International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 12 December 2025
DOI: 10.47191/ijcsrr/V8-i12-04, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

Soil Actinomycetes as Potential Producers of L-Asparaginase: Qualitative, Quantitative and Fermentation Studies

Hariharan Vijayakumar[1], Juby T R[2]
[1]Department of Quality Assurance, Life Cell international Pvt, Ltd, Chennai, Tamil Nadu
[2]Department of Microbiology, Sree Narayana Guru College, Coimbatore, Tamil Nadu

ABSTRACT: Actinomycetes are a group of filamentous, Gram-positive bacteria widely distributed in soil and recognized as prolific producers of bioactive secondary metabolites, including antibiotics and enzymes of therapeutic importance. Among these, L-asparaginase is a clinically significant enzyme used as an antineoplastic agent in the treatment of acute lymphoblastic leukemia. The enzyme catalyzes the hydrolysis of *L*-asparagine into *L*-aspartic acid and ammonia, thereby depriving tumor cells of an essential amino acid required for their growth and survival. Although *L*-asparaginase derived from *Escherichia coli* and *Erwinia carotovora* is commercially available, these bacterial sources often cause hypersensitivity and other side effects. Hence, there is a growing need to explore alternative microbial sources such as actinomycetes, which can produce the enzyme with better therapeutic efficiency and fewer side effects. This study aims to identify potent *L*-asparaginase-producing actinomycetes and their production under submerged fermentation conditions. The study was designed with four major objectives, to isolate actinomycetes from soil samples, to perform qualitative and quantitative assays for *L*-asparaginase production, and to evaluate the production of L-asparaginase by submerged fermentation using boiled rice water as the substrate. Soil sample was collected, and actinomycetes were isolated using standard microbiological techniques. The qualitative screening of isolates was carried out using a plate assay method to identify potential *L*-asparaginase-producing strains based on the formation of color zones. Quantitative estimation of enzyme activity was performed by measuring the amount of ammonia released during enzymatic reaction. For enzyme production, submerged fermentation was employed, utilizing boiled rice water as a nutrient-rich, cost-effective substrate to enhance enzyme yield.

KEYWORDS: Actinomycetes, L- Asparaginase, Qualitative and quantitative assay, submerged fermentation.

INTRODUCTION

Microorganisms produce various enzymes that are important in medicine. One enzyme, L-asparaginase, has gained attention for its role as a treatment for cancer. This enzyme breaks down the amino acid L-asparagine into L-aspartic acid and ammonia. It fights tumors by degrading extracellular L-asparagine, which is essential for the growth of cancer cells. By removing L-asparagine from the bloodstream, L-asparaginase affects malignant lymphoblast. Normal cells can create L-asparagine using the enzyme asparagine synthetase. However, some cancer cells, especially in acute lymphoblastic leukemia, do not have this ability. This means that L-asparaginase treatment deprives these leukemic cells of asparagine, which stops protein synthesis and leads to cell death. Healthy cells can still grow normally without external L-asparagine. Commercial L-asparaginase mostly comes from bacteria like Escherichia coli and *Erwinia carotovora* [1]. Although these enzymes are effective, their clinical use often faces challenges such as allergic reactions, anaphylaxis, liver problems, and immune responses. Because of this, there is growing interest in exploring other microbial sources that can produce L-asparaginase with better biochemical and therapeutic characteristics, like improved substrate specificity, higher stability, and reduced immune responses. In recent years, actinomycetes have shown promise as an alternative source for L-asparaginase production [2]. Their natural ability to produce extracellular enzymes, along with their genetic and metabolic flexibility, is a strong basis for discovering new enzyme variants with desirable effects.

Several studies have found L-asparaginase activity in different species of Streptomyces and related actinomycetes, indicating that these microorganisms can effectively produce the enzyme with unique molecular and kinetic traits [3]. Additionally, since actinomycete L-asparaginase is produced extracellularly, it simplifies downstream processing and purification, which are important for large-scale enzyme production. Besides therapeutic effectiveness, temperature tolerance is now a key factor in assessing microbial L-asparaginase. Enzymes that remain effective across a wide temperature range are valuable for clinical and

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industrial uses, where stability under various conditions is crucial. Thermostable or temperature-tolerant L-asparaginase tend to have a more rigid structure, resist changes, and last longer than those from mesophilic sources. These traits make them especially suitable for industrial applications and therapeutic products that require greater stability and durability. Actinomycetes, particularly heat-resistant species of Streptomyces, have been found to produce L-asparaginase that maintain good activity even at high temperatures, highlighting their potential as superior producers [4]. Another key aspect of enzyme production is the choice of fermentation substrate. Traditional enzyme production media can be expensive and increase overall production costs. Therefore, using low-cost, nutrient-rich agricultural and household waste as alternatives has gained interest. One such option is boiled rice water, which is an easily accessible byproduct from food preparation. It serves as an affordable and sustainable source of carbon and nitrogen for microbial growth. Its nutrient profile supports the growth of actinomycetes and may increase enzyme yields like L-asparaginase during submerged fermentation. Taking these factors into account, this study aimed to isolate effective L-asparaginase-producing actinomycetes from soil samples and assess their enzyme production capabilities. The research also sought to evaluate the qualitative and quantitative activities of the isolates and optimize L-asparaginase production under submerged fermentation using boiled rice water as a low-cost substrate. This investigation strives to identify new actinomycete strains that may be temperature-tolerant and capable of producing therapeutically significant L-asparaginase with improved properties. The goal is to contribute to developing an economical, efficient, and environmentally friendly enzyme production process.

Considering these perspectives, the present study was undertaken with the objective of isolating potent L-asparaginase-producing actinomycetes from soil samples and assessing their enzyme production potential. The study further aimed to evaluate the qualitative and quantitative enzyme activities of the isolates and to optimize the production of L-asparaginase under submerged fermentation using boiled rice water as a cost-effective substrate. Through this investigation, an attempt has been made to identify novel, potentially temperature-tolerant actinomycete strains capable of producing therapeutically significant L-asparaginase with improved properties and to contribute toward the development of an economical, efficient, and environmentally friendly process for enzyme production.

MATERIALS AND METHODS

Sample collection

The soil sample was collected from the brick-kiln in the nearby the area of Bodinayakkanur, Theni district, Tamil Nadu. Sample was collected aseptically from a depth of 5-10 inches from the surface of the brick – kiln ground. The sample was stored in a sterile polythene bag and sealed tightly and shade dried under the room temperature. Collected soil sample was air-dried for 1 week, crushed and sieved. The pretreated soil sample was used for the further procedure.

Isolation of Actinomycetes from the soil sample and Morphological characterization

One gram of the pretreated soil sample was subjected for decimal dilution up to 10^{-5} dilutions. Spread plate technique was employed on actinomycetes isolation agar (L-asparagine - 0.1 g/L, Dipotassium phosphate - 0.5 g/L, Ferrous sulfate - 0.001 g/L, Magnesium sulfate - 0.1 g/L, Sodium caseinate - 2 g/L, Sodium propionate - 4 g/L, Agar -15 g/L.) for the isolation of Actinomycete's. To minimize the bacterial and fungal growth, nalidixic acid 100 mg/land cycloheximide 20 mg/l were added respectively in to sterile medium. The plates were incubated at 30°C for 10 days [5]. The plates were observed for the growth of actinomycetes at every 24hr during incubation. Gram staining was performed to study the cell wall nature of the selected actinomycetes.

Preparation and Preservation of Pure culture of the Actinomycetes Isolates

After incubation, morphologically distinct actinomycetes colonies were selected from the actinomycetes isolation agar plates and further purified by streak plate method. T he pure colonies were further analyzed based on its characteristics such as earthy like the smell, colony morphology, the color of hyphae and aerial and substrate mycelium. The selected colonies of actinomycetes were transferred to starch casein agar slant and incubated at 27 °C for 7 days. After incubation, the slants containing pure actinomycetes isolates were stored at-4 °C for further studies.

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Qualitative assay of the L – asparaginase produced by the actinomycetes isolates

Qualitative assay for the L Asparaginase production by the selected actinomycete sp was done by the plate assay method. Asparagine Dextrose Salts agar (ADS) ((L-asparagine (0.5%), Dextrose (1%), K2 HPO4 (0.1%), Trace salts solution (0.1%), pH – 7.2) medium with L – asparagine as sole nitrogen source with phenol red as pH indicator was used for the Screening of L-Asparaginase producing actinomycetes strains. The change of coloration from yellow to pink around bacterial colonies indicates the alkalinisation of the medium due to the presence of ammonia produced by L-Asparagine hydrolysis reaction catalyzed by L-Asparaginase. The pink color corresponds to the turn of the pH indicator. Alkalinisation is shown by a color change of the Phenol Red The plates were observed for the colour change during the incubation period.

Production of L- Asparaginase by submerged fermentation using boiled rice water as carbon source

Seed culture was prepared by inoculating 1 ml spore suspension (25.8×10^6 CFU/ml) into 100 ml sterile starch casein nitrate broth in an Erlenmeyer flask. The flask was then incubated in a shaking incubator at 28±2°C for 7 days at 200 rpm. 2 ml of the culture was used to inoculate 50 ml of fermentation medium.

Submerged Fermentation

Boiled rice water was used as carbon source for the production of L – asparaginase by submerged fermentation. The production medium (Boiled rice water – 10.0 ml/l, Casein – 0.3 g/l, KNO3 – 2 g/l, NaCl – 2 g/l, K2HPO4 – 2 g/l, MgSO4.7H2O – 0.05 g/l, CaCO3 – 0.02 g/l FeSO4.7H2O – 0.01 g/l, L – Asparagine – 1 g/l pH – 7) was taken in 100ml of Erlenmeyer flask. The contents were thoroughly mixed and sterilized at 100°C for 20 minutes. The 2ml of 7 days old inoculum was introduced into the sterilized components. The inoculated flasks were incubated on a rotatory shaker at 30 – 37°C with shaking at 150-200 rpm [6].

Quantitative Estimation of L- Asparaginase produced by the submerged fermentation.

Quantitative estimation of the L- asparaginase enzyme was determined by optical density method. The amount of ammonia released by adding Nessler's reagent (Nesslerization process) is quantitatively determined which indirectly indicate the quantity of the enzyme. The reaction mixture containing 1.5ml of 0.04 M L- asparagine was prepared in 0.05 M Tis-HCl buffer, Ph 8.6 and added 0.5 ml of the crude enzyme to make up the volume of 2 ml. The tubes were incubated at 37°C for 30 minutes. The reaction was stopped by adding 0.5 ml of 1.5 M trichloroacetic acid (TCA). The blank was prepared adding the enzyme after adding the TCA. The precipitate was removed by centrifugation at 10,000 X g for 5 minutes and the the supernatant was collected. 0.5 ml of clear supernatant was taken in the test tubes and added 5 ml of distilled water. 1ml of Nessler's reagent was added into the diluted enzyme and incubated at room temperature for 20 minutes. Development of yellow colour indicates the presence of ammonia and at higher concentrations, a brown precipitate may form. The spectrophotometric measurements were carried out at 425 nm. Samples were compared to a control reaction where enzymatic activity was stopped with TCA before incubation.

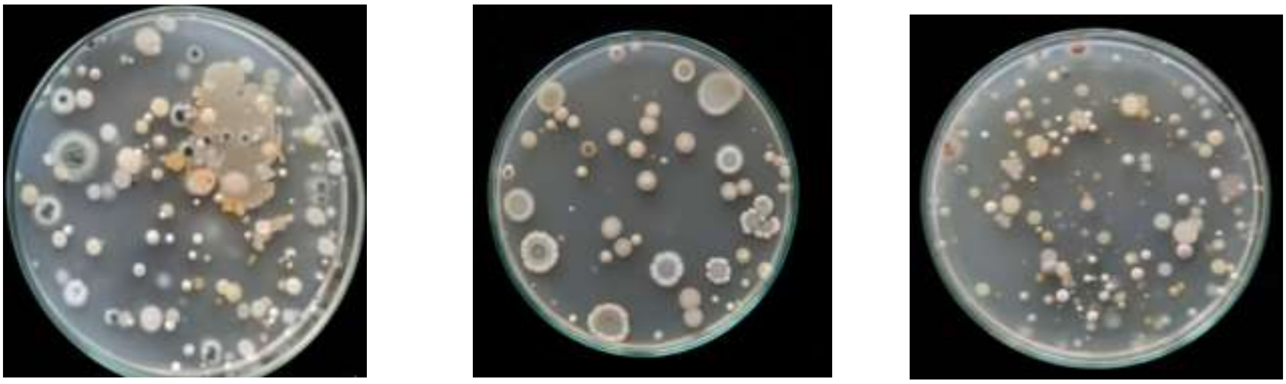
A standard curve was prepared using ammonium sulphate solution in 0.2N H2SO4. Enzyme activity (EA) was calculated as the amount of ammonia released during the enzymatic degradation of L-asparagine from the substrate, as shown in the following equation [7]

EA (U/ml) = Amount of NH_4 liberated/Incubation Time (min) x Volume of enzyme (ml).

RESULTS

Isolation of Actinomycetes from the soil sample and Morphological characterization

After the initial incubation period of 7 days the actinomycetes growth were spotted on the actinomycetes isolation agar in different colour and size as shown in Fig 1. Totally 13 distinct colonies of actinomycetes were spotted on AIA in the dilution of 10^{-3}, 10^{-4} and 10^{-5} and five species were selected for the study based on their morphological characteristics (Table.1). The selected species were named as SNGCHH1, SNGCHH-2, SNGCHH-3, SNGCHH-4, and SNGCHH-5 respectively.



(a) 10^{-3} Dilution (b) 10^{-4} Dilution (c) 10^{-5} Dilution

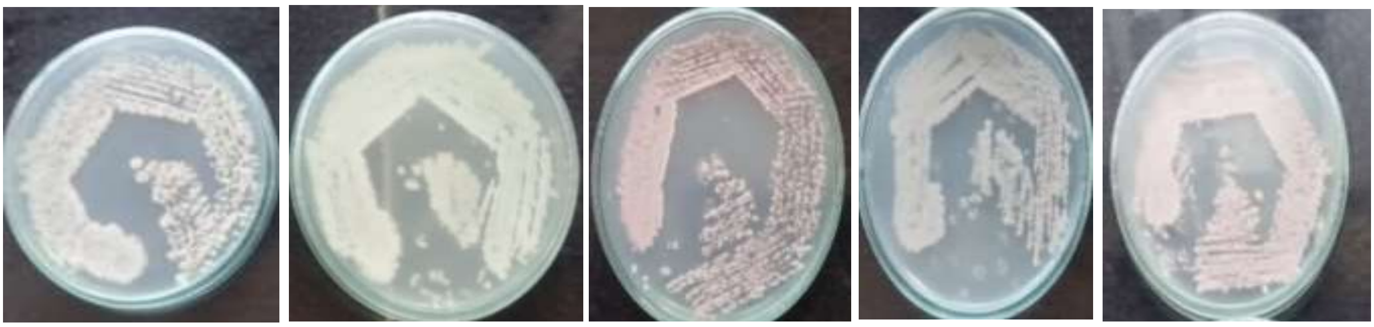
Fig.1 Isolation of actinomycetes from soil sample

Table 1. Morphological Characterization of the Actinomycetes isolated from the Kiln soil

S. No.	Actinomycete Isolates	Gram staining	Substrate mycelium	Aerial mycelium
1	SNGCHH-1	Gram positive	Ash black	Off White
2	SNGCHH-2	Gram positive	Purple	White
3	SNGCHH-3	Gram positive	Bright white	White
4	SNGCHH- 4	Gram positive	Pink	White
5	SNGCHH- 5	Gram positive	Lavender	Pink

Preparation and Preservation of Pure Culture of the Isolated Actinomycetes

The selected colonies were streaked on Starch casein Nitrate agar plate technique and obtained the pure culture colonies of selected actinomycetes after the incubation period of 7 – 14 days were kept under refrigeration for further use (Fig.2).



(a) (b) (c) (d) (e)

Fig.2 Pure culture of isolated actinomycetes on SCA medium

Qualitative assay of the L – asparaginase produced by the actiomycetes isolates

After 3 days of incubation, Pink colour started to develop on the assay medium around the line of streaking and after 7 days of incubation the entire medium changed the color from yellow to pink which are shown in Fig 3. This indicates the

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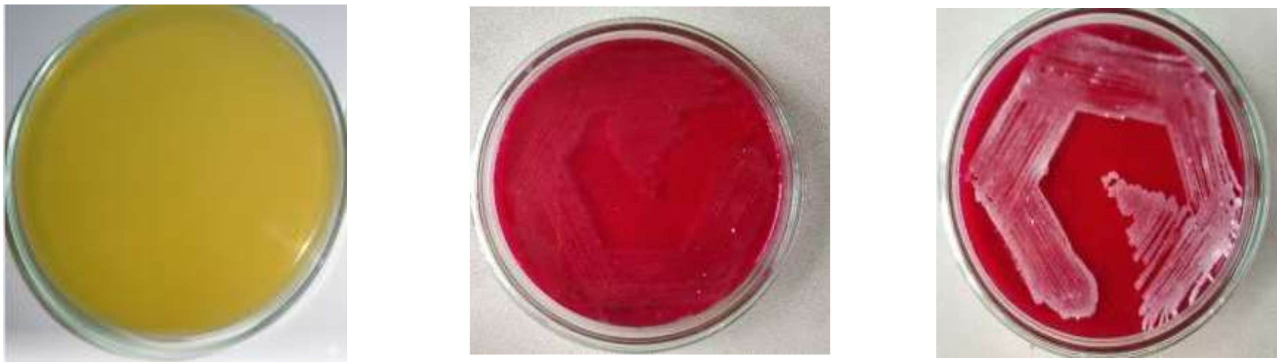
positive result for the qualitative assay of L- Asparaginase by the selected strain of actinomycetes. Out of five actinomycete isolates, two strains, SNGCHH-1 and SNGCHH-2 showed the positive result. After 7 days of incubation both the strains changed the colour of the entire medium in to deep pink. Both the actinomycetes strains which showed positive results were used for the quantitative analysis.



(a) Control plate (b) SNGCHH-1- positive result (c)SNGCHH-3- positive result

Fig.3 Qualitative assay for the production of L – asparaginase

Quantitative Estimation of L- Asparaginase produced by the submerged fermentation.

The quantitative estimation of L- asparaginase was done by Nesslerization process. The strains showed positive results in qualitative study namely SNGCHH-1 and SNGCHH-3 were shows the positive results in the Nesslerization test which means that the selected strains released ammonia while adding the Nessler's reagent. And the brown precipitation was formed in the test tubes contains L – asparaginase enzyme at room temperature. After 7 days of incubation SNGCHH-1 produced 40.18 ul/ml and SNGCHH-3 produced 34.45 ul/ml.

DISCUSSION AND CONCLUSION

The present study reveals that the isolated actinomycetes were produce L – asparaginase enzyme qualitatively and quantitatively. The isolated strain of SNGCHH – 1, SNGCHH – 3 were showing enormous amount of the production of L – asparaginase. The obtained results reveals the suitability of using soil actinomycetes isolates in pharmaceutical and medical industries. Results obtained showed higher intensity of pink coloration [8]. Production of L-asparaginase began after 24 h of cultivation and reached to maximum levels after 72 h of incubation. Maximum biomass production obtained with 72 h old culture and yeast extract as the best nitrogen source for maximum yield of L-asparaginase. The isolated strains were produced L – asparaginase in the pH of 7.2-8.0 at room temperature. The results were matched with the statement of [10] reported that the 6-day old culture of *Streptomyces collinus* produced high amount of L- asparaginase when grown at pH 8.5 and temperature 28–30°C. But in the present study, 3-day old culture of *S. albidoflavus* was found to produce maximum amount of enzyme when cultured at pH 7.5 and temperature 35°C [9]. The substrate assessment shows the positive result for the production of L – asparaginase enzyme. Natural raw materials and by-products generated by industry are widely used as culture medium due to their low cost, once the components of the medium can represent from 38 to 73% of the total production cost. Agro-industrial residues are untreated and underutilized, while being rich in nutrient components, such as carbohydrates, proteins, fibers, minerals, and vitamins. The utilization of this waste not only eliminates the disposal problems, but also the environment pollution (toxicity to aquatic life, pollution of surface and ground waters, altered soil quality, phytotoxicity, odorous, and colored natural waters) and negative impact on human and animal health [11].

From this study it has been concluded that the isolated actinomycetes were demonstrated the production of the enzyme L – asparaginase. For further work needed for the complete analysis of the enzyme produced for further purification and cytotoxicity for the anti-cancerous activity.

REFERENCES

1. El-Refai, H. A., Abdel Naby, M. A., Gaballa, A. A., El-Araby, M. H., & Ammar, H. S. (2020). L-Asparaginase from microbial sources: Production, optimization, and applications. Journal of Genetic Engineering and Biotechnology, 18(1), 1–12.
2. Sasidharan, S., & Priya, K. (2021). Actinomycetes as a promising source of novel L-asparaginase with improved biochemical properties. Biotechnology Reports, 30, e00615.
3. Manisha, M., Kumar, S., & Singh, P. (2022). Exploration of Streptomyces species for extracellular L-asparaginase production and characterization. Journal of Applied Microbiology, 133(1), 45–56.
4. Verma, N., & Kumar, K. (2018). Thermostable L-asparaginase from actinomycetes: Production, characterization, and industrial relevance. International Journal of Biological Macromolecules, 117, 1084–1092.
5. Sheik, G. B., M. S. Maqbul, G. S. S., and R. M. S. Isolation and characterization of actinomycetes from soil of ad-dawadmi, saudi arabia and screening their antibacterial activities. International Journal of Pharmacy and Pharmaceutical Sciences, vol. 9, no. 10, Oct. 2017, pp. 276-9.
6. Noura EE, Hassan M, Nancy ME, Sara ME. (2015) Optimization of culture conditions for production of the anti-leukemic glutaminase free Lasparaginase by newly isolated Streptomyces olivaceus NEAE-119 using response surface methodology. BioMed Res Int 2015; 2015:627031.
7. Ashok, A., Doriya, K., Rao, J. V., Qureshi, A., Tiwari, A. K., & Kumar, D. S. (2019). Microbes producing L-asparaginase free of glutaminase and urease isolated from extreme locations of Antarctic soil and moss. Scientific Reports, 9 (1), 1423.
8. Deshpande, N., Choubey, P., & Agashe, M. (2014). Studies on optimization of growth parameters for L-asparaginase production by Streptomyces ginsengisoli. The Scientific World Journal, 2014, Article ID 895167.
9. Narayana KJP et al. (2007) L-asparaginase production by Streptomyces albidoflavus. Indian J.Microbiol. 48(3): 331-336.
10. Mostafa SA and Salama M S (1979) L-asparaginase producing Streptomyces from soil of Kuwait. Zentralbl Bakterol 134:325–334.
11. Lopes Fernanda Cortez, Ligabue-Braun Rodrigo. 2021., Agro-Industrial Residues: Eco-Friendly and Inexpensive Substrates for Microbial Pigments Production Frontiers in Sustainable Food Systems, VOL 5: 2021.589414.

Cite this Article: Vijayakumar, H., Juby T R (2025). Soil Actinomycetes as Potential Producers of L-Asparaginase: Qualitative, Quantitative and Fermentation Studies. International Journal of Current Science Research and Review, 8(12), pp. 5904-5909. DOI: https://doi.org/10.47191/ijcsrr/V8-i12-04

 *Corresponding Author: Juby T R