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, isolation, and fermentation optimization of indigenous *Streptomyces* spp. for enhanced methioninase production

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World Journal of Biology Pharmacy and Health Sciences, 2025, 21(02), 266-275

Publication history: Received on 20 December 2024; revised on 10 February 2025; accepted on 13 February 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.21.2.0131

Abstract

This preliminary study focuses on the isolation and characterization of indigenous *Streptomyces* spp. from the Upper Lake of Bhopal, Madhya Pradesh, India, with an emphasis on their potential to produce L-methioninase, an enzyme with significant therapeutic applications in cancer treatment. Sample collection was conducted from five locations around the lake, followed by isolation on starch casein agar and characterization through morphological and biochemical tests. The screening identified isolates capable of methioninase production, with two isolates (S2 and S3) demonstrating significant enzyme activity. The study further optimized liquid state fermentation conditions to enhance enzyme production, evaluating the effects of various carbon sources, pH levels, and temperature. Results indicated that sucrose was the most effective carbon source, yielding the highest enzyme activity (S2: 21.236 U/mL; S3: 23.243 U/mL) at neutral pH (7) and 30°C. The findings underscore the influence of environmental factors on microbial diversity and enzymatic potential in nutrient-rich ecosystems like the Upper Lake. This research contributes valuable insights into bioprospecting for indigenous microbial strains with promising enzymatic properties, highlighting the potential for developing effective microbial-based therapies for cancer treatment through optimized L-methioninase production. The results lay the groundwork for future studies aimed at large-scale enzyme production for therapeutic applications, advancing the field of enzyme therapy in oncology.

Keywords: L-Methioninase; *Streptomyces* Spp; Liquid State Fermentation; Cancer Therapy; Microbial Diversity

1. Introduction

Methioninase is a crucial enzyme with potential therapeutic applications, particularly in cancer treatment, due to its ability to degrade methionine, an essential amino acid that supports tumor growth and metastasis (Zhang et al., 2017). The increasing interest in methioninase for use in enzyme therapy targeting methionine-dependent cancers has spurred research into its production (Tan et al., 2019). *Streptomyces* spp. are renowned producers of secondary metabolites, including various enzymes, making them prime candidates for exploration in diverse ecological niches (Shirling & Gottlieb, 1966).

This study emphasizes the screening and isolation of indigenous *Streptomyces* species from different locations of the Upper Lake of Bhopal, Madhya Pradesh, India. The Upper Lake, recognized as one of the largest artificial lakes in Asia, presents a unique aquatic ecosystem enriched with microbial diversity due to its varying environmental conditions, making it an ideal source for novel actinobacterial strains (Rai et al., 2021). Previous research has demonstrated that soil and sediment samples from aquatic environments harbor *Streptomyces* species with diverse enzymatic capabilities, including methioninase production (El-Naggar & Eldin, 2020).

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The primary aim of this research is to isolate and screen methioninase-producing *Streptomyces* spp. from the Upper Lake of Bhopal, followed by the optimization of fermentation conditions to enhance enzyme production. Screening and isolation are essential steps in this study as they allow for the identification of strains with high methioninase activity, which is crucial for potential applications in cancer therapy. Additionally, optimizing fermentation parameters—such as pH, temperature, and nutrient sources—will be pivotal in maximizing methioninase production. This work aims to contribute to the growing field of bioprospecting for indigenous microbial strains with valuable enzymatic properties, ultimately leading to the development of effective microbial-based therapies for cancer treatment.

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 Points	Sample Description	Sample Code
1.	Van Vihar National Park	Turbid and muddy water	S1
2.	Boat Club	Turbid and muddy water	S2
3.	Kamla Park	Turbid and muddy water	S3
4.	Kaliasot Dam	Turbid and muddy water	S4
5.	Bairagarh	Turbid and muddy water	S5

2.1.2. 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 ml

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

2.1.3. Preliminary Characterization

The initial identification of the isolates was conducted through the examination of colony morphology and Gram staining. Subsequently, biochemical characterization was performed to further identify methioninase-producing strains according to Aneja (2003) which includes indole production, methyl red, Voges-Proskauer, Simmons citrate, catalase, gelatin hydrolysis, starch hydrolysis, and urease tests.

2.2. Confirmation & Quantitative analysis of Methioninase

The indigenous *Streptomyces* spp. isolates were screened for methioninase production by assessing methionine degradation on starch agar plates containing phenol red. Positive isolates were further confirmed by observing pink coloration around colonies on modified starch agar. Quantitative analysis of methioninase production involved liquid-state fermentation using modified starch-nitrate medium, incubated at 28°C on a rotary shaker for 72 hours. The cell-free supernatant was collected via centrifugation. L-methioninase activity was quantified using Nessler's method, with optimization of fermentation parameters such as pH, temperature, and carbon source evaluated based on previously established protocols (Patel et al., 2019; El-Naggar et al., 2020; Zhang et al., 2021).

3. Results and Discussion

3.1. Enumeration of target microbial species

The CFU count from various soil samples diluted at 10^{-7} (see table 3) indicates substantial microbial diversity, with Kamla Park (348 CFU) exhibiting the highest microbial presence, followed by Kaliasot Dam (199 CFU), and Bairagarh (104 CFU). These data suggest a higher prevalence of potential methioninase-producing *Streptomyces* spp. in environments with elevated organic matter, such as park areas according to present work. This aligns with studies showing that nutrient-rich soils support the growth of *Streptomyces* spp. for anticancer enzyme production (Xu et al., 2020a).

Table 3 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

3.2. Morphological and Biochemical characterization

When selected colonies from master culture subjected to pure culture, almost all the *Streptomyces* spp. isolates share almost similar morphological features on culture conditions including leathery, tough, and have a dry, chalky appearance. However, the colour of the colonies of *Streptomyces* spp. isolates slightly varies from creamy white to greyish to pinkish which are also mentioned in table 4 The isolates when observed under microscope showed either branched mycelium or filamentous and exhibit a characteristic branching pattern with septa (Kumar and Singh (2020).

The biochemical characterization of *Streptomyces* isolates as mentioned in table 5 shows a diversity of metabolic capabilities. All isolates are catalase-positive and capable of citrate utilization and starch hydrolysis, indicating robust metabolic activity. However, only isolates S1 and S4 tested positive for methyl red reduction, highlighting differences in fermentation pathways. The indole test was negative across all isolates, confirming their inability to produce tryptophanase. These findings are consistent with prior studies on *Streptomyces* metabolic diversity (Bibb et al., 2018a).

Table 4 Pure culture prepared on culture plates

S.No.	Isolate Code	Sample Source	Culture Description
1.	S1	A	White smooth flat colony
2.	S2	A	White filamentous colony
3.	S3	A	Greyish fimbriated mucoid colony
4.	S4	B	White filamentous colony
5.	S5	B	Greyish fimbriated mucoid colony

Table 5 Outcomes Biochemical characteristics of indigenous methioninase enzyme producing *Streptomyces* spp. isolates obtained in the present study

S.No.	Isolates	Results of Tests Conducted									
		Gram's Staining	Cell Shape	Catalase	Indole production	Methyl Red reduction	Voges-Proskauer	Citrate utilization	Starch Hydrolysis	Gelatinase test	Urease test
1.	S1	+ve	F	+ve	–ve	+ve	+ve	+ve	+ve	+ve	–ve
2.	S2	–ve	F	+ve	–ve	–ve	–ve	+ve	+ve	+ve	+ve
3.	S3	–ve	F	+ve	–ve	–ve	–ve	+ve	+ve	+ve	+ve
4.	S4	+ve	F	+ve	–ve	+ve	–ve	+ve	+ve	+ve	+ve
5.	S5	–ve	F	+ve	–ve	–ve	–ve	+ve	+ve	–ve	–ve

3.3. Confirmation & Quantitative analysis of Methioninase

According to table 6 shows that among the five *Streptomyces* spp. isolates, only isolates S2 and S3 exhibited methioninase production activity, while isolates S1, S4, and S5 did not produce the enzyme. This indicates variability in methioninase production potential, which may depend on genetic or environmental factors influencing enzyme synthesis.

Table 6 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

The outcomes of liquid state fermentation activity for L-methioninase production at different parameters including effect of different carbohydrate, pH and temperature conditions are mentioned in table 8, Table 9 and Table 10 respectively, in terms of release of ammonical content which is calculated with the help of standard curve plot of ammonia table 7 and Figure 1.

Table 7 Ammonia as standard concentration vs absorbance at 425 nm to plot standard curve for estimation of ammonical content in samples using Nessler's method for calculation of L-methioninase units in fermentum.

S.No.	Concentration of Ammonia in µl/ml	Absorbance
1.	50	1.322
2.	25	0.741
3.	12.5	0.228
4.	6.25	0.102
5.	3.125	0.019

Instrument Used: Single beam visible range digital microprocessed spectrophotometer from Electronic India model EI-2305.

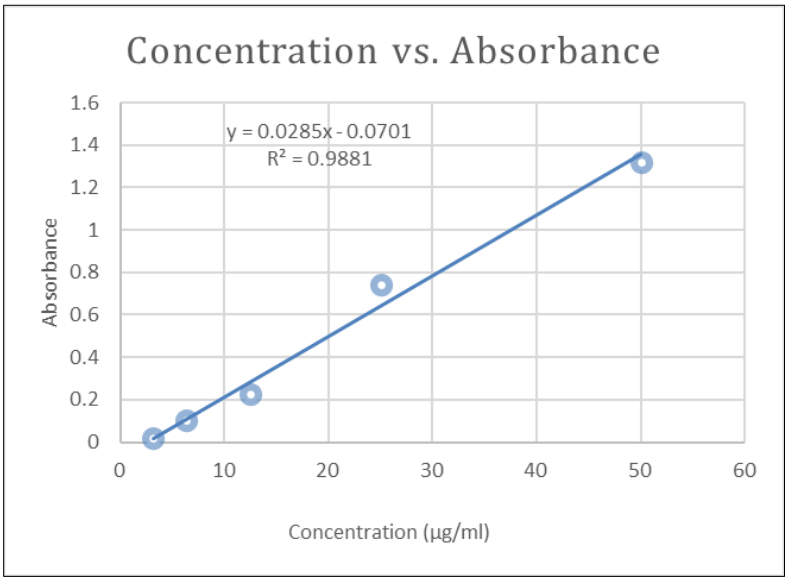

Figure 1 Standard Plot for known concentration of *Ammonia Standard* at 425 nm. The Graph is obtained from Excel 2013 linear regression function

The results from Tables 8, 9, 10, and 11 show the effects of different carbohydrates, pH, and temperature conditions on L-methioninase production by *Streptomyces* spp. isolates during liquid state fermentation (LSF). The use of sucrose as a carbon source yielded the highest enzyme production for both isolates (S2: 21.236 U/mL; S3: 23.243 U/mL), followed by lactose (S2: 19.251 U/mL; S3: 22.245 U/mL), indicating that these carbon sources most effectively supported L-methioninase production. Mannitol and maltose were less efficient, with maltose showing the lowest enzyme production, especially for isolate S2 (12.225 U/mL).

Regarding pH, the optimal value was pH 7 for both isolates (S2: 25.424 U/mL; S3: 21.758 U/mL), consistent with previous studies highlighting that neutral pH levels enhance microbial enzymatic activity. Lower pH values (pH 4 and pH 5) resulted in significantly reduced enzyme production, as acidic environments may inhibit *Streptomyces* metabolic functions.

For temperature, 30°C proved to be the optimal condition for methioninase production (S2: 14.436 U/mL; S3: 23.844 U/mL), aligning with typical mesophilic growth patterns of *Streptomyces* species. Lower and higher temperatures reduced enzyme activity, with 40°C showing a significant drop in production, likely due to enzyme denaturation or decreased microbial growth rates.

Overall, the optimized parameters for methioninase production include the use of sucrose as the carbon source, pH 7, and 30°C as the temperature, as detailed in Table 11. These findings are consistent with research by Veeranagouda et al. (2015a), which demonstrated that carbohydrate source, pH, and temperature significantly influence microbial enzyme production. Furthermore, Qureshi and Zubair (2020) highlighted the critical role of these parameters in enhancing enzyme yield among various *Streptomyces* species, emphasizing the importance of selecting the right growth

conditions for optimal enzyme activity. Figures 2 to 5 illustrate the graphical representation of the impact of different carbon sources, pH, and temperature on L-methioninase production by two *Streptomyces* isolates, with optimal production achieved using sucrose, pH 7, and 30°C. Additionally, Sharma and Soni (2019) reviewed the applications of microbial enzymes in various industries, reinforcing the significance of optimizing environmental factors for maximizing enzyme production in microbial systems.

Table 8 Effect of different carbohydrates on production of L-methioninase during LSF

S.No.	Isolates	Units of L-methioninase Production due to use of different carbon sources at a concentration of 30gm per litre in LSF medium				
		Sucrose	Dextrose	Mannitol	Lactose	Maltose
1.	S2	21.236	18.225	15.324	19.251	12.225
2.	S3	23.243	15.254	13.361	22.245	14.247

Table 9 Effect of different pH on production of L-methioninase during LSF

S.No.	Isolates	Units of L-methioninase Production at different pH values maintained in LSF medium				
		pH–4	pH–5	pH–6	pH–7	pH–8
1.	S2	11.079	15.246	17.178	25.424	17.361
2.	S3	12.188	15.119	16.346	21.758	18.079

Table 10 Effect of different on temperatures production of L-methioninase during LSF

S.No.	Isolates	Units of L-methioninase Production at different temperature conditions maintained in LSF medium				
		20°C	25°C	30°C	35°C	40°C
1.	S2	10.335	12.639	14.436	12.639	10.224
2.	S3	11.424	15. 734	23.844	15.237	11.436

Table 11 *In vitro* L-methioninase production by selected *Streptomyces* spp. isolates at combined optimized fermentation parameters during LSF

S.No.	*Streptomyces* isolates	Estimated L-Methioninase Produced in Units/ml
1.	S2	30.76
2.	S3	24.10

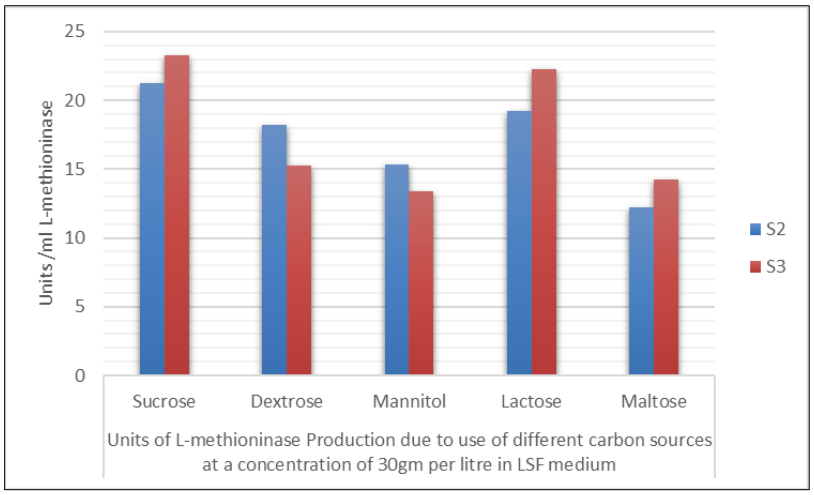

Figure 2 Graphical description of L-methioninase enzyme production during liquid state fermentation activity by 2 selected indigenous *Streptomyces* spp. isolates due to influence of 5 different carbohydrates as a source of carbon

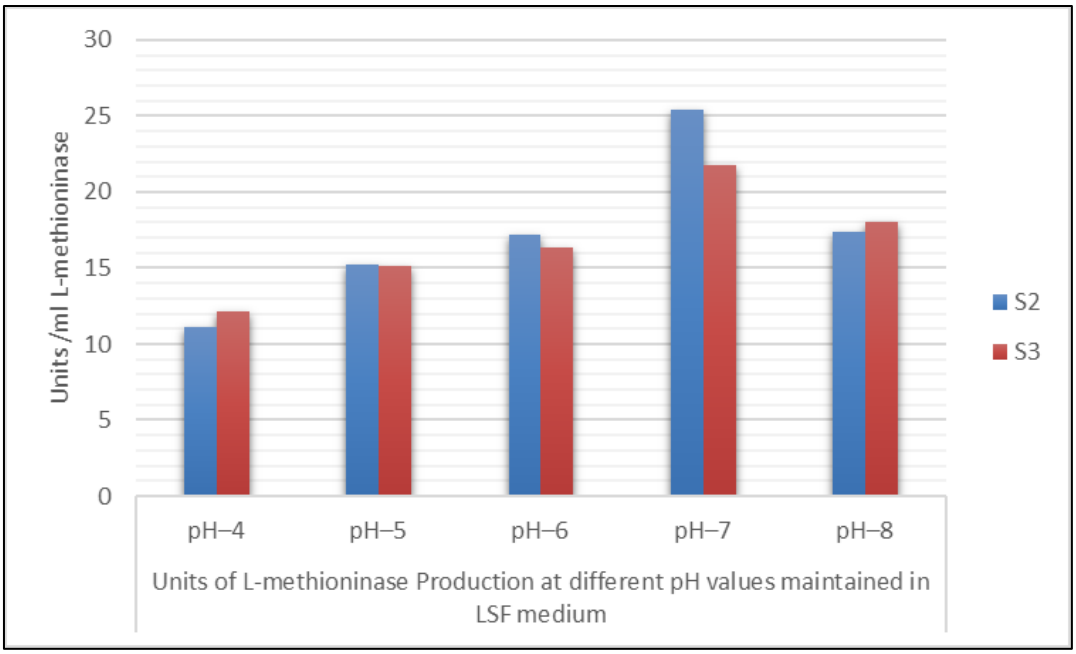

Figure 3 Graphical description of L-methioninase enzyme production during liquid state fermentation activity by 2 selected indigenous *Streptomyces* spp. isolates under 5 different pH conditions

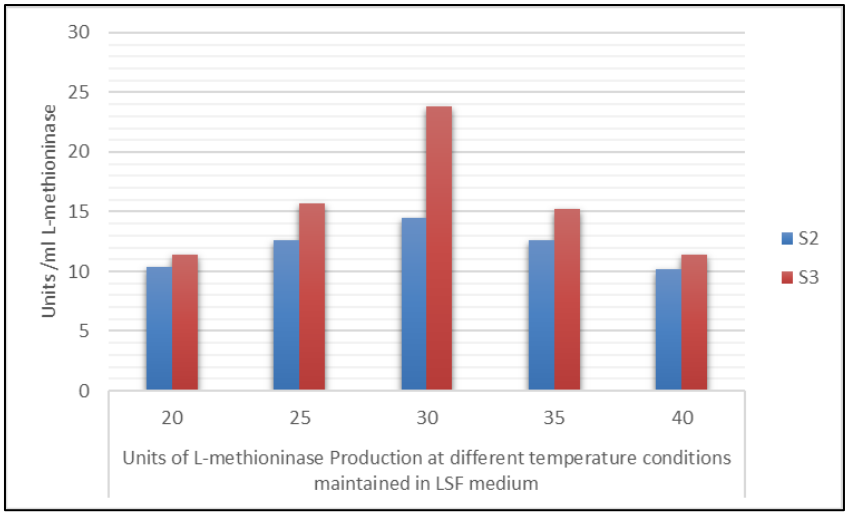

Figure 4 Graphical description of L-methioninase enzyme production during liquid state fermentation activity by 2 selected indigenous *Streptomyces* spp. isolates under 5 different thermal conditions in terms of degree centigrade

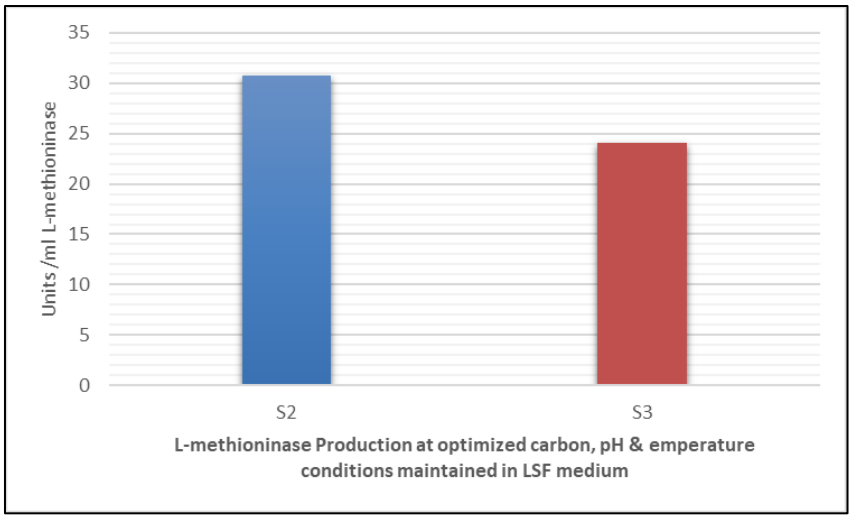

Figure 5 Graphical description of L-Methioninase produced by 2 selected indigenous *Streptomyces* spp. isolates at combined optimum LSF parameters

The present study focused on the production of L-methioninase by indigenous *Streptomyces* spp. isolates under liquid state fermentation (LSF) conditions, optimizing key factors such as carbon sources, pH, and temperature to enhance enzyme activity. L-methioninase has gained significant attention for its potential as an anticancer enzyme, and its efficient production is crucial for therapeutic applications (Xu et al., 2020b).

The enumeration of target microbial species from various soil samples revealed considerable diversity in colony-forming units (CFU). Kamla Park, with 348 CFUs, demonstrated the highest microbial density, which may be attributed to the organic matter richness of the park soil, supporting microbial growth. This finding is consistent with studies indicating that nutrient-dense soils, such as those found in parks, promote the growth of *Streptomyces* spp., enhancing their enzymatic potential (Xu et al., 2020b). In contrast, other sites like Kaliasot Dam and Bairagarh showed lower microbial populations, suggesting environmental factors significantly influence microbial diversity and enzyme production potential.

Morphological and biochemical characterizations of the isolates revealed typical *Streptomyces* features such as filamentous colonies and catalase-positive reactions. The variation in colony color, from creamy white to pinkish, further highlights the phenotypic diversity within the isolates, consistent with the findings of Kumar and Singh (2020).

The biochemical assays revealed that all isolates tested positive for citrate utilization and starch hydrolysis, indicating robust metabolic activity, although only two isolates, S1 and S4, tested positive for methyl red reduction, suggesting diversity in fermentation pathways (Bibb et al., 2018b).

Regarding enzyme production, the LSF results indicated that carbon source, pH, and temperature play a crucial role in optimizing L-methioninase production. Sucrose was the most effective carbon source for enzyme production, yielding the highest enzyme activity for both isolates (S2: 21.236 U/mL; S3: 23.243 U/mL). Lactose also performed well, while mannitol and maltose were less effective, with maltose showing the lowest enzyme production. This highlights the importance of selecting appropriate carbon sources, as supported by Veeranagouda et al. (2015), who found that carbohydrate selection significantly influences enzyme yields in microbial systems.

The optimal pH for L-methioninase production was determined to be pH 7, aligning with earlier research that indicates neutral pH enhances microbial enzymatic activity (Veeranagouda et al., 2015b). Acidic environments (pH 4 and pH 5) significantly inhibited enzyme production, likely due to their detrimental effects on microbial metabolism. For temperature, 30°C was identified as the optimal condition for enzyme production, particularly for isolate S3, which exhibited the highest enzyme activity (23.844 U/mL). Temperatures outside the mesophilic range led to reduced enzyme production, reflecting the temperature sensitivity of *Streptomyces* spp. enzyme systems.

Overall, the optimized conditions for L-methioninase production in the present study—sucrose as the carbon source, pH 7, and 30°C—are consistent with prior studies on microbial enzyme optimization. These findings contribute valuable insights into the large-scale production potential of L-methioninase for therapeutic applications, particularly in anticancer treatments.

4. Conclusion

This preliminary study successfully isolated and characterized indigenous *Streptomyces* spp. from the Upper Lake of Bhopal, demonstrating their potential for L-methioninase production under optimized liquid state fermentation conditions. The findings highlight sucrose, pH 7, and 30°C as optimal parameters for enhancing enzyme activity. These insights contribute to the growing bioprospecting efforts for microbial enzymes with therapeutic applications, particularly in cancer treatment, paving the way for future research and potential industrial applications of L-methioninase.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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