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Molecular Docking and Antibacterial Potential of Marine *Streptomyces laurentii* Derived L-Asparaginase against ESBL-Producing Pathogens

[1]Vindhuja Viswan, [2]T.Shankar, [3]Lakshmanan Ashokkumar

[1]Research Scholar, Dept. of Microbiology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Tiruchengode - TK, Namakkal District, Tamilnadu - 637 205, India.

[2]Professor, Dept. of Microbiology, Excel College for Commerce and Science, Komarapalayam-637303, Namakkal Dt, Tamilnadu, India.

[3]Dept. of Biotechnology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Tiruchengode - TK, Namakkal District, Tamilnadu - 637 205, India.

Corresponding author: Lakshmanan Ashokkumar, Dept. of Biotechnology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Tiruchengode - TK, Namakkal District, Tamilnadu - 637 205, India.

E-mail : *ashokmicro2010@gmail.com,* ORCID: *https://orcid.org/0000-0003-2616-4751*

Abstract

Emergence of multiple drug-resistant bacteria capable of producing β-lactamase enzymes that can degrade a wide range of pharmaceuticals has generated an emergent need for new therapeutic agents. The objective of the current work was to screen marine *Actinomycetes* for their potential to produce L-asparaginase, a compound that could potentially exhibit antibacterial activity. A total of 32 *Actinomycete* isolates was obtained from marine sediment samples collected from Rameshwaram, India. Initial screening revealed 15 isolates capable of producing l-asparaginase, with isolate AI 17 exhibiting the highest enzyme activity (78.19 U/mg). Through the process of molecular identification using 16s rRNA sequencing, it was determined that belonged to the *Streptomyces laurentii* species. The enzyme was eluted using NH_3SO_4 precipitation, then purified through dialysis and Sephadex g-100 column chromatography, resulting in a specific activity of 127.4 u/mg**.** Antibacterial assays demonstrated dose-dependent inhibition of ESBL-producing clinical isolates, particularly *Pseudomonas aeruginosa*, *E. coli*, and *A. baumannii*. Molecular docking analysis using the HDOCK server revealed a strong binding affinity between L-asparaginase and the *P. aeruginosa* target protein 2YNU, with a docking score of –281.52 and a confidence score of

0.9328. Key interface residues involved in the interaction were identified, supporting a dual antimicrobial mechanism: structural interference with bacterial proteins and enzymatic depletion of L-asparagine, essential for bacterial survival. These findings highlight *S. laurentii*-derived L-asparaginase as a promising candidate for developing novel antimicrobial therapies against resistant bacterial strains.

Keywords: Multidrug-resistant (MDR) bacteria, Extended-spectrum β-lactamase (ESBL), L-asparaginase, Marine actinomycetes, *Streptomyces laurentii*, Antibacterial activity.

Introduction

The global healthcare community is grappling with the challenge of resistance to antibiotics, which poses a significant threat to patient care. The main reason for resistance is the excessive and improper use of antibiotics, which is not confined to hospitals but is also prevalent in the food, agriculture, and other industries. Antibiotics that are prescribed in treating infections caused by *Enterobacteriaceae* are mostly beta-lactam medications, including carbapenems, cephalosporins, monobactams, and extended-spectrum penicillins. The widespread administration of β-lactam antibiotics has led to a marked rise in resistance among Enterobacteriaceae, primarily driven by the enhanced production of β-lactamase enzymes (Teklu *et al.,* 2019).

The ESBL-producing enterobacteria on the WHO's 2017 priority list of pathogens and the CDC's 2019 list of resistant pathogens pose a critical and serious threat, necessitating the urgent development of alternative treatment sources for these resistant pathogens (Mancuso *et al.,*2021). L-asparaginase (EC 3.5.1.1), also identified as L-asparagine amidohydrolase (ASNase) is one of the most widely used non-toxic medical enzymes and supplies 40% of the world's enzyme needs (Jia *et al.,* 2021). Numerous plants, animals and microorganisms *viz.,*filamentous fungi, bacteria, and yeasts, are known to contain it. These are believed to be the best sources of this potent enzyme. It is crucial for the nutrition, drug, and pharmaceutical industries, particularly for the therapeutic management of cancers such as acute Hodgkin's lymphoma andlymphoblastic leukemia (Qeshmi *et al.,* 2018).

Over the past decades, L-asparaginase has been widely recognized for its various beneficial applications. Recent investigations have highlighted increasing interest in the antimicrobial potential of L-asparaginase, with multiple studies demonstrating its inhibitory activity against various pathogens when sourced from diverse biological origins especially reported the antibacterial properties of L-asparaginase isolated from both marine and terrestrial environments (Meganathan 2016); (Jeea and Sathiyamurthy, 2016); (Vimal and Kumar 2017); (Vimal and Kumar 2021) and (Meng *et al.,* 2011). However, reports on antimicrobial L-asparaginase produced specifically by marine *Streptomyces* species remain limited especially against ESBL pathogens.

Collectively, these findings underscore the antimicrobial effectiveness of L-asparaginase and support the need for further investigations to validate its applicability as a therapeutic agent. Since drug development is a lengthy process with multiple validation stages, L-asparaginase emerges as a promising candidate. Therefore, its development should be accelerated with a focus on safety and understanding its mechanism of action. Molecular docking is a crucial computational tool in drug design, predicting how a drug binds to its target protein. It helps identify binding sites, estimate binding strength, and evaluate drug efficacy. By enabling virtual screening, it accelerates drug discovery, reduces costs, and supports the development of safer, more effective drugs (Balan 2013). This research seeks to explore the antimicrobial activity of L-asparaginase synthesized by marine *Streptomyces* species against ESBL-producing bacterial

pathogens and to explore the enzyme's mechanisms of action against bacteria through molecular docking.

Methodology

Collection of clinical isolates

For this study, clinical isolates were obtained from Microtech Clinical Laboratory in Coimbatore, Tamil Nadu, India. The isolates were confirmed as ESBL (Extended Spectrum Beta-Lactamase) producers based on the method described by (Balan 2013). To detect ESBL production, discs containing Amoxicillin (30 µg) and a combination of Amoxicillin (30 µg) with Clavulanic acid (10 µg) were positioned at an edge-to-edge distance of 15 mm on MHA (Mueller-Hinton Agar). After a 24hr incubation period, the inhibitory zone around both discs was measured. An increase of ≥5 mm in the zone area surrounding the Amoxicillin–Clavulanic acid disc in relation to that of the Amoxicillin disc alone was interpreted as a positive indication of ESBL production.

Soil sampling

Soils from marine environments were collected from five geographically distinct locations in Rameshwaram, maintaining a minimum inter-site distance of 200 meters. Sampling was performed at a depth of approximately 15 cm below the surface thereby minimizing external factors in analysis. The samples were transported to the laboratory in sterile polythene bags. To selectively inhibit non-spore-forming microbes, the samples dried at 65 °C for 1 hr, following the protocol outlined by (Kashif *et al.,* 2016).

Isolation of Actinomycetes

A 1-gram portion of the pre-treated sediment was measured and added to 100 mL of saline (0.9%). The mixture was agitated at RT for 10 min in an orbital shaker to ensure uniform suspension, which was then used as the stock culture. Serial dilutions were carried out from 10^{-1} to 10^{-6}.

From each dilution, 1 mL was dispensed into sterile Petri dishes, and molten SCNA (Starch Casein Nitrate Agar) medium, augmented with 50 µg/mL of cycloheximide and nystatin as for suppress fungal contamination. Once the medium solidified, incubation was performed for 7 to 15 days at 28°C to allow for colony development, following the protocol described by (Raja and John 2014).

The colonies that developed showed a variety of morphological features, often characterized by a rough or powder-like texture and a dry, wrinkled surface. They were tightly attached to the agar medium and exhibited branched filaments, sometimes with aerial mycelium, in line with earlier reports. Individual colonies were isolated and transferred to ISP2 slants for storage at 4°C and were also preserved in glycerol (20%) at -20°C for future analysis.

Detection of L-asparaginase activity among isolates

L-asparaginase-active bacterial isolates were identified using the method of (Mostafa *et al.,* 2019) with slight modifications. M9 medium (HiMedia) was supplemented with 0.1% L-asparagine, 2% agar, and 0.03% phenol red, with the pH adjusted to 6.5. Following incubation at 37°C for 3 days, the presence of a pink halo surrounding each bacterial colony demonstrates L-asparaginase activity.

Isolation of potential isolates with enzyme assay

Inoculum Preparation

Inoculum was prepared in an Erlenmeyer flask (250 ml) with sterile 50 mL of asparagine dextrose salts medium (1.0% L-asparagine, 0.1% K_2HPO_4, 0.2% dextrose, and 0.05% $MgSO_4$) The medium was seeded with 3 agar discs (8 mm) of 7-day-old starch nitrate agar cultures. Incubation (150 rpm, 72 hrs, and 30°C) was carried out in a rotary shaker.

L-asparaginase biosynthesis under submerged conditions

The screened microbial strains were propagated in 50 mL of the same medium (adjusted to the desired pH) in 250 mL flasks. The incubation was performed with agitation at 35°C on a rotary platform shaker (150-250 rpm). After incubation, mycelium was extracted by centrifugation (5000g, 4°C for 30 min).

L-asparaginase Assay

L-asparaginase activity was evaluated by estimating the release of NH_3 during the enzymatic hydrolysis of L-asparagine, following a modified protocol adapted from (Basha *et al.,* 2009). The reaction mixture (2 mL) comprised 1.5 mL of 0.04 M L-asparagine prepared in 0.05 M Tris-HCl buffer (pH 8.6) and 0.5 mL of the enzyme extract. The reaction was incubated at 37°C for 30 min and terminated by the addition of 0.5 mL of 1.5 M trichloroacetic acid (TCA). A control was prepared by adding the enzyme after TCA to eliminate non-enzymatic background. The samples were centrifuged at 10,000 × g for 5 min, and 0.5 mL of the resulting supernatant was mixed with 1 mL of Nessler's reagent and 7 mL of distilled water. After incubation at RT for 20 min, absorbance was recorded at 480 nm. The concentration of NH_3 was calculated using a standard curve generated with ammonium chloride. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of NH_3per min under the assay conditions. Enzyme activity was expressed in units per gram of dry substrate (U/gds).

Identification of potential isolate

The isolate exhibiting the maximum L-asparaginase activity was subjected to molecular identification through PCR amplification followed by partial 16S rRNA gene sequencing. Sequencing was outsourced to Barcode Biosciences (Bangalore, India). The obtained sequence was aligned manually and analyzed with related sequences of *Actinomycete* available in the GenBank database using the BLAST tool for species-level identification.

Purification of L-asparaginase from potential isolate:

The crude-extract of the enzyme underwent stepwise ammonium sulfate precipitation, beginning with 45% saturation through overnight at 4°C maintained at pH 8.4. The mixture was then centrifuged for 10 min at 8000 rpm, and the resultant supernatant was further treated to reach 85% saturation. The precipitated protein was separated and redissolved in 1 M Tris-HCl buffer. This solution was subjected to dialysis using tubing pre-equilibrated with Tris-HCl buffer to remove residual salts. The enzyme was further purified using gel filtration chromatography on a Sephadex G-100 column pre-equilibrated with 0.05 M Tris-HCl buffer (pH 8.4) with 0.1 M KCl. Thirty fractions (5 mL each) were collected at 30min intervals, and those exhibiting high L-asparaginase activity were pooled for downstream analysis (Basha *et al.,*2009).

Antibacterial activity of L-asparaginase against ESBL producing isolates

To investigate the antibacterial efficacy of L-asparaginase, a modified protocol based on the method reported by (Kamar *et al.,* 2023).Clinical isolates producing extended-spectrum β-lactamases (ESBLs), obtained from a diagnostic laboratory, and were cultured overnight to obtain an inoculum strength 10^8 CFU/mL. A uniform lawn was achieved by spreading the suspensions evenly over Mueller-Hinton agar plates. Sterile stainless-steel borers were used to punch wells of 6 mm diameter into the agar. Various concentrations of the enzyme preparation were loaded into the wells using a micropipette. As controls, ciprofloxacin (5 µg) was employed for positive reference, and PBS was used to represent baseline (negative) conditions. Plates were maintained at 37 °C for a period of 24 hrs under standard incubation conditions., after which the antimicrobial effect was evaluated by measuring the inhibition zones surrounding the wells in mm.

Molecular docking analysis

Molecular docking analyses of protein–protein and protein–nucleic acid interactions were performed via the HDOCK platform. This tool employs a hybrid approach that combines template-driven prediction with ab initio simulations to enhance the accuracy of molecular interaction modeling. Several evaluation metrics were used to assess the quality and biological relevance of the docking results. The docking score was computed using an iterative, knowledge-driven scoring algorithm, IT Score PP for protein-protein interactions or IT Score PR for protein-DNA/RNA interactions. A lower docking score typically indicates a stronger predicted interaction; however, it does not directly correlate with experimental binding affinity, as the scoring function is not empirically calibrated. To further assess binding probability, a confidence score was calculated based on the docking score.

The score reflects the predicted binding affinity, with values above 0.7 indicating strong binding potential, scores between 0.5 and 0.7 suggesting moderate likelihood, and values below 0.5 denoting a low probability of interaction. It is vital to recognize that the confidence score is empirically derived and should be interpreted with caution. Additionally, ligand RMSD (Root Mean Square Deviation) was determined by comparing the ligand's conformation in the docking model with that of the input or modeled structure. In this context, ligand RMSD does not necessarily indicate the accuracy of the model but reflects the conformational variation. Finally, interface residues were identified based on residue pairs within a 5.0 Å distance among the ligand and receptor in the predicted complex. These residues provide insight into potential interaction sites and help infer binding specificity. The HDOCK server also offers downloadable data for further analysis of these interaction interfaces.

Results and Discussion

Pharmaceutical innovation has been a defining achievement in 20th-century biomedical science, with drugs like penicillin and streptomycin revolutionizing disease treatment. These therapies have extended lifespan and improved quality of life, making society increasingly dependent on effective medicines. However, many diseases—such as cancer, bacteria, viral and fungal infections, inflammatory, and neurodegenerative disorders—still lack effective treatments. Rising resistance in pathogens and cancer cells further demands ongoing drug innovation. Furthermore, drug resistance in microbes highlights the urgent need for continued investment in new antibiotics (Salam *et al.,* 2023).

Marine ecosystems span over 70% of planet's surface area, are rich in microorganisms, especially marine actinobacteria, which are valuable sources of bioactives with antimicrobial, anticancer, and antiviral properties. Among them, *Streptomyces* species are particularly known for producing diverse natural products. Although India's marine ecosystems remain underexplored, Indian researchers have made significant contributions in isolating marine actinomycetes (Sarkar and Suthindhiran, 2022). This study focuses on exploring marine *Streptomyces* for novel antimicrobial compounds effective against multidrug-resistant and ESBL-producing pathogens.

Marine sediment soils were sampled from five distinct locations within the Rameshwaram area, from which 32 actinomycete isolates (AI 1–AI 32) were obtained and characterized based on their morphological features on SCA medium. A large percentage of isolates demonstrated white, dry, circular colonies with variations in elevation and texture, including concave, convex, and flat morphologies. Some isolates showed brown and gray-colored colonies with concave and convex shapes, as well as both regular and irregular colony structures. These morphological characteristics are consistent with those reported in previous studies. For instance Ghanem *et al.,* 2000 were observed that actinomycetes isolated from marine sediments in Alexandria exhibited powdery, compact colonies with varying pigmentation. Similarly Meenakshi *et al.,* 2024 were noted that actinomycetes on SCA medium

typically form white to brown, gray colonies that are powdery in appearance, which were observed in marine sediment samples.

The phenol red agar assay was employed to screen all 32 actinomycete isolates for L-asparaginase activity. Among them, 15 isolates (approximately 46.8%) exhibited positive results, as evidenced by the development of a pink zone encircling the colonies, indicating L-asparaginase secretion. The remaining 17 isolates showed no such activity and were considered negative. While L-asparaginase from bacterial sources is effective, its prolonged use can cause hypersensitivity and allergic reactions. Actinomycetes, particularly *Streptomyces* species, are promising alternatives for safer enzyme production. Previous studies have identified several *Streptomyces* strains, including *S. venezuelae*, *S. karnatakensis*, *S. longisporoflavus*, and a marine *Streptomyces* sp. PDK2, as potent L-asparaginase producers (Dhevendaran and Annie 1999) and (Narayana *et al.,* 2008).The positively screened isolates were subjected to enzyme assays to determine L-asparaginase activity, and the isolate showing the highest enzyme production was recognized as the potential L-asparaginase producer. A total of fifteen microbial strains were assessed for the L-asparaginase, biosynthesis. Among the isolates, AI 17 displayed the highest specific activity of 78.19U/mg, followed closely by AI 19 and AI 18, with quantified enzyme activities of 67.18 U/mg and 67.03 U/mg, for the respective isolates (Table.1). These high values indicate a strong potential for L-asparaginase production. According to a prior report (Raja *et al.,* 2023). *Streptomyces antibioticus* KPMS7 was isolated from Gulf of Mannar sediments and showed substantial L-asparaginase activity. The study highlighted the potential of marine actinomycetes from Tamil Nadu as sources of L-asparaginase.

Table .1 Screening of L-asparaginase producing potential isolates

S.No	Isolates name	Enzyme (U/ml)	Protein (mg/ml)	Catalytic efficiency (U/ml)
1.	AI 2	84	2.37	35.44
2.	AI 8	121	2.19	55.25
3.	AI 12	128	2.38	53.78
4.	AI 13	115	1.89	60.85
5.	AI 14	110	2.19	50.23
6.	AI 17	165	2.11	78.19
7.	AI 18	124	1.85	67.03
8.	AI 19	131	1.95	67.18
9.	AI 23	124	1.94	63.92
10.	AI 24	114	1.87	60.96
11.	AI 25	111	1.78	62.36
12.	AI 28	106	2.14	49.53
13.	AI 30	94	2.11	44.55
14.	AI 31	116	2.88	40.28
15.	AI 32	121	2.74	44.16

Based on enzyme assay results, the isolate AI 14, which showed the maximal activity for L-asparaginase, was subjected for molecular identification. The 16S rRNA gene of isolate AI 17 was sequenced, yielding a fragment of approximately 1100 base pairs. BLAST analysis against the GenBank database indicated close similarity to several members of the *Streptomyces* genus. A phylogenetic tree (Fig. 1) was generated using the neighbor-joining algorithm to illustrate the evolutionary relationship of the isolate with related *Streptomyces* species. Specifically, strain AI 14 clustered within a clade that included *Streptomyces laurentii*, showing strong similarity to the reference sequence with GenBank accession number MH542246.1. This highlights the advantages of genotypic identification over phenotypic methods, which can be influenced by environmental factors, leading to misidentification (Petti *et al.,* 2005). The 16S rRNA gene employed as a well-established marker for bacterial phylogeny and taxonomy owing to its conserved regions interspersed with species-specific hypervariable segments (Woo *et al.,* 2008). While several studies have documented the recovery of *Streptomyces* strains that are capable of producing L-asparaginase from marine ecosystem, a survey of existing literature indicates that *Streptomyces laurentii* has not previously been reported as an L-asparaginase producer from marine environments..

The confirmed isolate of *Streptomyces laurentii* was subjected to L-asparaginase enzyme synthesis. After production, enzyme was purified with 3 steps. The initial crude enzyme extract demonstrated an activity of 165 U/mL with a protein content of 2.11 mg/mL, resulting in a specific activity of 78.19 U/mg. Precipitation using NH_3SO_4 reduced the enzyme activity to 121 U/mL but led to an increase in specific activity to 85.81 U/mg, corresponding to a 1.09-fold purification and a yield of 73.33%. Further purification by dialysis enhanced the specific activity, yielding a 1.17-fold purification and a final recovery of 59.39%.Subsequent Sephadex G-100 chromatography resulted in a peak specific activity of 127.4 U/mg, reflecting improved enzyme purity. However, the yield declined to 39.39%, with a fold increase in purity of 1.26 (Table 2).

Table 2. Sequential Processing and Recovery Metrics of L-asparaginase from *Streptomyces laurentii*

S.No.	Purification steps	Enzymes value	Protein value	Specific enzyme (U/ml)	Purification fold	% of Yield
1	Crude	165	2.11	78.19	0	100
2	Ammonium sulphate	121	1.41	85.81	1.09	73.33
3	Dialysis	98	0.97	101.0	1.17	59.39
4	Sephadex	65	0.51	127.4	1.26	39.39

In this study, multiple bacterial genera were isolated, each represented by at least three individual strains. All isolates underwent screening for ESBL production using the double disc synergy method, and six genera tested positive for ESBL activity. In recent decades, ESBL-producing bacteria have emerged as a significant public health concern in both hospital and community settings. These organisms are associated with limited treatment options, severe infections, high mortality rates, and increased healthcare costs. In intensive care units (ICUs), they are frequently linked to ventilator-associated pneumonia. Risk factors for infection include prolonged hospital stays (Husna *et al.,* 2023). Hence, alternative therapeutic approaches are urgently required to combat ESBL-producing pathogens. Previous studies have demonstrated that marine-derived microbes and compounds effectively combat pathogens without adverse side effects and are considered safe (Ghosh *et al*., 2022; Bharathi and Lee, 2024). Building on this foundation, the present study was undertaken.

The enzyme, after purification, was assessed for its inhibitory effect on ESBL-producing pathogens. The L-asparaginase exhibited dose-dependent antibacterial efficiency against ESBL-producing isolates. *E. coli*, *E. faecalis*, and *A. baumannii* showed increasing inhibition zones up to 16 mm, while *P. aeruginosa* showed the highest response, reaching 18 mm at 40 U/ml. *S. aureus* was sensitive only at higher concentrations, and *K. pneumoniae* showed moderate activity. No inhibition was observed with PBS, and only selected isolates responded to ciprofloxacin. Highlight the antimicrobial potential of L-asparaginase for future therapeutic exploration, especially against *P. aeruginosa*, *E. coli*, and *A. baumannii*. Number of authors determined the antimicrobial activity of L-asparaginase from various bacterial isolates, but very scares the antimicrobial activity producing L-asparaginase from marine isolates of *Streptomycetes* (Table 3 and Fig.2).

Table 3.Antibacterial activity of *Streptomyces laurentii* producing L-asparaginase enzyme against ESBL isolates

S.No	Isolates	Different con. of enzyme (U/ml) Zone of measured in mm				PBS	Ciprofloxacin
		10	20	30	40		
1.	*E.faecalis*	10	12	14	16	-	-
2.	*K.pneumoniae*	-	10	11	13	-	10
3.	*E.coli*	-	12	14	16	-	-
4.	*S.aureus*	-	-	12	15	-	-
5.	*A. baumannii*	10	12	14	16	-	17
6.	*P.aeruginosa*	10	13	15	18	-	12

In alignment with our results, Ayyanar (2016) revealed that L-asparaginase exhibited significant inhibitory activity against both Gram-negative and Gram-positive bacterial isolates. Their research highlights the role of L-asparaginase in inhibiting growth by disrupting essential metabolic pathways, which is particularly beneficial against hospital-associated infections caused by resistant bacteria. In their study, 10 U/ml of the enzyme suppressed 3 bacterial isolates, while 30 U/ml inhibited 6 types of bacteria. A comparable observation was reported by Sivasankar *et al.* (2013), where a Streptomyces isolate from Bay of Bengal sediments documented an enzyme production of 25.90 IU and demonstrated antimicrobial potential.

Furthermore, mechanisms of antimicrobial activity of L-asparaginase enzyme were determined with molecular docking study. Among the 6 isolates tested *P.aeruginosa* was highly suppressed, hence such isolate was carryout to docking study. The potein-protein docking analysis was conducted using the HDOCK server to investigate the interaction between the L-asparaginase-producing receptor protein A0A3D5DV37 from *Actinomycetes bacterium* (369 amino acids) and the ligand protein 2YNU from *Pseudomonas aeruginosa* (233 amino acids). The input was provided in PDB format, and the server generated 4392 possible docking conformations. As shown in Figure 3, the docking process revealed a significant interaction between the two proteins,

and the best-ranked model, presented in Figure 3, showed a docking score of-281.52 kcal/mol and a high confidence score of 0.9328, indicating a strong binding affinity.

The analysis identified several critical interface residues contributing to the receptor-ligand binding. Key residues on the receptor protein A0A3D5DV37 involved in the interaction included GLU 124A, ASN 128A, PHE 121A, PHE 125A, ASN 129A, PHE 6A, TYR 120A, MET 127A, SER 4A, GLU 5A, GLY 7A, TYR 10A, ARG 68A, ARG 11A, TYR 8A, and LYS 116A. These residues were repeatedly observed in the docking interface, indicating their essential role in stabilizing the complex. Figure 4 validated the high quality of the input structures used for docking, supporting the reliability of the predicted interactions. Overall, the docking study suggests a strong and biologically meaningful interaction between L-asparaginase and the Pseudomonas ligand, which may have implications in therapeutic or antibacterial applications.

The inhibition mechanism of *Pseudomonas aeruginosa* by the L-asparaginase enzyme can be understood through its binding interference and enzymatic activity. The molecular docking analysis indicates that L-asparaginase may bind to a vital protein (2YNU) in *P. aeruginosa*, which is likely involved in essential metabolic or regulatory processes. This binding may block the protein's active site, interfere with critical protein-protein interactions necessary for bacterial survival, or induce conformational changes that deactivate the target protein. In addition to this structural interference, if the L-asparaginase enzyme retains its catalytic function upon binding, it can enzymatically degrade L-asparaginea key amino acid required for protein biosynthesis in *P. aeruginosa*, particularly under stress conditions. The depletion of L-asparagine would subsequently inhibit bacterial protein synthesis, leading to reduced bacterial growth or cell death (Meghavarnam *et al.,* 2017). This dual mechanism of action structural inhibition through binding and functional suppression *via* substrate depletion supports the therapeutic promise of L-asparaginase in combating *P. aeruginosa*.

This work demonstrates that *Streptomyces laurentii* from marine origin holds promise as a dual-purpose source of L-asparaginase and antibacterial agents. Among 32 marine actinomycete isolates, AI 17 exhibited the highest L-asparaginase activity, while AI 17 was identified through 16S rRNA sequencing as *Streptomyces laurentii*. Sequential purification steps resulted in a final enzyme preparation exhibiting a catalytic efficiency of 127.4 U/mg. Antibacterial assays demonstrated dose-dependent inhibition against ESBL-producing pathogens, particularly *Pseudomonas aeruginosa*. Molecular docking confirmed a strong interaction between L-asparaginase and a key protein of *P. aeruginosa*, indicating a dual antimicrobial mechanism involving both structural interference and L-asparagine depletion. These findings underscore the therapeutic value of marine actinomycetes in addressing multidrug resistance. Furthermore, L-asparaginase from *S. laurentii* represents a promising alternative to conventional antibiotics and may serve as a complementary agent in combination therapies to improve treatment outcomes and mitigate resistance development.

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