCG AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG TTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGT GCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTA CGGCCGCAAGGCTAAAACTCAAAGGAATTGACGCGGGC CCGCACAAGCAGCCCAGCATGTGGCTTAATTCGACGCA ACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAA AGCATCAGAGATGGTGCCCGGGTTGTGGTCGGTGTACA GGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATG TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTG TGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACA GGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACG ACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAC ACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCG CAAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAG

Table 7 Closest resemblance of indigenous *Streptomyces* spp. isolates with reference to 16S rRNA region sequence analysis using NCBI BLAST tool.

S. No.	*Streptomyces* spp. Isolate Code	Closest Neighbour upon Alignment	Accession	Percentage Resemblance
1.	S2	*Streptomyces mutabilis strain NBRC 12800*	NR_112281.1	99.65%
2.	S3	*Streptomyces thinghirensis strain S10*	NR_116901.1	98.36%
3.	S6	*Streptomyces rectiviolaceus strain NBRC 100765*	NR_112590.1	99.49%
4.	S7	*Streptomyces albus strain DSM 40313*	NR_025615.1	99.13%
5.	S9	*Streptomyces griseus strain KACC 20084*	NR_042791.1	99.51%
6.	S13	*Streptomyces griseolus strain NBRC 3719*	NR_112493.1	98.62%
7.	S17	*Streptomyces niveus strain NRRL 2466*	NR_115784.1	99.00%

Table 7 shows the closest relatives of indigenous *Streptomyces* spp. isolates based on 16S rRNA sequence analysis using the NCBI BLAST tool. The isolates, including S2 (*Streptomyces mutabilis*), S3 (*Streptomyces thinghirensis*), and others, exhibit high sequence similarity, ranging from 98.36% to 99.65%, with the highest similarity observed for isolate S2. These results confirm the close genetic relationship of the isolates with known *Streptomyces* strains.

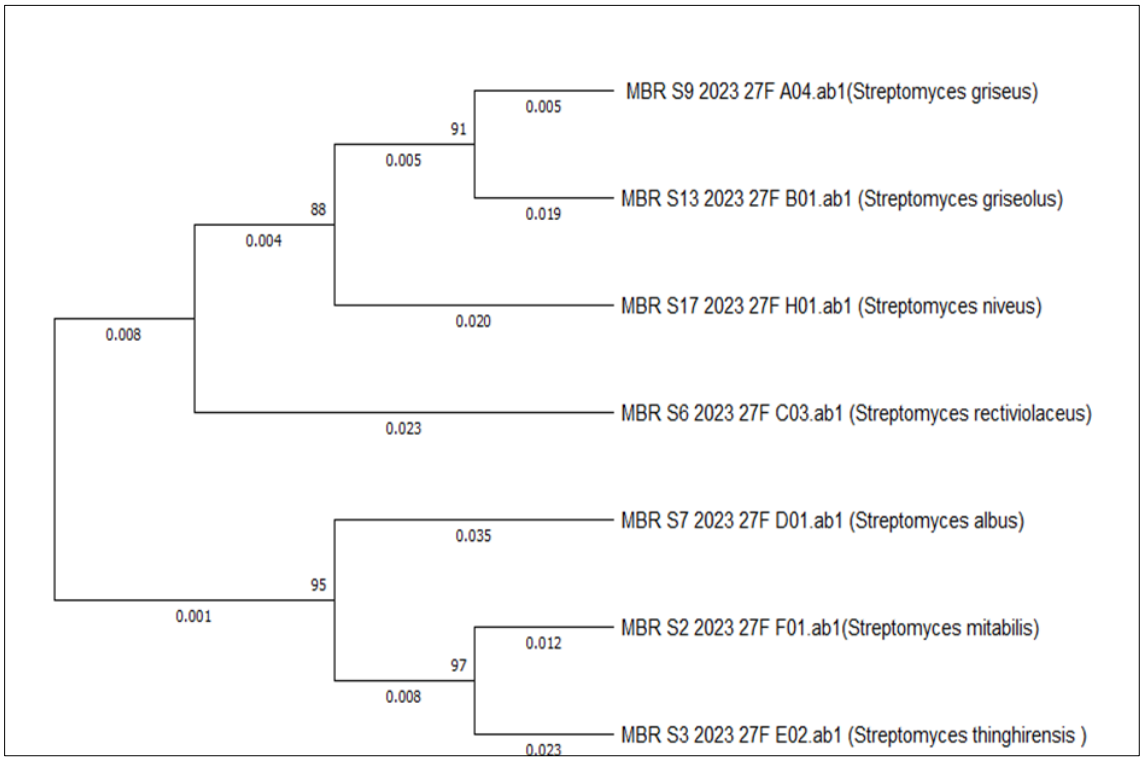

Figure 1 Phylogenetic tree generated among 7 different indigenous *Streptomyces* spp. isolates from pooled data of nucleotides by Test Neighbor-Joining statistical method through MEGA 11 software version 11.0.13 / CLUSTAL W

The phylogenetic analysis of seven indigenous *Streptomyces* spp. isolates was conducted using MEGA 11 software, employing the neighbor-joining method with 1000 bootstrap replications. The isolates, identified as *Streptomyces griseus*, *S. griseolus*, *S. niveus*, *S. rectiviolaceus*, *S. albus*, *S. mitabilis*, and *S. thinghirensis*, formed two major clades. The first clade included *S. griseus* and *S. griseolus*, which exhibited a close evolutionary relationship, supported by high bootstrap values (91). *S. griseus* is known for its methioninase production, which has biotechnological relevance, especially in cancer therapy (Tan et al., 2010). The second clade consisted of *S. albus*, *S. mitabilis*, and *S. thinghirensis*,

with high bootstrap support (95–97), highlighting their genetic and functional similarities. *S. albus* is noted for its bioactive compound production (Baltz, 2017). This phylogenetic diversity, depicted in Figure 1, underscores the potential of these isolates for methioninase production and biotechnological applications (Chen et al., 2016).

4. Conclusions

This study successfully isolated and identified methioninase-producing *Streptomyces* spp. from various locations around the Upper Lake of Bhopal. Screening identified seven potent isolates, which were further characterized using molecular methods, including 16S rRNA gene sequencing. The findings highlight the potential of these indigenous *Streptomyces* strains as a source of methioninase, an enzyme with promising therapeutic applications, especially in cancer treatment. Further optimization and detailed exploration of these strains could contribute significantly to enzyme-based cancer therapies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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