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Research Article

MODULATION OF CULTURE CONDITIONS ON LIPASE PRODUCTION BY *LYSINIBACILLUS FUSIFORMIS* PM4 ISOLATED FROM THE GUT OF *PENAEUS MONODON*

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ABSTRACT

Microbial symbionts inhabiting the gastrointestinal tract of *Penaeus monodon* constitute a crucial reservoir of functional traits that influence host nutrition, immunity, and disease resistance. This study evaluated the effects of key environmental parameters including temperature, pH, and salinity on extracellular lipase production by a potent gut-associated bacterial isolate, *Lysinibacillus fusiformis* PM4. The isolate was cultured under varying physicochemical conditions, and lipase activity was quantified across incubation periods. Results revealed that *L. fusiformis* PM4 is a halotolerant, mesophilic, and alkaliphilic strain exhibiting peak lipase activity under defined stress conditions. Time-course assessments further indicated a consistent and progressive increase in enzyme production, highlighting its stable and sustained catalytic potential. The adaptive resilience, enzymatic competence, and antagonistic properties of *L. fusiformis* PM4 underscore its promise as a probiotic candidate for improving nutrient assimilation, pathogen suppression, and overall productivity in shrimp aquaculture systems.

Keywords: *Penaeus monodon*, Gut microbes, *Lysinibacillus*, Lipase, Optimization, Aquaculture.

INTRODUCTION

Aquaculture has emerged as a vital sector in global food production, contributing substantially to food security, employment generation, and economic growth. Among farmed species, the black tiger shrimp *Penaeus monodon* holds high commercial significance due to its market demand, rapid growth rate, and consumer preference (AOUN, 2018). However, sustainable production of *P. monodon* is frequently constrained by poor feed utilization, disease outbreaks, and environmental fluctuations. The shrimp gut microbiome comprises a diverse and dynamic community of microorganisms that play crucial roles in host nutrition, immunity, and overall physiological performance. Gut-associated bacteria aid in the digestion and assimilation of nutrients by secreting extracellular enzymes such as proteases, amylases, lipases, and cellulases (Zhou *et al*., 2009; Xie *et al*., 2021). These enzymes enhance feed conversion efficiency and support metabolic processes, thereby promoting growth and productivity. In addition to their digestive functions, many gut bacteria possess probiotic properties that provide multiple benefits to the host. These include the inhibition of pathogenic microbes through competitive exclusion, production of antimicrobial compounds, improvement of gut integrity, and modulation of immune responses (Balcázar *et al*., 2006). The use of probiotics in aquaculture has been associated with enhanced stress tolerance, improved water quality, and reduced disease incidence, making them a promising strategy for sustainable shrimp farming.

Recent advances in microbiome research have emphasized the importance of isolating and characterizing functionally active gut bacteria, particularly those with enzymatic and probiotic potential. Such microorganisms can be incorporated into functional feeds or microbial formulations to enhance aquaculture performance. Metagenomic tools have further revolutionized microbiome assessment by enabling comprehensive analysis of gut microbial diversity, early detection of pathogens, and real-time monitoring of microbial community dynamics in

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aquaculture systems (Dhanush *et al*., 2025). Members of the genus *Lysinibacillus*, including *L. fusiformis* and *L. sphaericus*, have been recognized for their biocontrol attributes in plants, owing to their ability to produce organic acids, hydrolytic enzymes, and metal-chelating compounds that transform insoluble minerals into bioavailable forms (Bravo *et al*., 2011; Ahmad *et al*., 2014; Sule *et al*., 2020). Although *L. fusiformis* has been isolated from diverse environments such as soil and industrial effluents, its presence and enzymatic potential in estuarine organisms remain underexplored. This study was therefore undertaken to isolate a potent lipase-producing bacterium from the Vellar estuary and evaluate the effects of temperature, pH, and salinity on its extracellular lipase production. We hypothesized that the estuarine-derived strain would exhibit optimal enzymatic activity under moderate temperature, alkaline pH, and elevated salinity conditions reflecting its natural habitat and adaptive physiology.

MATERIALS AND METHODS

Sample collection

Live specimens of *P. monodon* were collected from the Vellar estuary (Lat: 11.4872219; Lon: 79.7644497) along the southeast coast of India. Immediately after collection, the shrimp were transported to the Fish Genetics Laboratory, Centre of Advanced Study in Marine Biology, Annamalai University, under chilled and sterile conditions to minimize microbial contamination.

Isolation of bacteria

Upon arrival, the shrimp were surface-sterilized using 70% ethanol and dissected aseptically. The entire gut was excised using sterile instruments and homogenized in 1 mL of sterile double-distilled water. The homogenate was serially diluted ten-fold up to 10^{-5}. From each dilution, 0.1mL was spread-plated onto Zobell Marine Agar (ZMA) and incubated at 37 ± 0.5°C for 24h. Following incubation, morphologically distinct colonies were selected and purified by repeated streaking on fresh ZMA plates. Five purified isolates were obtained and designated PM1, PM2, PM3, PM4, and PM5.

Screening of enzyme activity

The extracellular enzyme-producing ability of the isolates was assessed qualitatively using plate-based assays for protease, amylase, and lipase activity. Protease activity was evaluated on Skim Milk Agar (SMA), amylase activity on Starch Agar, and lipase activity on Tributyrin Agar (TBA). All media were sterilized at 121°C for 15 min, inoculated with individual isolates, and incubated at 37 ± 0.5°C for 24h. Enzyme activity was indicated by clear hydrolysis zones: casein degradation on SMA (protease), starch hydrolysis after flooding starch agar plates with 1% iodine (amylase), and tributyrin degradation on TBA (lipase).

Antagonistic activity and antibiotic susceptibility test

Antagonistic activity of the five isolates was evaluated in vitro against selected clinical bacterial and fungal pathogens. Each isolate was cultured in Zobell Marine Broth or Potato Dextrose Broth at 37 ± 0.5°C under shaking conditions for 24 h. Cultures were centrifuged at 5,000rpm for 5 min at 4°C, and the resulting cell-free supernatants were tested for antimicrobial activity using the agar well diffusion method. Mueller–Hinton Agar (MHA) or Potato Dextrose Agar plates previously spread with the test pathogens were used, and 6 mm wells were filled with 100 µL of supernatant. Plates were incubated at 37 ± 0.5°C for 24h, and zones of inhibition (mm) were recorded. Antibiotic susceptibility profiles of the isolates were determined by the Kirby–Bauer disc diffusion method using commercial antibiotic discs on MHA plates.

Assessment of potential strain

Based on extracellular enzyme production and antagonistic activity, isolate PM4 was identified as the most promising strain and selected for further characterization.

DNA isolation and PCR

Genomic DNA was extracted from a pure culture of the PM4 strain. Approximately 0.1g of freshly harvested bacterial biomass was homogenized in 300 µL of lysis buffer (200mM Tris-HCl, pH5.8; 250mM NaCl; 25mM EDTA; 0.5% SDS) using a glass–glass homogenizer. The homogenate was centrifuged at 12,000 rpm to remove cellular debris, and the supernatant was transferred to a fresh microcentrifuge tube. An equal volume of absolute ethanol was added, mixed gently, and the mixture was incubated overnight at 4°C. The precipitated DNA was collected by centrifugation at 13,000 rpm, air-dried briefly, and dissolved in 100µL of TE buffer (pH 8.0). PCR amplification of the 16S rRNA gene was carried out using 10ng of purified genomic DNA in a thermal cycler (Applied Biosystems). Each 25µL reaction contained 1× EmeraldAmp® GT PCR Master Mix, 0.4µM of each primer (533F: 5′-GTGCCAGCAGCCGCGGTAA-3′; 1100R: 5′-AGGGTTGCGCTCGTTG-3′), template DNA, and nuclease-free water. The thermal cycling program consisted of an initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30s, annealing at 56°C for 45s, and extension at 72°C for 50s; followed by a final extension at 72°C for 10 min. PCR amplicons were resolved on a 1% agarose gel containing ethidium bromide and visualized under UV illumination. Bidirectional Sanger sequencing of the purified amplicons was performed at the Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkazhi.

Optimization of lipase production

Optimization of extracellular lipase production by *L. fusiformis* PM4 was conducted by evaluating the effects of pH, salinity, and temperature. Cultures were grown under varying conditions: pH levels of 5, 6, 7, 8, and 9; salinity concentrations of 10, 20, 30, 40, and 50ppt; and incubation temperatures of 25, 30, 35, and 40°C. Lipase activity was

measured at 12h intervals over a 96h fermentation period. To enable direct quantitative comparison of production efficiency across conditions, average volumetric productivity was calculated by dividing the final lipase activity at 96h by the total fermentation time.

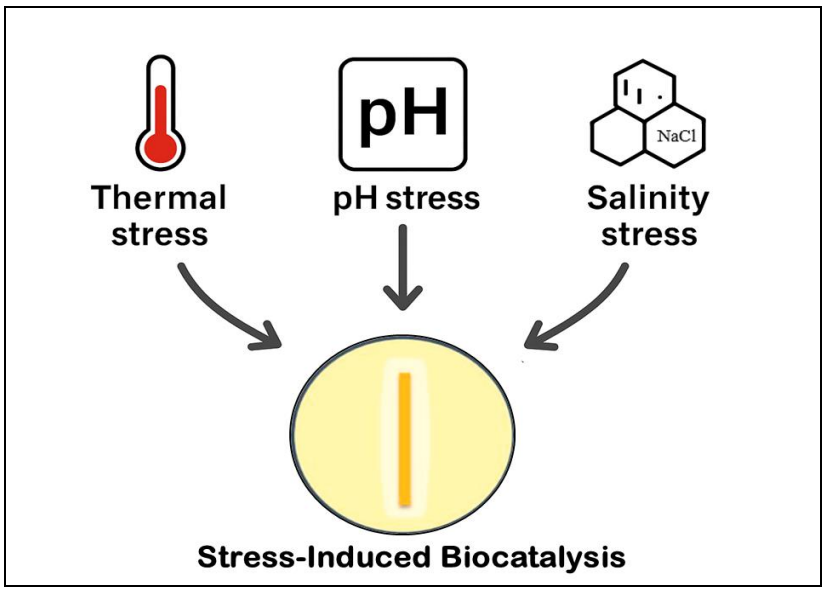

Figure 1. Environmental stress on lipase secretion by *L. fusiformis*

Antagonistic and antibiotic susceptibility test in *L. fusiformis* PM4

The antagonistic activity of *L. fusiformis* PM4 was evaluated using the agar well diffusion assay. A 100µL aliquot of the cell-free supernatant was dispensed into 6mm wells on pathogen-inoculated agar plates, while Penicillin G (10 units) served as the positive control. Plates were incubated at 37 ± 0.5°C for 24h. After incubation, the antimicrobial activity was quantified by measuring the diameter of the zone of inhibition (mm) surrounding both the wells and control discs. Susceptibility was interpreted according to standard guidelines.

RESULTS AND DISCUSSION

The PM4 strain exhibited distinct colony characteristics on Zobell Marine Agar, forming cream-coloured, convex colonies with smooth margins and an opaque appearance. Microscopic examination and Gram staining confirmed that the isolate was a Gram-positive, rod-shaped bacterium. Amplification and sequencing of the 16S rRNA gene followed by BLASTn analysis revealed 100% sequence similarity and full query coverage with *Lysinibacillus fusiformis*. The sequence was deposited in the NCBI GenBank database under the accession number PV875865, confirming the taxonomic identity of the isolate. Identification of *L. fusiformis* from an estuarine shrimp gut is noteworthy, as members of this genus are widely recognized for their enzymatic versatility, ecological adaptability, and microbial antagonistic properties, supporting their potential use in probiotic applications (Bravo *et al*., 2011; Sule *et al*., 2020). Temperature significantly influenced extracellular lipase production by *L. fusiformis* PM4. A time-dependent increase in enzyme activity was observed across all tested temperatures, indicating stable and continuous secretion of lipase during incubation. Maximum hydrolysis activity was recorded at 35°C, exhibiting a clear zone diameter of 16.08 mm at 48h. This was followed by moderate enzyme production at 40°C (13.42 mm) and 30°C (10.09 mm), while the lowest activity was recorded at 25°C (4.40 mm).

These findings clearly demonstrate that 35°C is the optimal temperature for lipase production by *L. fusiformis* PM4. The decline in activity at lower temperatures can be attributed to reduced metabolic and catalytic rates, whereas temperatures above the optimum may impair protein stability or cellular integrity. Similar temperature-dependent trends have been reported for other *Lysinibacillus* species and marine-derived lipase-producing bacteria, which often exhibit mesophilic behaviour with peak activity between 30–37°C. The strong temperature tolerance exhibited by *L. fusiformis* PM4 suggests adaptive resilience consistent with its estuarine origin, where thermal fluctuations are common. Lipase production by *L. fusiformis* PM4 was strongly influenced by the pH of the culture environment. Among the pH levels tested, pH8 supported the highest enzyme activity, establishing it as the optimal pH for extracellular lipase synthesis. Moderate activity was observed at pH5 and pH6, whereas pH7 yielded comparatively lower hydrolysis zones throughout the incubation period. Notably, no lipase activity was detected at pH9, indicating that highly alkaline conditions inhibit either enzyme expression or catalytic function in *L. fusiformis* PM4. These observations clearly demonstrate

that the strain favours mildly alkaline conditions for lipase production, consistent with the physiology of several halotolerant and estuarine bacteria known to secrete alkaline lipases. The elevated enzyme output at pH 8 may be linked to optimal protein folding, gene regulation, and membrane-associated secretion processes that are pH-sensitive. The reduced or absent activity at excessively high pH may be attributed to energetic constraints. Highly alkaline environments substantially decrease external proton concentration, impairing the organism's ability to maintain a stable proton motive force (PMF). This imbalance disrupts metabolic pathways, energy transduction, and enzyme synthesis machinery, ultimately suppressing lipase production. Overall, the pH-dependent pattern observed in this study highlights the ecological and physiological adaptation of *L. fusiformis* PM4 to mildly alkaline estuarine conditions while underscoring the critical role of pH homeostasis in regulating microbial enzyme expression.

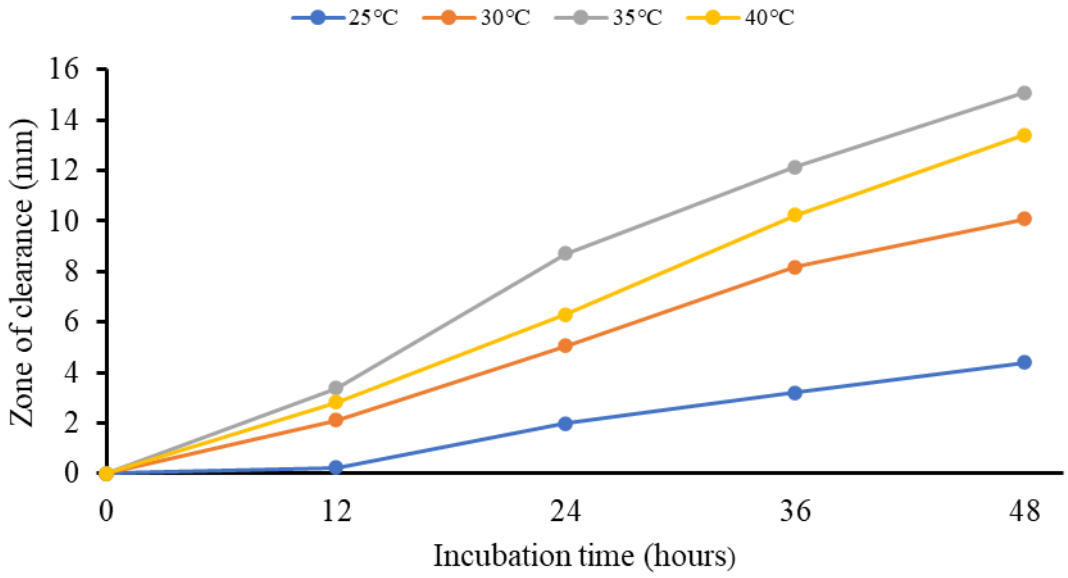

Figure 2. Effect of temperature on lipase secretion by *L. fusiformis* PM4

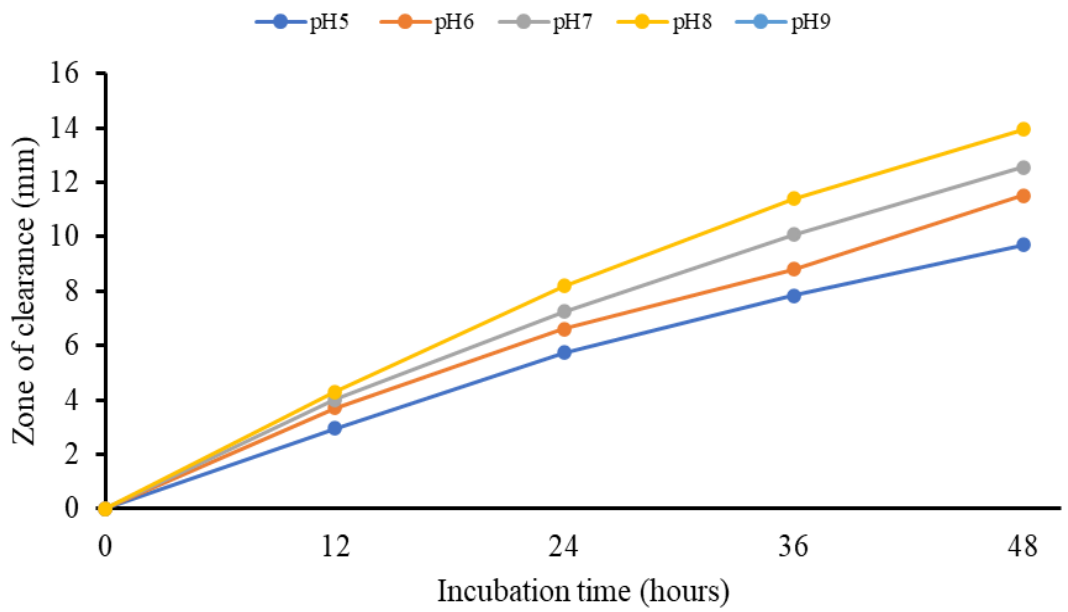

Figure 3. Effect of pH on lipase secretion by *L. fusiformis* PM4

Salinity played a decisive role in modulating the extracellular lipase production of *L. fusiformis* PM4. Lipase activity was quantified by measuring the diameter of the clearance zones (mm) formed on lipid-containing agar over a 48h incubation period. The salinity levels tested were 10, 20, 30, 40, and 50ppt. Among the tested concentrations, 20ppt supported the highest enzyme production, corresponding to an average reaction rate of 0.121 mm h^{-1}, indicating that this salinity level represents the optimal condition for lipase expression under the given experimental setup. Enzyme activity declined markedly at 30ppt, and no lipase activity (0.0 mm) was detected at 40 and 50ppt, demonstrating a complete loss of catalytic function at elevated salinity. The pronounced decline in activity beyond the optimal salinity suggests substrate inhibition and osmotic stress at high salt concentrations. Excessive salinity can impair membrane integrity, disrupt protein folding, alter ion balance, and compromise overall metabolic efficiency, ultimately inhibiting enzyme synthesis and secretion. Conversely, the strong performance at 20ppt aligns well with the estuarine origin of the strain, which is naturally adapted to moderate salinity fluctuations. These results highlight that *L. fusiformis* PM4 is a moderate halotolerant bacterium capable of substantial lipase production at optimal salt concentrations, but unable to sustain enzymatic activity under highly saline conditions.

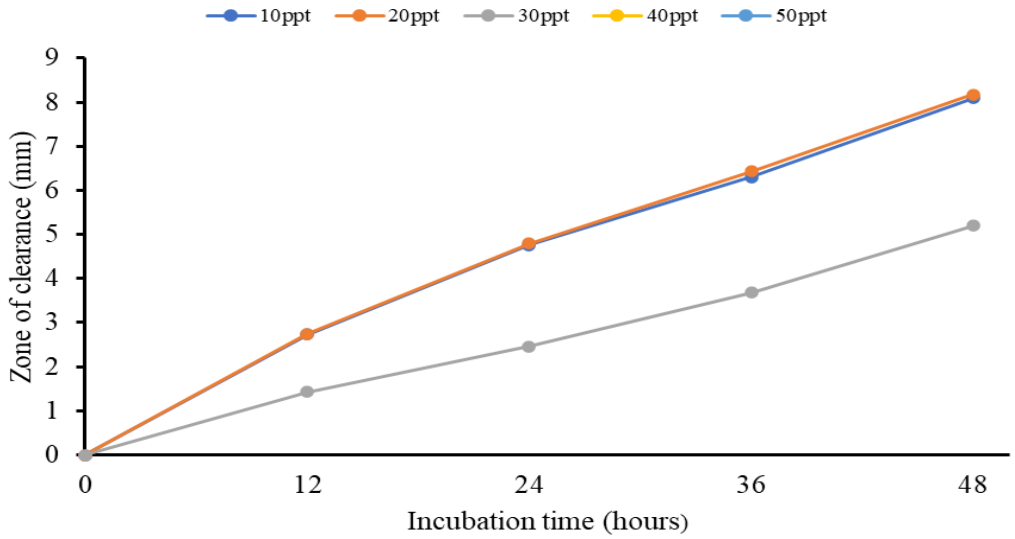

Figure 4. Effect of salinity on lipase secretion by *L. fusiformis* PM4.

The antimicrobial potential of *L. fusiformis* PM4 was assessed in comparison with Penicillin G (10 U) against a panel of clinical bacterial and fungal pathogens. Penicillin G exhibited broad antifungal activity, effectively inhibiting *Candida* sp., *Aspergillus flavus*, and *Aspergillus niger*. In contrast, *L. fusiformis* PM4 demonstrated more selective antifungal capabilities, exhibiting inhibition only against *A. niger* and *A. fumigatus*. Notably, the inhibitory effect of PM4 against *A. fumigatus* was slightly greater than that produced by Penicillin G, indicating a degree of strain-specific antifungal potency.

Among bacterial pathogens, both Penicillin G and *L. fusiformis* PM4 displayed limited and selective inhibition. Penicillin G showed activity against *Salmonella paratyphi* and *Klebsiella* sp., whereas *L. fusiformis* PM4 inhibited *Escherichia coli*, a strain that did not respond to Penicillin G. These contrasting inhibition profiles suggest that *L. fusiformis* PM4 produces antimicrobial compounds with a distinct spectrum of activity, likely reflecting differences in cell wall targeting mechanisms or metabolite composition (Table 1). The selective antagonistic activity observed supports the potential application of *L. fusiformis* PM4 as a probiotic candidate with targeted antimicrobial effects, particularly against specific fungal pathogens and Gram-negative bacteria such as *E. coli*.

Table 1. Antagonistic activity of *L. fusiformis* PM4 and Penicillin G against selected pathogens.

Group	Clinical pathogens	*L. fusiformis* PM4 (100µl) (mm)	Penicillin G (10U) (mm)
Fungal	*Aspergillus niger*	1.10	2.20
	Aspergillus fumigatus	1.20	1.00
	Aspergillus flavus	0.00	1.40
	Candida sp.	0.00	8.40
	Salmonella paratyphi	1.10	1.30

	Escherichia coli	0.50	0.00
Bacterial	*Klebsiella sp.*	0.30	0.40

Time-course monitoring of extracellular lipase activity across the optimized parameter sets showed a steady and continuous increase in enzyme accumulation throughout the 96h incubation period. Among the tested conditions, the highest activity was recorded at 35°C and pH 8, confirming these as the optimal physiological parameters for lipase synthesis by *L. fusiformis* PM4. In contrast, the 20ppt salinity condition supported only restricted enzyme production. The optimal temperature of 35°C falls within the mesophilic range (20–45°C), which is typically associated with maximal bacterial growth and enzyme biosynthesis. This observation is consistent with previous reports indicating that temperatures close to 37°C often favour high lipolytic activity in microbial enzymatic extracts. The superior activity at pH 8 further suggests that *L. fusiformis* PM4 is adapted to alkaline environments and likely secretes an alkaline-type lipase, a trait commonly observed in halotolerant and estuarine bacteria. In comparison, the 20ppt salinity condition resulted in the lowest lipase yield (11.80 mm), representing approximately a 40% reduction relative to the optimal temperature condition. This significant decline highlights the strong inhibitory effect of elevated osmotic pressure, indicating that 20ppt acts as a physiological stressor that disrupts metabolic efficiency and severely limits volumetric productivity. The time-course pattern demonstrates that lipase secretion by *L. fusiformis* PM4 is sustained over prolonged incubation but remains highly dependent on environmental parameters, with temperature and pH exerting strong positive influences and salinity imposing a substantial constraint on enzyme output.

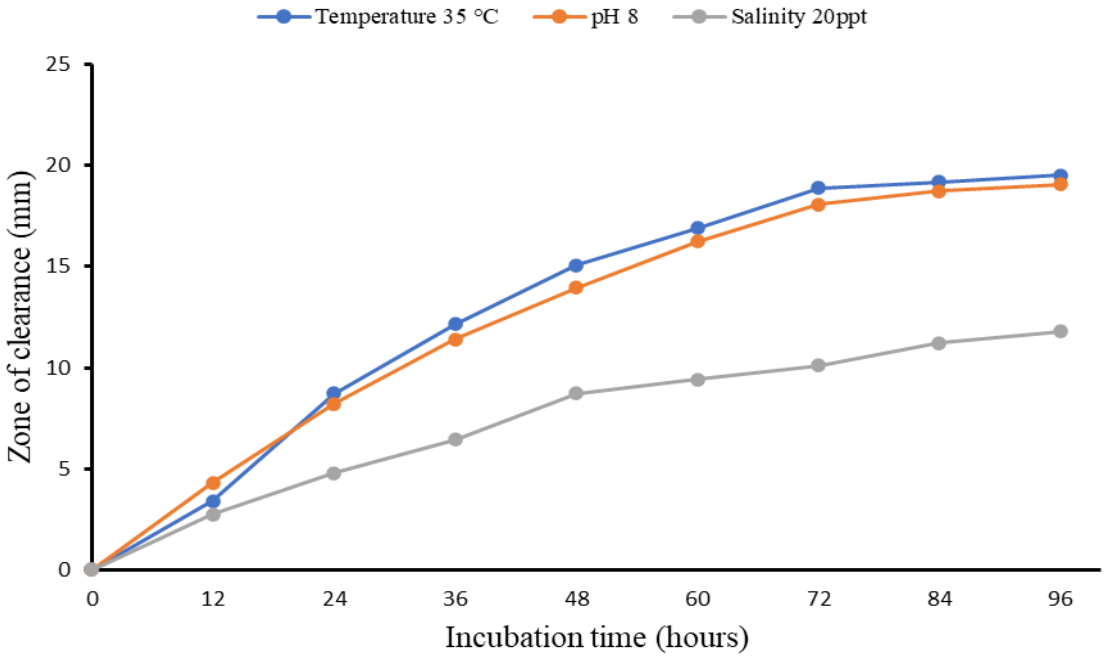

Figure 5. Optimum lipase secretion by *L. fusiformis* PM4 for 96h

To evaluate the operational efficiency of the three optimized parameter conditions, average volumetric productivity over the 96h fermentation period was calculated (Table 2). The kinetic assessment revealed that the 35°C temperature condition yielded the highest productivity, averaging 0.203mm h^{-1}, marginally surpassing the pH8 condition, which recorded 0.198mm h^{-1}. In contrast, the 20ppt salinity condition exhibited a markedly lower productivity of 0.123mm h^{-1}, reflecting a substantial reduction in enzymatic output. The comparatively low productivity observed at 20ppt underscores the strong inhibitory effect of osmotic stress on cellular metabolism and enzyme secretion. These findings confirm that while temperature and pH exert favourable influences on lipase biosynthesis, elevated salinity imposes a physiological limitation that significantly constrains volumetric yield in *L. fusiformis* PM4.

Table 2. Comparative performance metrics and kinetic designation at 0–96h.

Condition	Final activity at 96h (mm)	Average productivity (mm/h)	Productivity phase (12-48h)	Kinetic curve trajectory	Primary physiological influence
Temperature (35 °C)	19.50	0.203	Rapid exponential growth	Highest curve; sustained, rapid ascent toward 96h. Did not plateau	High suitability due to minimal mesophilic stress and efficient translation.
pH 8	19.05	0.198	steady exponential/transition	Second highest curve; steepest initial slope (12h–48h)	Optimal efficiency via alkali tolerance and metabolic adaptation
Salinity (20ppt)	11.80	0.123	Stress-limited Growth/synthesis	Visibly lowest curve; significantly flattened slope throughout the duration	Severe osmotic stress limiting resource allocation for synthesis

The results of the present study clearly demonstrate that the estuarine isolate *L. fusiformis* PM4 is a potent producer of extracellular lipase, and its enzyme expression is strongly regulated by environmental conditions that mirror its natural habitat. The combined influence of temperature, salinity, and pH highlights the strain's ecological adaptability and biotechnological potential. The optimal temperature for lipase production was recorded at 35°C, placing *L. fusiformis* PM4 within the mesophilic category a pattern characteristic of microorganisms inhabiting tropical and subtropical estuarine waters. This optimal temperature, slightly lower than the conventional laboratory incubation temperature of 37°C, resulted in a pronounced enhancement of enzyme productivity (19.50 U/mL). A decline in activity at 40°C suggests the onset of protein denaturation or reduced efficiency of the cellular machinery responsible for enzyme synthesis. These findings closely align with reports by Mohan *et al*. (2008), who identified 35°C as the optimal temperature for lipase production in *Bacillus* spp., noting that temperatures outside the 35–40°C range lead to enzyme instability. Strain-level variation in thermal tolerance has also been acknowledged by Hassan and Hameed (2001), while Ohnishi *et al*. (1994) emphasized that environmental parameters such as temperature and pH exert regulatory control over microbial growth and enzyme synthesis. Even though *Bacillus* spp. are capable of growth over a broad thermal range (28–60°C), lipase production does not always coincide with the optimal growth temperature (Gupta *et al*., 2004). Reduced enzyme yields at low temperatures can be attributed to membrane rigidification, hampering nutrient transport and cellular activity (Noman *et al*., 2010).

Salinity exerted a distinct influence on lipase production, with 20ppt supporting the maximum activity. This preference reflects the natural estuarine environment, where salinity fluctuates between freshwater and marine conditions. *L. fusiformis* PM4 thus demonstrates moderate halotolerance rather than obligate halophilicity. The results are consistent with the broader understanding that halophilic enzymes often require high ionic strength for stability and activity, with Mevarech *et al*. (2000) reporting that 1– 4M NaCl enhances activity of halophilic proteins through improved hydration and surface charge adaptation. However, unlike extreme halophiles, *L. fusiformis* PM4 exhibited complete inhibition at 40–50ppt, confirming that excessive ionic stress compromises enzyme synthesis. Previous studies support this pattern: *Chromohalobacter canadensis* lipase exhibited optimal activity at 17.5% NaCl (Ai *et al*., 2018), while *Bacillus* spp. achieve peak production under moderate salinity (Singh *et al*., 2019; Goswami and Tipre, 2025).

The optimal pH for lipase production was pH 8, consistent with the profile of many commercially relevant alkaline-active lipases used in detergents and industrial applications. The strong activity at pH 8 indicates the adaptation of *L. fusiformis* PM4 to slightly alkaline conditions typical of coastal and estuarine systems. Similar results have been reported in *Bacillus stearothermophilus* (Achaamman *et al*., 2003; Berekaa *et al*., 2009) and *Bacillus megaterium* (Anurag *et al*., 2006), where maximum lipase production occurred near neutral to mildly alkaline conditions. Deviations from optimal pH can reduce enzyme yield due to altered nutrient ionization, membrane instability, disrupted metabolism, or inhibition of enzyme-secreting pathways (Gupta *et al*., 2004; Sahu and Martin, 2011). pH also governs substrate transport across the membrane, which directly affects metabolic flux and enzyme production efficiency (Kanimozhi *et al*., 2011).

Beyond enzymatic performance, the antagonistic activity demonstrated by *L. fusiformis* PM4 highlights its potential as a probiotic candidate for shrimp aquaculture. The strain showed selective antimicrobial effects against fungal and bacterial pathogens, including activity against *Aspergillus fumigatus* and *Escherichia coli*, the latter not inhibited by Penicillin G. This selectivity could be attributed to the

production of bacteriocin-like or antifungal metabolites, consistent with broader observations on *Lysinibacillus* spp. known for their insecticidal, biocontrol, and antimicrobial properties (Jamal and Ahmad, 2022). The genomic uniqueness of *Lysinibacillus*, particularly the lysine-rich peptidoglycan structure (Ahmed *et al*., 2007), may contribute to the distinct antimicrobial spectrum observed. Numerous studies have documented the effectiveness of *Bacillus* spp. in shrimp farming due to their roles in nutrient assimilation, pathogen suppression, and water quality improvement (Zokaeifar *et al*., 2014; Nemutanzhela *et al*., 2014; Majhool *et al*., 2025), suggesting that *L. fusiformis* PM4 may offer similar benefits.

Temporal profiling revealed that maximal lipase expression occurred at 96h, which corresponds to the late stationary phase—consistent with secondary metabolite production patterns. The final yield of 19.50 U/mL positions *L. fusiformis* PM4 among high lipase producers. Previous studies have reported wide variability in optimal incubation times: 24h in *Bacillus stearothermophilus* (Massadeh and Sabra, 2011), 30h in *Geobacillus stearothermophilus* (Berekaa *et al*., 2009), 34h in *B. megaterium* (Anurag *et al*., 2006), and 96h in *Penicillium notatum* (Rehman *et al*., 2010). The decline beyond the optimum incubation period in some species can be attributed to nutrient depletion, enzyme denaturation, or pH shifts in the medium (Mahanta *et al*., 2008). The variation across taxa underscores the species-specific nature of microbial enzyme kinetics. This study clearly establishes *L. fusiformis* PM4 as a promising lipase-producing strain with substantial relevance for shrimp aquaculture. Its optimal functioning under moderately alkaline, mesophilic, and brackish conditions, combined with its antagonistic properties, supports its potential application as a probiotic and enzyme-secreting bioadditive, capable of enhancing nutrient assimilation and contributing to disease mitigation in *Penaeus monodon* culture systems.

CONCLUSION

This study successfully isolated and characterized *Lysinibacillus fusiformis* strain PM4, an efficient extracellular lipase producer derived from the gut of *Penaeus monodon* in the Vellar Estuary. Optimal conditions for maximal enzyme production were determined to be 35°C**,** pH 8**,** and 20ppt salinity, with temperature emerging as the most influential factor, yielding the highest volumetric productivity (0.203mm h^{-1}). The strain's ability to perform effectively under moderately saline and alkaline conditions highlights its adaptation to estuarine environments and underscores its potential as a reliable source of salinity- and alkali-tolerant lipases. These attributes position *L. fusiformis* PM4 as a promising candidate for applications across industrial biocatalysis**,** environmental biotechnology**,** and aquaculture**,** particularly in the development of probiotic formulations and enzyme-enriched feeds aimed at improving shrimp health and nutrient assimilation.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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