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Research Article

Optimization of Protease Production and Antimicrobial Activity of *Pseudomonas aeruginosa* RGT01 Isolated from Mangrove Sediments of Vellar Estuary

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Abstract

In this study, bacteria were isolated from mangrove sediments of Vellar estuary and screened for extracellular enzyme production. Among the twenty bacterial isolates obtained, *Pseudomonas aeruginosa* RGT01 isolate exhibited the highest proteolytic activity and was selected for further optimization. The highest proteolytic activity was observed at 1.5% NaCl concentration, pH 8, 35°C and 48 h incubation period, with lactose serving as the most suitable carbon source. Under optimized conditions, the isolate produced a maximum zone of clearance of 9.7 ± 0.4 mm on skim milk agar plates. The crude extract obtained from *Pseudomonas aeruginosa* RGT01 exhibited broad-spectrum antimicrobial activity against several clinical bacterial and fungal pathogens. The highest antibacterial activity was observed against *Streptococcus sp.* (23 mm), while the strongest antifungal activity was recorded against *Aspergillus fumigatus* (31 mm). Fourier Transform Infrared (FTIR) analysis revealed the presence of various functional groups, including alcohols, phenols, alkanes, carbonyl compounds and aromatic derivatives, indicating the presence of bioactive metabolites. The findings of this study demonstrate that mangrove-associated *Pseudomonas aeruginosa* RGT01 is a promising source of extracellular protease and antimicrobial compounds with potential industrial and pharmaceutical applications.

Keywords: Marine Bacteria; *Pseudomonas aeruginosa*; Mangrove Sediment; Enzyme Optimization; Antimicrobial Activity; FTIR Analysis

Introduction

Proteases, also known as peptidases or proteinases, constitute one of the most important groups of industrial enzymes because of their extensive applications in diverse industrial sectors. Owing to their versatility and catalytic efficiency, proteases account for nearly 60-75% of the global enzyme market and are widely utilized in detergent, food processing, pharmaceutical, leather, brewing, photography and waste treatment industries [1,2]. Among them, alkaline proteases are of industrial significance due to their high stability and activity under alkaline conditions, making them highly suitable for applications in detergents and leather processing industries [2,3].

Microorganisms, especially bacteria, are extensively exploited for protease production because of their rapid growth and their ability to secrete extracellular enzymes directly into the culture medium, thereby simplifying downstream processing. Bacterial genera such as *Bacillus* and *Pseudomonas* are recognized as efficient producers of industrially important proteases [4,5].

In recent years, increasing attention has been focused on halophilic and marine microorganisms as potential sources of industrial enzymes. Enzymes derived from these microorganisms often exhibit optimal activity at high salt concentrations and remarkable

stability under extreme environmental conditions, where conventional enzymes frequently lose their functionality [6]. Furthermore, several studies have demonstrated that protease-producing bacteria possess antimicrobial properties through the degradation of structural proteins in pathogenic microorganisms, thereby broadening their potential applications in biotechnology and medicine [7].

Mangrove sediments are rich in organic detritus and harbour diverse microbial communities, particularly proteolytic microorganisms that play a crucial role in nutrient recycling, ecosystem productivity and ecological balance [8]. With the rapid advancement of biotechnology and the growing demand for eco-friendly and efficient industrial processes, there is an increasing need to explore novel microorganisms capable of producing enzymes with unique and improved characteristics. In this context, bacterial isolates obtained from mangrove sediments of the Vellar estuary were screened for extracellular enzyme production. Among the isolates, *Pseudomonas aeruginosa* exhibited significant proteolytic enzyme production along with antimicrobial activity against various human pathogens. The present study highlights the potential of mangrove-associated bacteria as promising sources of industrial enzymes and bioactive compounds with potential biotechnological applications.

Materials and Methods

Isolation Of Bacteria

Approximately 100 g of sediment samples were collected from a dense mangrove region of the Vellar estuary, Parangipettai, Tamil Nadu, India (11.491134°N, 79.765664°E). The samples were aseptically transferred to the Microbiology Laboratory, Centre of Advanced Study (CAS) in Marine Biology, Annamalai University, for further processing.

1g of sediment sample was serially diluted from 10^{-1} to 10^{-6} using sterile saline solution. Aliquots from appropriate dilutions were inoculated onto nutrient agar plates by the spread plate method and incubated at 37°C for 48 h. Distinct bacterial colonies obtained after incubation were selected and purified through repeated streaking on fresh agar plates.

Identification of Bacterial Isolates

Preliminary identification of the bacterial isolates was carried out based on colony morphology and Gram staining characteristics following the guidelines provided in Bergey's Manual of Systematic Bacteriology [9]. A total of 20 morphologically distinct bacterial colonies were selected for further screening.

Screening for Extracellular Enzyme Production

All 20 bacterial isolates were screened for extracellular enzyme production, including amylase, protease and lipase activities, using starch agar, skim milk agar and tributyrin agar media, respectively. The inoculated plates were incubated at 37°C for 24 h.

Amylase activity was detected by flooding starch agar plates with 1% iodine solution, where the formation of clear halos around colonies indicated starch hydrolysis. Protease and lipase activities were determined by the appearance of clear zones surrounding the bacterial colonies on skim milk agar and tributyrin agar plates, respectively. Based on the zone clearance, the present study concentrated only on protease optimization.

Molecular *Identification* of *Iso*late RGT01

Among the 20 isolates screened, isolate RGT01 exhibited the highest protease-producing ability and was therefore selected for molecular characterization. Genomic DNA of isolate RGT01 was extracted from an overnight-grown culture following the method described by [10]. The extracted DNA was used as a template for amplification of the 16S rRNA gene using the primers 533F (5′-GTGCCAGCAGCCGCGGTAA-3′) and 1100R (5′-AGGGTTGCGCTCGTTG-3′). Polymerase Chain Reaction (PCR) amplification was performed with an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 40 s, with a final extension at 72°C for 10 min. The amplified PCR products were purified and subjected to bidirectional Sanger sequencing at the Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkazhi.

Optimization of Protease Production

The protease-producing isolate, *Pseudomonas aeruginosa* RGT01, was subjected to optimization studies to determine the conditions for maximum protease production. The effect of salinity was evaluated at different NaCl concentrations (0.5%, 1%, 1.5%, 2% and 2.5%). The influence of incubation time was studied at intervals of 12, 24, 36 and 48 h. Different carbon sources,

including dextrose, sucrose, maltose, lactose, glucose and fructose, were individually supplemented into the medium at a concentration of 0.2 g per plate to assess their effect on enzyme production. The effect of pH was evaluated by adjusting the medium to pH values ranging from 5 to 9. Similarly, the influence of temperature was studied by incubating the cultures at 20°C, 25°C, 30°C, 35°C and 40°C. Following incubation, protease production was assessed by measuring the diameter of the clear zone formed on skim milk agar plates, which indicated casein hydrolysis and proteolytic activity.

Estimation of Protease Activity

Under optimized conditions (1.5% salinity, pH 8, 1.0% lactose, 35°C, 48 h), protease activity was evaluated on skim milk agar by comparing clearance zones to those produced by a Proteinase K standard (Hi-Media). A 10 µL aliquot of the Proteinase K standard was added to agar wells; plates were incubated at 35°C for 48 hours and the zones of clearance were measured.

Extraction of Bioactive Compounds

The cultured broth of *Pseudomonas aeruginosa* RGT01 was mixed with an equal volume of ethyl acetate (1:1, v/v) and agitated at 100 rpm for 1 h in a shaking incubator to facilitate extraction of bioactive compounds. The mixture was subsequently transferred to a separating funnel and the organic phase was collected. The collected extract was filtered through Whatman No. 1 filter paper to remove impurities and concentrated using a rotary evaporator (IKA® RV10) to obtain crude extracts. The dried residues were dissolved in the respective solvent and stored at 4°C for further analysis.

Antimicrobial Activity Assay

The antimicrobial activity of the crude extracts was evaluated against selected bacterial pathogens, namely *Bacillus subtilis*, *Escherichia coli*, *Proteus sp.*, *Pseudomonas sp.*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella sp.* and *Vibrio harveyi*, as well as fungal pathogens including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Rhizopus sp.* Mueller-Hinton Agar (MHA) plates were prepared and the test pathogens were uniformly swabbed onto the agar surface using sterile cotton swabs. Wells were aseptically made in the agar plates and 100 µL of crude extract was added into each well. The plates were incubated at 37°C for 24 h, after which the zones of inhibition were measured in millimetres (mm). All experiments were carried out in triplicate.

Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to characterize the functional groups present in the crude extracts [11,12]. The spectra were recorded over the range of 650-4000 cm^{-1} using an Agilent Cary 630 FTIR spectrometer at the Department of Chemistry, Annamalai University. All samples were analysed in triplicate to ensure reproducibility and accuracy of the results.

Results

Identification of the Bacterial Isolate

The obtained 16S rRNA gene sequence of the bacterial isolate was analysed using the BLASTn in the NCBI database. The analysis revealed 100% sequence similarity with *Pseudomonas aeruginosa*. Based on this high sequence identity, the isolate was confirmed as *Pseudomonas aeruginosa* RGT01(Fig. 1). The nucleotide sequence of the isolate was subsequently submitted to the NCBI GenBank database and assigned the accession number (PX954249).

Optimization of Protease Production

The protease-producing bacterial isolate was subjected to optimization studies to determine the conditions for maximum enzyme production. The 1.5% NaCl medium revealed the highest zone of clearance (8.4 ± 0.7mm) in skim milk agar plate (Fig. 2). Protease activity increased with incubation time, showing the highest zone of clearance (8.0 ± 0.9 mm) at 48 hours (Fig. 2), indicating enhanced enzyme production with prolonged incubation. Among the tested carbon sources, lactose supported the highest protease activity with a zone of clearance of (7.6 ± 0.3 mm) (Fig. 2), followed by maltose and glucose, while dextrose showed comparatively lower activity. The effect of pH revealed that protease production increased from pH 5 to pH 8, with maximum activity observed at pH 8 (8.9 ± 0.3 mm) (Fig. 2), after which it declined at pH 9. Temperature also significantly influenced enzyme production, where the highest proteolytic activity was recorded at 35°C (9.5 ± 0.7 mm) (Fig. 2), followed by a slight decrease at higher temperatures. These results indicate that 48 hours incubation, lactose as the carbon source, pH 8 and 35°C temperature are the optimal conditions for maximum protease production by the bacterial isolate.

Estimation of Protease Activity Under Optimized Conditions

Under optimized culture conditions (1.5% NaCl, pH 8, 1% lactose, 35°C for 48 h), the RGT01 isolate exhibited its highest proteolytic activity, producing a clearance zone of 9.7 ± 0.4 mm on skim milk agar. This activity was evaluated against the standard enzyme control, Proteinase K (Table 1).

Antimicrobial Activity

The antimicrobial activity of the crude extract obtained from *Pseudomonas aeruginosa* RGT01 was evaluated against selected clinical bacterial and fungal pathogens and the results are given, the crude extract exhibited considerable antibacterial activity against all tested bacterial pathogens (Fig. 3). Among them, the highest zone of inhibition was observed against *Streptococcus sp.* (23.4 ± 0.5 mm), followed by *Pseudomonas sp.* (17.8 ± 0.mm) and *Proteus sp.* (16.8 ± 0.5 mm). Moderate inhibition was recorded against *Escherichia coli* (16.5 ± 0.mm) and *Salmonella paratyphi* (15.4 ± 0.4 mm), whereas comparatively lower activity was observed against *Staphylococcus sp.* (14.1 ± 0.4 mm), *Salmonella typhi* (12.6 ± 0.7 mm) and *Klebsiella* sp. (12.4 ± 0.2 mm).

Similarly, the crude extract of *Pseudomonas aeruginosa* RGT01 also showed significant antifungal activity against the tested clinical fungal pathogens (Fig. 3). The highest antifungal activity was observed against *Aspergillus fumigatus* with a zone of inhibition of 31.1 ± 0.6 mm, followed by *Candida sp.* (17.6 ± 0.6 mm), *A. niger* (14.7 ± 0.9 mm) and *A. flavus* (14.1 ± 0.7 mm). These results indicate that the crude extract of *Pseudomonas aeruginosa* possesses strong broad-spectrum antimicrobial activity against both bacterial and fungal clinical pathogens.

FTIR Analysis

The FTIR spectrum of the crude extract of *Pseudomonas aeruginosa* RGT01 revealed the presence of several functional groups corresponding to different bioactive compounds (Fig. 4). A broad absorption peak observed at 3296 cm^{-1} indicates the presence of O-H stretching vibrations, which are typically associated with alcohols or phenolic compounds. The strong peaks at 2922 cm^{-1} and 2855 cm^{-1} correspond to C-H stretching vibrations of alkanes, suggesting the presence of aliphatic compounds.

A peak at 2124 cm^{-1} is attributed to C≡C stretching vibrations, indicating the presence of alkyne groups. The absorption band at 1714 cm^{-1} corresponds to C=O stretching vibrations, which are characteristic of carbonyl compounds such as ketones, aldehydes or carboxylic acids. The peak observed at 1461 cm^{-1} is associated with C-H bending vibrations of alkanes. Further peaks at 1269 cm^{-1} and 1043 cm^{-1} indicate C-O stretching vibrations, suggesting the presence of alcohols, esters or ethers. Additional absorption bands at 916 cm^{-1}, 797 cm^{-1} and 700 cm^{-1} correspond to C-H bending vibrations of aromatic or alkene groups. These functional groups indicate that the crude extract contains various bioactive compounds such as alcohols, phenols, alkanes, carbonyl compounds and aromatic derivatives, which may contribute to its antimicrobial activity.

Standard / Sample	Concentration (mg/ml)	Zone of clearance (mm)
Standard Proteinase K (Hi-media)	0.10	11
Pseudomonas aeruginosa RGT01	0.909	10

Table 1: Comparison of standard Proteinase K and protease produced by *Pseudomonas aeruginosa* RGT01.

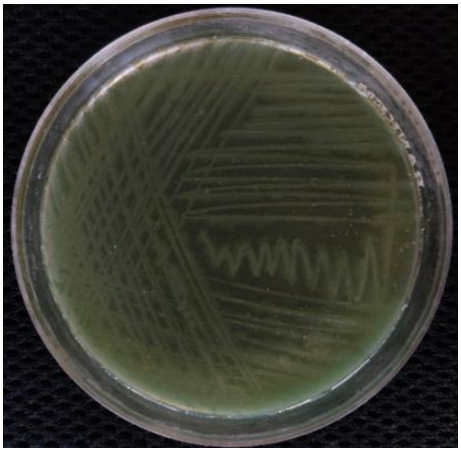

Figure 1: Pure culture of *Pseudomonas aeruginosa* RGT01.

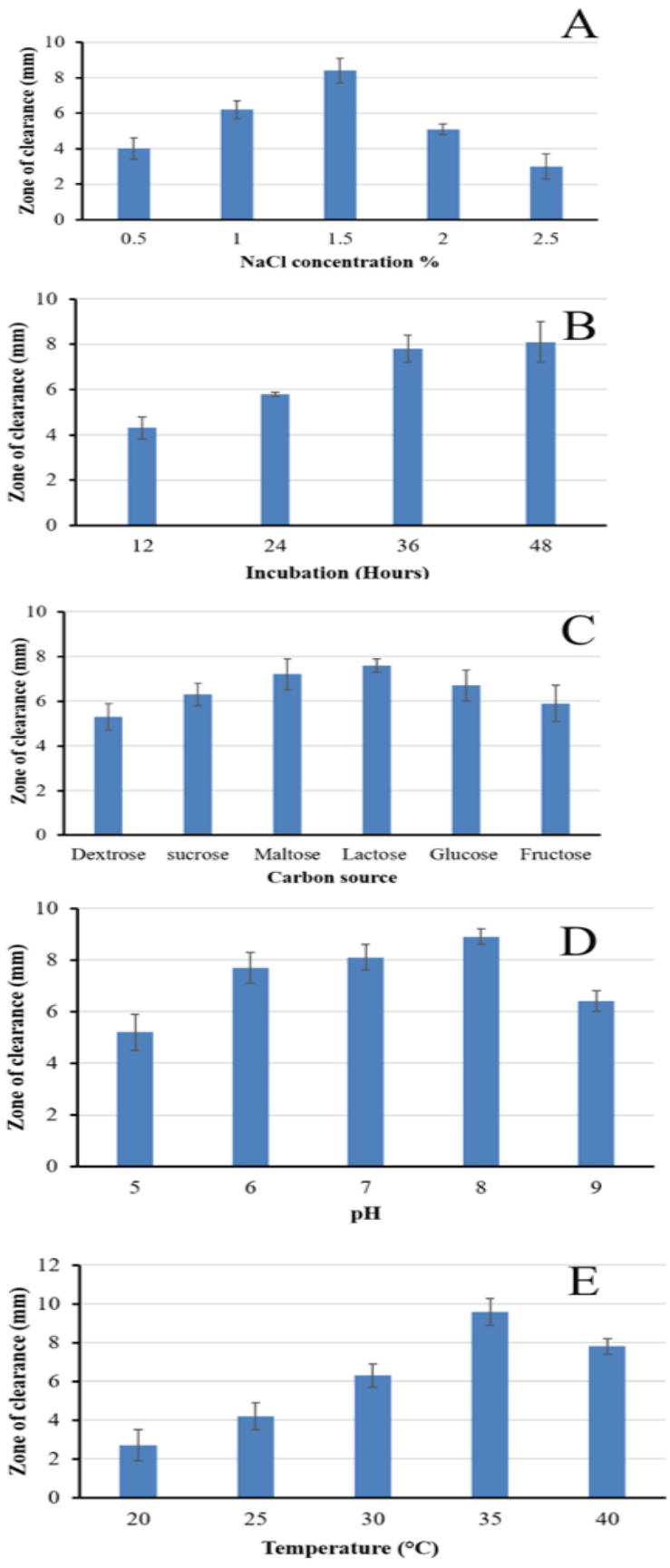

Figure 2: Protease activity of *Pseudomonas aeruginosa* RGT01 at various culture conditions.

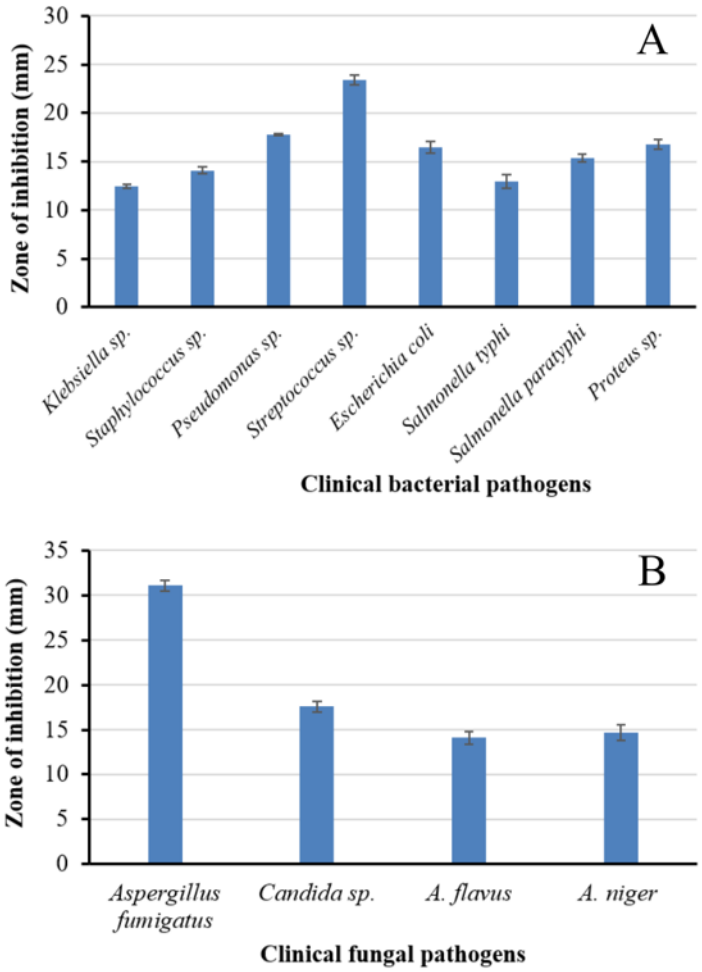

Figure 3: Antagonistic effect of *Pseudomonas aeruginosa* RGT01 against clinical pathogens.

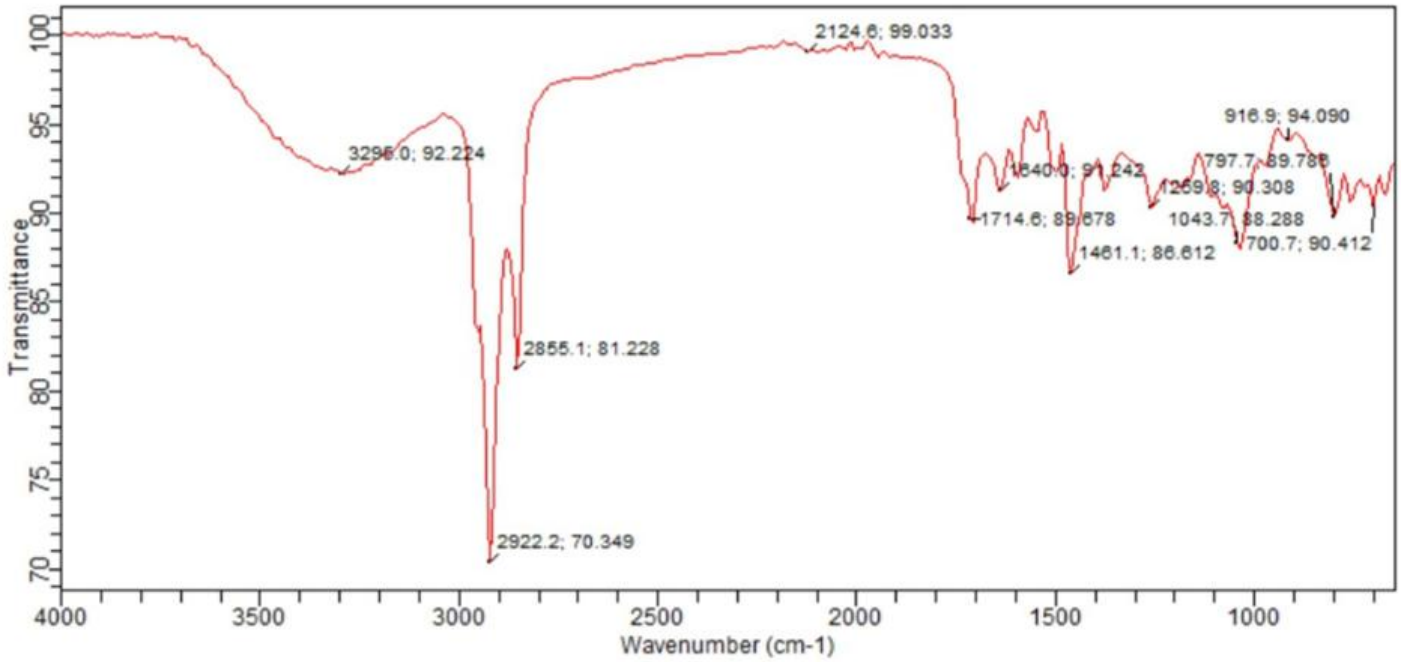

Figure 4: FTIR spectrum of ethyl acetate extract of *Pseudomonas aeruginosa* RGT01.

Discussion

Marine microorganisms have recently emerged as an important source for the isolation of industrial enzymes [13]. Marine bacterial enzymes offer several advantages for industrial utilisation [14]. In the present study, *Pseudomonas aeruginosa* RGT01, isolated from mangrove sediment, exhibited marked extracellular protease activity. The appearance of well-defined clear zones of proteolysis during primary screening confirmed the isolate's ability to synthesize and secrete extracellular protease enzymes. Similar observations have been reported that proteolytic bacteria isolated from soil samples approximately 40% of the isolates showed significant proteolytic activity, exhibiting clearance zones greater than 3 mm on skim milk agar plates after incubation for 20-30 hours at 37°C [15]. The current findings are also consistent with earlier studies reported that protease-producing bacteria are commonly distributed in environmental samples and play an important role in organic matter degradation [16,17]. Likewise, *Bacillus* firmus LE02 and *CytoBacillus firmus* LE03, isolated from the ponyfish *Leiognathus equula*, exhibited considerable extracellular protease production [18]. Similarly, *Bacillus subtilis* produced a maximum proteolytic zone of clearance measuring 22 mm, indicating strong protease activity [19]. In addition, *Bacillus cereus* MSU, isolated from *Sardinella longiceps*, exhibited protease production, as evidenced by the formation of a 17 mm zone of hydrolysis [20].

Optimization of culture conditions is an essential step to enhance enzyme production. Salinity is a factor which can increase or supress the protease production. Being mangrove ecosystem as a euryhaline area the strain may have salinity adaptations. The study demonstrated that 3% NaCl has highest production [21]. In contrast our study had 8.4 ± 0.7 mm in 1.5%. Similarly, a haloalkaliphilic *Bacillus sp.* which produce maximum protease production at 2% NaCl concentration [22]. Other than *Bacillus sp.*, maximum protease production at 2% NaCl concentration by *Salinivibrio sp.* [23]. In the present study, protease production increased with incubation time and reached maximum activity after 48 hours, producing a zone of clearance of 8.1 ± 0.9 mm. The increase in enzyme production with incubation time may be attributed to the gradual increase in bacterial biomass and metabolic activity during the exponential growth phase.

Carbon source acts as a regulator in enzyme production by microorganisms. Among the different carbon sources tested in this study, lactose supported the highest protease production (7.6 ± 0.3 mm) compared with other carbon sources such as glucose, maltose and dextrose which is similar to 1% lactose as the most effective carbon source for maximum protease production by *Bacillus* firmus LE02 [18]. Likewise, lactose was the most suitable carbon source for enhanced protease production [24]. In contrast, glucose as the optimal carbon source for protease production by *Bacillus subtilis* [25]. Similarly, glucose to be the most favourable carbon source for protease production by *Pseudomonas aeruginosa* [21]. Furthermore, glucose and maltose supported maximum protease production in *Pseudomonas fluorescens* [26,27].

The factors such as pH influences microbial growth and enzyme activity. In this study, the maximum protease production of 8.9 ± 0.3 was observed at pH 8, while enzyme activity declined at higher pH levels. This indicates that the protease produced by the bacterial isolate functions optimally under slightly alkaline conditions. Similar observations were reported that protease produced by *Bacillus* species showed optimum activity around neutral to slightly alkaline pH [22,28]. Likewise, pH 9 as the favourable condition for protease production by *Pseudomonas fluorescens* [26,27]. Maximum protease production at pH 9.0 by *Bacillus firmus* TAP5, *Bacillus sp.* [30]. *Bacillus macerans* IKBM-11, *B. licheniformis* IKBL-17 and *B. subtilis* IKBS-10 [29,31].

Temperature is an essential parameter in enzyme production and microbial metabolism. In the present study, the highest proteolytic activity of 9.6 ± 0.7 was recorded at 35°C, after which enzyme production gradually declined at higher temperatures. The decrease in activity at elevated temperatures may be associated with thermal denaturation of enzymes and disruption of bacterial metabolic processes. Several studies reported that the optimum temperature for protease production by *Pseudomonas aeruginosa* was 36°C and 37°C. The temperature affects bacterial growth in two ways. Moderate temperature increases enzymatic reactions and microbial growth rate, whereas higher temperatures may damage cellular proteins [32].

The antimicrobial activity observed in the present study was comparable with previous reports on *Pseudomonas species*. In the present study, inhibition zones against bacterial pathogens ranged from about 12-23 mm, which is similar to the results observed by the zones between 15.0 ± 1.41 and 21.5 ± 0.70 mm against pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* [33]. Previous reports indicate that marine bacteria produce antifungal metabolites that contribute to microbial balance and disease prevention in aquatic systems [34,35]. *Pseudomonas aeruginosa* RGT01 displayed slightly higher activity against *A. fumigatus* and *Candida sp.*, suggesting more potent antifungal metabolite production. The experiment proved that the

Bacillus sp. has the probiotic potential against the pathogens [36]. Earlier study has demonstrated that probiotic *Bacillus* and *Vibrio* strains can suppress pathogenic bacteria through competitive exclusion, production of antimicrobial compounds and modulation of host immune responses [37]. Previously moderate inhibition (9.5-15.2 mm) against several bacterial species and fungi such as *Aspergillus flavus* and *A. niger* was observed by the same species [38]. Comparatively higher inhibition zones (26.6-36.1 mm) against *E. coli* and *S. aureus* and moderate antibacterial activity of *Pseudomonas fluorescens* against *Escherichia coli* (16.33 mm), *Klebsiella pneumoniae* (18.67 mm) and *Staphylococcus aureus* (11.33 mm) [39,40].

Conclusion

The bacterial isolate, *Pseudomonas aeruginosa* RGT01 exhibited a remarkable extracellular protease production along with broad-spectrum antimicrobial activity against several clinical bacterial and fungal pathogens. Optimization studies revealed that maximum protease production was achieved under moderately saline and slightly alkaline conditions, specifically at 1.5% NaCl, pH 8, 35°C and 48 h incubation period with lactose as the preferred carbon source. The crude extract of *Pseudomonas aeruginosa* RGT01 showed considerable antibacterial and antifungal activities, indicating the presence of potent bioactive metabolites. FTIR analysis further confirmed the occurrence of various functional groups associated with biologically active compounds, including alcohols, phenols, carbonyl compounds and aromatic derivatives. Further purification and characterization of the enzyme and bioactive metabolites are recommended to explore their commercial and pharmaceutical applications.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

The project did not meet the definition of human subject research under the preview of the IRB according to federal regulations and therefore was exempt.

Informed Consent Statement

Informed consent was taken for this study.

Authors' Contributions

All authors contributed equally to this paper.

References

1. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev. 1998;62(3):597-635.
2. Gupta R, Beg QK, Lorenz P. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol. 2002;59(1):15-32.
3. Abdel-Naby MA, Ismail AS, Ahmed SA, Fattah AFA. Production and immobilization of alkaline protease from *Bacillus mycoides*. Bioresour Technol. 1998;64(3):205-10.
4. Caballero AR, Moreau JM, Engel LS, Marquart ME, Hill JM, O'Callaghan RJ. *Pseudomonas aeruginosa* protease IV enzyme

assays and comparison to other *Pseudomonas* proteases. Anal Biochem. 2001;290(2):330-7.
5. Sakata K, Yajima H, Tanaka K, Sakamoto Y, Yamamoto K, Yoshida A, et al. Erythromycin inhibits the production of elastase by *Pseudomonas aeruginosa* without affecting its proliferation in vitro. Am Rev Respir Dis. 1993;148:1061.
6. Baweja M, Tiwari R, Singh PK, Nain L, Shukla P. An alkaline protease from *Bacillus pumilus* MP27: functional analysis of its binding model toward its applications as detergent additive. Front Microbiol. 2016;7:1195.
7. Mushtaq H, Ganai SA, Jehangir A, Ganai BA, Dar R. Molecular and functional characterization of protease from psychrotrophic *Bacillus sp.* HM49 in North-western Himalaya. PLoS One. 2023;18(3):e0283677.
8. Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ. *Bacillus* species in soil as a natural resource for plant health and nutrition. J Appl Microbiol. 2020;128(6):1583-94.
9. Bergey DH, Sneath PHA, Holt JG. Bergey's manual of systematic bacteriology. Vol. 2. Baltimore: Williams & Wilkins; 1986. p. 1105-39.
10. Neumann B. Rapid isolation of genomic DNA from gram-negative bacteria. Trends Genet. 1992.
11. Anbuselvan S, Sanjaikumar V, Nabisha S, Ramesh T, Thangaraj M. *In vitro* antimicrobial potentials of halophilic *Fusarium oxysporum* and *Aspergillus niger* extracts against various clinical pathogens. J Pure Appl Microbiol. 2025;19(1).
12. Sanjaikumar V, Anbuselvan S, Nabisha S, Ramesh T, Thangaraj M. Effect of salinity on hyphal growth and antimicrobial potential of two halophilic fungi. J Adv Microbiol. 2025;25(7):155-65.
13. Chandrasekaran M. Industrial enzymes from marine microorganisms: the Indian scenario. J Mar Biotechnol. 1997;5:86-9.
14. Ventosa A, Nieto JJ. Biotechnological applications and potentialities of halophilic microorganisms. World J Microbiol Biotechnol. 1995;11:85-94.
15. Singh SK, Tripathi VR, Jain RK, Vikram S, Garg SK. An antibiotic-, heavy metal-resistant and halotolerant *Bacillus cereus* SIU1 and its thermoalkaline protease. Microb Cell Fact. 2010;9:59.
16. Asha B, Palaniswamy M. Optimization of alkaline protease production by *Bacillus cereus* FT1 isolated from soil. J Appl Pharm Sci. 2018;8(2):119-27.
17. Kotb E, Alabdalall AH, Alsayed MA, Alghamdi AI, Alkhaldi E, AbdulAzeez S, et al. Isolation, screening and identification of alkaline protease-producing bacteria and application of the most potent enzyme from *Bacillus* spp. Mar64. Fermentation. 2023;9(7):637.
18. Vasanthapriya P, Ajith N, Ramesh T, Thangaraj M. Optimization of extracellular alkaline protease production by *Bacillus firmus* LE02, isolated from a ponyfish, *Leiognathus equula*. Res J Pharm Technol. 2025;18(5):2032-6.
19. Pant G, Prakash A, Pavani JV, Bera S, Deviram GV, Kumar A, et al. Production, optimization and partial purification of protease from *Bacillus subtilis*. J Taibah Univ Sci. 2015;9(1):50-5.
20. Ananthi SU, Ramasubburayan R, Palavesam A, Immanuel G. Optimization and purification of lipase through solid-state fermentation by *Bacillus cereus* MSU isolated from the gut of the marine fish *Sardinella longiceps*. Int J Pharm Pharm Sci. 2014;6(5):291-8.
21. Kumar R, Sathees D, Prabhu T, Shankar S, Sankaralingam K, Anandapandian KTK. Optimization of alkalophilic protease production by *Pseudomonas aeruginosa*. World J Fish Mar Sci. 2011;3(5):371-5.
22. Patel R, Dodia M, Singh SP. Extracellular alkaline protease from a newly isolated haloalkaliphilic *Bacillus sp.*: production and optimization. Process Biochem. 2005;40(11):3569-75.
23. Lama L, Romano I, Calandrelli V, Nicolaus B, Gambacorta A. Purification and characterization of a protease produced by an aerobic haloalkaliphilic species belonging to the *Salinivibrio* genus. Res Microbiol. 2005;156(4):478-84.
24. Karbalaei-Heidari HR, Amoozegar MA, Hajighasemi M, Ziaee AA, Ventosa A. Production, optimization and purification of a novel extracellular protease from the moderately halophilic bacterium *HaloBacillus karajensis*. J Ind Microbiol Biotechnol. 2009;36(1):21-7.
25. Gomma MA, Mostafa MM, Abouzied MM, El-Habashy M. Egypt J Food Sci. 1990;16:9.
26. Vinoth J, Murugan S, Stalin C. Optimization of alkaline protease production and its fibrinolytic activity from the bacterium *Pseudomonas fluorescens* isolated from fish waste discharged soil. Afr J Biotechnol. 2014;13(30):3052-61.
27. Kalaiarasi K, Sunitha PU. Optimization of alkaline protease production from *Pseudomonas fluorescens* isolated from meat waste contaminated soil. Afr J Biotechnol. 2009;8(24):7035-40.
28. Patil RC, Jadhav BL. Isolation and characterization of protease-producing *Bacillus* species from soil of dairy industry. Int J Curr Microbiol Appl Sci. 2017;6(6):853-60.
29. Joshi BH. Purification and characterization of a novel protease from *Bacillus firmus* Tap5 isolated from tannery waste. J Appl

Sci Res. 2010;6(8):1068-76.
30. Boominadhan U, Rajakumar R, Sivakumaar PK, Joe MM. Optimization of protease enzyme production using *Bacillus sp.* isolated from different wastes. Bot Res Int. 2009;2(2):83-7.
31. Olajuyigbe FM, Ajele JO. Production dynamics of extracellular protease from *Bacillus* species. Afr J Biotechnol. 2005;4(8):776-9.
32. Dissanayaka S, Rathnayake I. Effect of temperature, pH, carbon and nitrogen sources on extracellular protease production by four *GeoBacillus* species isolated from Maha Oya geothermal springs in Sri Lanka. BMC Res Notes. 2021;14:250.
33. Amankwah FKD, Gbedema SY, Boakye YD, Bayor MT, Boamah VE. Antimicrobial potential of extract from a *Pseudomonas aeruginosa* isolate. Scientifica (Cairo). 2022;2022:4230397.
34. Thomas TRA, Kavlekar DP, LokaBharathi PA. Marine drugs from sponge-microbe association: a review. Mar Drugs. 2010;8(4):1417-68.
35. Sayem SMA, Manzo E, Ciavatta L, Tramice A, Cordone A, Zanfardino A, et al. Anti-biofilm activity of an exopolysaccharide from a sponge-associated strain of *Bacillus licheniformis*. Microb Cell Fact. 2011;10:74.
36. Elshaghabee FMF, Rokana N, Gulhane RD, Sharma C, Panwar H. *Bacillus* as potential probiotics: status, concerns and future perspectives. Front Microbiol. 2017;8:1490.
37. Padmavathi AR, Pandian SK. Antibiofilm activity of biosurfactant-producing coral-associated bacteria isolated from Gulf of Mannar. Indian J Microbiol. 2014;54(4):376-82.
38. Marrez DA, Abdel-Rahman GN, Salem SH. Evaluation of *Pseudomonas fluorescens* extracts as biocontrol agents against some foodborne microorganisms. Jordan J Biol Sci. 2019;12(5):603-8.
39. Alzahrani OM. Isolation, identification and antimicrobial activities of four bacteria associated with entomopathogenic nematodes, *Steinernema* spp. J Pure Appl Microbiol. 2023;17(1):198-209.
40. Sharma D, Gupta M, Gupta S, Jaglans S, Mallick SA. Characterization of secondary metabolites produced during interaction of *Pseudomonas fluorescens* with *Fusarium oxysporum*. Indian J Agric Sci. 2019;89(6):998-1004.

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