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ISOLATION, MOLECULAR IDENTIFICATION, AND ASSESSMENT OF THE BIOTECHNOLOGICAL POTENTIAL OF A NOVEL LIPASE-PRODUCING ASPERGILLUS FLAVUS ISOLATE FROM RIJAL ALMAA, SAUDI ARABIA

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ABSTRACT
Background: The increasing depletion of fossil fuels has created a global demand for renewable and environmentally friendly alternative energy sources. The outcomes of this study are expected to contribute to the development of a cost-effective, locally sourced lipase preparation with potential applications in detergent, food, and biofuel industries.
Methods: A lipase-producing fungal isolate was obtained from Rijal Almaa, Saudi Arabia. Lipase activity was screened using Tween 80 agar, Tributyrin agar, and Phenol Red agar media. Molecular identification was performed through 18S rDNA sequencing, identifying the isolate as Aspergillus flavus (MH279408.1). Lipase production was optimized by varying carbon, nitrogen, mineral sources, and pH conditions. The culture medium was inoculated with fungal spores and incubated at 30 ± 1°C for 5 days. Enzyme activity was determined using p-nitrophenyl laurate (p-NPL). The enzyme was partially purified by ammonium sulfate precipitation and characterized biochemically.
Results: The isolate exhibited strong lipolytic activity on all screening media. partially Purified lipase showed a molecular weight of 44 kDa on SDS-PAGE analysis. The enzyme demonstrated optimal activity at 20°C and pH 5, with kinetic parameters of Km = 0.4 mM and Vmax = 3.125 µmol/min/mg. Lipase activity was enhanced by Hg^{2+} and inhibited by Cu^{2+}, Fe^{3+}, and EDTA. Aspergillus flavus (MH279408.1) showed significantly high lipase production under optimized conditions.
Conclusion: The obtained results demonstrate the biotechnological potential of Aspergillus flavus lipase for biodiesel production and enzymatic bioremediation of lipid-rich wastewater. The study also highlights the importance of optimizing physicochemical parameters, particularly pH, for improving lipase efficiency in industrial and environmental applications.

KEYWORD: Screening, Lipase, Producing Aspergillus flavus**,** Isolate and Purification

Introduction

Lipases (triacylglycerol acyl-hydrolases EC3.1.1.3) are enzymes that break down the ester bonds of insoluble substrates in water at the substrate-water interface (Sharma et al., 2001). Lipases' most common industrial applications include detergents (Saisubramanian et al., 2006), pharmaceuticals (Hasan et al., 2006), and meals (Sharma et al., 2001). Their applications in the food industry include cheese maturing (Omar, and Awda 2024), fragrance synthesis (Ado et al., 2025), and the generation of lipids rich in unsaturated fatty acids (Reshma et al., 2008; Wang et al., 2008). The synthesis of methyl esters of fatty acids (biodiesel) (Park et al., 2006) is a recent and widely used application.
Because of its extracellular secretion, wide substrate specificity, and stability in harsh environments, fungi, yeast, and bacteria are the three main types of microbial lipase (Vishnoi et al., 2020). Fungal lipases have advantages over bacterial lipases, including easier recovery, greater stability over a broad pH and temperature range, and increased activity in organic solvents. Because of these qualities, they are very well suited for industrial uses, such as the bioremediation of lipid-rich pollutants such as palm oil mill effluent (POME) (Ahmed et al., 2023; Karim et al., 2023; Kumar et al., 2023;).
Titrimetry, interfacial tensiometry, spectroscopy, chromatography, immunochemistry, and conductimetry are among the various lipase assay techniques that have been categorized (Region, and Forecasts 2025). Titrimetry, also referred to as the pH stat method, is the most straightforward of all, even though it necessitates a continuous pH meter readout (Lfta et al., 2026).
Precipitation, hydrophobic interaction chromatography, gel filtration, ion exchange chromatography, and affinity chromatography are frequently employed methods for purification (Shakir and Bayar 2026). For instance, ammonium sulfate precipitation, Sepharose CL-6B chromatography, isoelectric focusing, and acidification of culture supernatant were used to purify lipases from different Pseudomonas species. It was discovered that the pure lipases from

Pseudomonas aeruginosa, Pseudomonas fragi, and Pseudomonas fluorescens were monomeric. P. fragi's lipase gene has been cloned and sequenced. A 29 kDa lipase from P. aeruginosa MB5001 was also isolated (Oeillade et al., 2025). This represents the first report on the isolation, OFAT-based optimization, and partial purification of a lipase from from [Rijal Almaa]. We will isolate, identify, optimize via OFAT, and partially purify lipase from a new A. flavus strain to fill this gap.

MATERIALS AND METHODS

Collection of Sample

Soil samples weighing 200 g were collected from different locations in Saudi Arabia, including the Rijal Almaa rhizosphere soil. Samples were taken 4 inches below the surface using a sterile hand trowel treated with 70% alcohol. The samples were transported to the Microbiology Laboratory at Jeddah University for analysis ((Wadia and Jain 2017; Ado et al., 2025).

3 Fungal isolation and Identification

Soil fungi were isolated using the pour plate technique. A 0.1g soil sample was spread on a Petri dish, and Potato Dextrose Agar with antibiotics was poured in. Plates were incubated for 5 days at 30°C. Colonies were counted after 24 hours, and pure cultures were obtained by sub-culturing distinct colonies. Fungal isolates were identified based on macroscopic and microscopic characteristics (Abdulmumini et al., 2022; Bharathi and Rajalakshmi 2019).

4 Screening of lipolytic fungal species

Ilesanmi et al. developed a chemically defined medium called Tween-80 agar to screen fungal isolates for lipase production. The medium was prepared by mixing peptone, agar, NaCl, and CaCl2·2H2O in distilled water, sterilized by autoclaving, and supplemented with Tween 80. Pure cultures were streaked onto Tween-80 agar plates and incubated at 37°C for 48 hours. Lipase activity was detected by a white precipitate around the colony, and opaque zones indicated lipase production by the organisms (Ilesanmi et al., 2020).

Potential Fungi Identification

Five potent fungal cultures were selected and identified using microscopic examination with Lactophenol cotton blue staining. The identified fungi were maintained on Potato Dextrose Agar (PDA) medium following laboratory manuals (Roy et al., 2018).

Identification of lipase-producing fungal

The isolated fungus was examined under a microscope at 40X magnification after staining with lactophenol cotton blue (LPCB). The most efficient fungal isolate, R1, was identified based on phenotypic characteristics and confirmed by 18S rRNA sequencing with the help of Solgent Company in Daejeon, South Korea. The sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) from the NCBI website (Ray et al., 2018; Fetyan et al., 2023).

Preparation of Medium for Lipase Production

The medium used for lipase production optimization composed of: sucrose (10, 15 and 20 g/L^{-1}), soybean oil (2, 4 and 6 g/L^{-1}), yeast extract (0, 1 and 2 g/L^{-1}), $(NH_4)^2SO_4$ (2, 4 and 6 g/L^{-1}), KH_2PO_4 (2 g/L^{-1}), $MgSO_4.7H_2O$ (0.5 $g.L^{-1}$), KCl (0.5 g/L^{-1}), $ZnSO_4$ (5 mg/L^{-1}), $FeSO_4.7H_2O$ (23 mg/L^{-1}), CuSO4. $5H_2O$ (6 mg/L^{-1}), $MgSO_4$. $5H_2O$ (20 $\mu g/L^{-1}$), and pH (6, 7 and 8).

Each flask contained 50 mL of the medium and was sterilized before inoculation. The flasks were then incubated for 5 days in a shaking incubator at 120 rpm and 30°C. After incubation, the cells were harvested, and the filtrate was used to assess enzyme production. All experiments were carried out in triplicate (Aly et al., 2012).

Optimization of fermentation medium using one factor at-a-time method

Incubation period

The most effective fungus was cultured on a nutrient-rich medium for 1-9 days at 30°C and 120 rpm (Wadia and Jain 2017).

Nutritive parameters

Numerous substrates and carbon sources were tested to determine their impact on lipase activity. Fresh oils (olive oil, coconut oil, tar oil, and used cooking oil) were used to identify the optimal concentration for lipase production. Additionally, six carbon sources (sucrose, glucose, fructose, dextrose, maltose, and lactose) were tested to determine the best conditions for lipase production.

Cultural environments
The potential strain was tested for lipase production at pH levels ranging from 3.0 to 9.0 and temperatures from 5°C to 80°C. Various inoculum sizes (1 to 6 mL) were also evaluated.

Achievement of Enzymatic Extracts
The fermented medium obtained from the fungal isolate's fermentation was filtered through cotton to retain hyphae and then frozen at -20°C. It was later used for the determination of enzymatic activities (Golani and Hajela 2024).

Measurement of growth:
Microbial growth in the culture filtrate was assessed by measuring the optical density at 550 nm using a UV spectrophotometer. SPECTRO UV-VIS AUTO UV-2602 (LaboMed, Inc.) (Ado et al., 2025).

Lipase assay by the titrimetric procedure
The enzyme assay used the cell-free supernatant as the enzyme source. A sample solution was mixed with an assay substrate containing olive oil, CaCl2, and phosphate buffer. The mixture was incubated at 37°C for one hour, then stopped with an ethanol/acetone mixture. The released fatty acids were titrated with NaOH to determine enzyme activity. One unit of lipase activity was defined as the amount of enzyme that produced 1 µmole of free fatty acids per min under the standard assayed conditions (Aly et al., 2012).

Determination of protein content:
The total protein concentrations of the cell-free extract were determined using the Bradford method with bovine serum albumin (BSA) as a standard, as described by Lowry et al (1951).

Partial Purification of Extracellular Lipases from isolate fungal
The enzymes collected in the permeates and retentates were measured for lipase activity. The fraction that exhibited lipase activity was dissolved in 30 mL of 50 mM phosphate buffer (pH 7.0).
Fungal culture was centrifuged to obtain cell-free supernatant, which was then treated with ammonium sulfate and dialyzed. Enzyme fractions were collected and tested for lipase activity. The fraction with the highest activity was dissolved in phosphate buffer for further analysis. (Romo-Silva et al., 2024).

Molecular characterization of lipase by SDS-PAGE analysis
The purified lipases were separated by electrophoresis (SDS-PAGE) following Priyanka et al method of Priyanka et al., (2020) and Romo-Silva et al., (2024).

Partial Characterization of Extracellular Lipase from fungal isolate
Characterization of extracellular lipase from a fungal isolate was conducted using p-nitrophenyl palmitate (pNPP) as the substrate (Sailwal et al., 2023).

Impact of pH on Lipase yield
The effect of pH on lipase activity was studied using 50mM phosphate buffers at pH 3, 5, 7, and 9 at 30°C. Lipase activity was measured relative to the maximum activity (100%).

Impact of Temperature on Lipase production
The optimal temperature of the purified lipases was determined by measuring their activity at different temperatures (5°C, 20°C, 40°C, and 60°C) in a 50mM phosphate buffer at the optimal pH. The activity at 60°C was set as 100%, and the relative enzyme activity was calculated based on this maximum activity.

Effect of metal ions and inhibitors on Lipase yield
The impact of various metal ions and inhibitors on lipase activity was assessed with 1mM of, Cu^{2+}, Fe^{3+}, Hg^{2+} and EDTA, in 0.05M phosphate buffer (pH 7), at 30 ºC.

Lipase Reaction Kinetics Determination
The reaction velocities of purified fungal lipases were measured using pNPP as a substrate at concentrations from 5 to 20 µM. Enzyme activities were measured at optimal pH and temperature. The Michaelis-Menten constant (KM) and apparent maximum velocity (Vmax) were calculated using the Lineweaver-Burk method. Maximum specific growth rate (µmax), biomass yield (YXS), and volumetric biomass productivity were also determined.

Statistical Analysis
The data was analyzed using analysis of variance followed by the Tukey test in Statistical 6.0 (Statsoft Inc., Tulsa, OK, USA) (Romo-Silva et al., 2024).

RESULTS and DISCUSSION
we first conducted morphological, microscopic, and molecular identification of the isolated fungal strain to confirm its species-level taxonomy. Subsequently, we employed a sequential OFAT strategy to optimize: (i) carbon sources (glucose, sucrose, Dextrose, maltose, Lactose and Fractose), (ii), lipid inducers (olive oil, Tra oil, coconut oil and used cooking oil), (iii) incubation temperature, (iv) initial pH of the medium, (v) fermentation time, and (vi) inoculum size.

Isolation of Lipase Producing Fungal
All fungi exhibiting lipase activity were isolated. A wide range of lipolytic activity was observed. The change in pH of the medium due to the release of fatty acids resulted in the formation of a prominent yellow zone on the Phenol red agar plate, indicating lipase production (Figure 1).

Characterization and Identification of the most lipase producing isolate:
Taxonomical identification of the fungal isolates
The light microscopic images of the hyphae and spores of the isolated fungal strain are depicted in Figure 2. Examination of SEM images of the isolated fungi show swollen vesicles and ellipsoidal to subglobose or globose conidia, resembling Aspergillus flavus as reported by Wadia and Jain (2017)

Molecular identification of dominant fungal
Genomic DNA was successfully amplified using the method described by Thegarathah et al through PCR with the 18S rRNA primer pair. The PCR products from soil samples were analyzed on a 2% agarose gel. The target amplicon size of the isolated fungus was determined to be 600 bp, as indicated by the 100 bp DNA ladder molecular size marker (Invitrogen, North America) (data not shown). The species of the fungus was identified by aligning the sequenced 18S rRNA regions with sequences in the NCBI (National Center for Biotechnology Information) database. Subsequently, a BLAST (Basic Local Alignment Search Tool) search was conducted to confirm the species identity, revealing the fungus to be Aspergillus flavus. The BLAST search showed a 97% identity and 92% query coverage for the isolated fungus (Thegarathah et al., 2024).
Phylogenetic analysis based on the sequenced data indicated a close relationship between the query fungus and various Aspergillus flavus strains. Maximum Parsimony (Figure 3) tree showed a bootstrap value of 100%, indicating high reproducibility. This suggests that the isolated fungus is a strain of Aspergillus aculeatus. The sequencing report confirmed the fungus as Aspergillus flavus and was designated as strain MH279408.1.

Effect of different factors on growth and lipase production by fungi:
One-factor-at-a-time fermentation medium optimization
OFAT methodology is essential for establishing the individual influence of critical parameters such as carbon source, nitrogen source, inducer concentration, incubation time, temperature, pH, and inoculum size. As noted by Ahmed et al. (2023), the OFAT approach is considered an easy convenience and has been the most preferred choice among researchers for designing medium composition in the initial stages of diverse strategies.

Lipase activity time course
As a result, the nine-day fermentation period was taken into account in subsequent research on the Aspergillus flavus strain's ability to produce lipase. The extracellular lipase activity during the fermentation period was depicted in Fig. 4. It was shown that the ideal time to achieve the maximal yield was amongst the 3rd and 5th day (log phase), peaked at 2.936 U/mL after four days, and then began to decline. After the first four days, the specific lipase production rate was 0.27/day, and there was a positive association ($r = 0.43$) between the incubation time and enzyme activity.
These findings are consistent with those of (Romo-Silva et al., 2024), who found that Yarrowia lipolytica's lipase activity peaked on the fourth day. The ensuing drop-in activity could be caused by metabolic changes like nutrient consumption, pH shifts, or the buildup of final products. Additionally, the most potent isolate exhibited the maximum lipase productivity after five days of cultivation in mineral medium. According to Roy et al (2018), the incubation time is a crucial factor in the generation of lipase from various microbes.

The impact of the carbon source
Figure 5 depicted the impact of carbon sources on the lipase activity of the Aspergillus flavus strain. Fructose (2.911 U/mL) and glucose (2.902 U/mL) were found to have the highest lipase activity. This could be because lipases are inducible enzymes that are secreted in the medium when activated with particular (lipid) substrates. The decrease in

lipase activity (2.885 U/mL) is caused by catabolite suppression Abdulmumini et al (2022), which happened while employing dextrose as a carbon source.

Effect of different substrates (oil types) on growth and lipase production

The results shown in Fig. 6 indicated that a medium supplemented with olive oil, coconut oil, tar oil, and used cooking oil as individual carbon sources had a highly noteworthy effect on lipase activity by Aspergillus flavus compared to other carbon sources, resulting in an increase of about 5%, relative to olive oil, respectively. These findings are in line with those of Verma et al (2021), who found that Aspergillus oryzae lipase activity dropped as soybean oil concentration increased. Furthermore, Wongfaed et al (2020) revealed that a medium enriched with 1% olive oil had the highest lipase activity, which was eight times higher than the basal medium used as a control.

The length of fatty acid carbon chains and degree of unsaturation, as well as the substrate concentration, were found to stimulate lipase activity (Karim et al., 2023; Kumar et al., 2023) demonstrated that A. niger favored sugar substrates (glucose) solely for fungal growth, not lipase production. They employed several substrates as carbon sources, including olive oil, refined oil, rice bran oil, gingelly oil, and leather fleshing oil. The lipase productivity ranged from 140 to 165 U/mL, and cumulative the oil content reduced the yield (Anita et al., 202023). In general, a 1% concentration of fish frying oil was shown to be the optimal carbon source for Aspergillus flavus' lipase synthesis.

Cooking oil and glycerol, so offering excellent resource productivity and supporting sustainability and the circular economy. Optimizing the fermentation conditions for enhanced lipase synthesis and prospective biotechnological applications should be the main focus of future research.

Impact of initial pH

The lipase synthesis by Aspergillus flavus varies significantly ($p < 0.05$) at various pH values between 3 and 9, as shown by the results presented in Fig. 7. At pH 3, high lipase activity values were recorded. In comparison to its value at the control pH of 7, the highest enzyme activity (2.869 U/mL) was attained at pH 3, which was the most advantageous parameter. The initial basic media may increase the intracellular pH of the cells and break the enzyme formation network, which might be the reason for the rapid reduction in lipase activity at pH 8 (Roy et al., 2018).

These results are consistent with those reported by Abdulmumini et al (2022), who demonstrated that the maximum lipase production by Penicillium expansum occurred at an initial pH of 5.5-6.0. The optimum pH for Lipase produced by Aspergillus flavus (MH279408.1) was found to be 3.0, with the optimum temperature being 30°C (Priyanka et al., 2020). Pseudomonas-KM110 extracellular lipase showed maximum lipolytic activity at 45°C, with the enzyme also being stable at this temperature.

In contrast, an initial pH of 7 was found to be the optimal concentration for lipase activity by A. niger, resulting in a 1.4 times greater increase over the basal medium that had been confirmed to pH 5.0. Furthermore, Singh et al (2023) found that Fusarium oxysporum produced the greatest lipase productivity at pH values between 6.5 and 7.5.

The impact of incubation temperature

Figure 8 illustrates how varied incubation temperatures affected Aspergillus flavus's production of lipase. It was found that 30°C was the ideal temperature for lipase production, with an activity of 2.845 U/mL. Excessive heat denaturates the enzyme protein's tertiary structure, responsible for the sharp decrease in activity at 40°C (Roy et al., 2018). Thegarathah et al (2024) published similar findings, showing that F. oxysporum's lipase activity maximized around 30°C. A. niger M4A135, on the other hand, demonstrated maximum lipase activity at 37°C, which was 4.3 and 3.2 times higher than its activity at 25°C and 30°C, respectively, according to Colin et al (2010).

The impact of inoculum size

The study showed that lipase activity increasingly improved with cumulative inoculum size up to 7% v/v, which resulted in the maximal activity of 2.931 U/mL (Fig. 9). However, increasing the inoculum size beyond 4% v/v led to a decrease in lipase yield. This could be attributed to enhanced cell mass formation and reduction of nutrients. The correlation coefficient (r) was extremely positive at 0.78. In contrast, it was established that a 10% inoculum size of A. japonicus was optimal for attaining maximal lipase yield (Roy et al., 2018).

It must be acknowledged that the optimization of lipase production in this study employed a one-factor-at-a-time approach. While this provides a clear picture of individual parameter effects, it does not account for synergistic or antagonistic factor interactions and therefore converges on a local optimum. The results presented herein serve as a validated baseline for advanced statistical optimization. A sequential experimental design strategy is currently planned, employing a Plackett-Burman design for initial factor screening, followed by Response Surface Methodology using a Central Composite Design to model interactions and predict a global production maximum.

Partial purification and enzyme characterization.
Solid ammonium sulfate ranging from 10% to 80% w/v was used to determine the optimal concentration for precipitating proteins from Aspergillus flavus. The best concentration identified was 30% salt, followed by filtration with gentle stirring at 4°C. The mixture was then centrifuged at 8000 rpm for 1 hour at 4°C.
The purification of lipase resulted in a two-fold purification with a 72% recovery rate through ammonium sulfate precipitation. Using SDS-PAGE, the lipase enzyme resulting from A. flavus was found to have a molecular weight of 44 kDa. According to Yao et al (2021), A. niger's protein mass ranged from 35 to 40 kDa (Fig. 10).

Partial Characterization of the Lipases from Aspergillus flavus: **Table 1**
Effect of pH and temperature on lipase
Lipase exhibited productivity within a pH range of 3 to 10, with maximal yield observed at pH 5 (Table 1). Bacillus thermoleovorans ID1 lipases were most active at pH 9. It has been found that the pH range of 7.2–8.5 is favorable for the process of lipases from thermophilic Bacillus species. Microbacterium sp. lipase was active between 10 and 90°C, with 50°C representing an optimal temperature. Comparable levels of lipase activity were identified in several strains, including Bacillus sp. A30-1 (60°C), Bacillus sp. J33 (60°C), and Bacillus thermoleovorans ID-1 (60–65°C) (Fetyan et al., 2023).
These lipases have an optimal pH of 8.0, which makes them useful for flavoring synthesis, bioremediation, and detergent production. When the pH was raised from 8.5 to 9.5 and lowered from 8.0 to 7.0, both yeasts' lipase activity drastically dropped. Reduced enzyme saturation with the substrate as a result of lower affinity could be the cause of this drop-in lipase activity. Fluctuations in solution pH can also affect enzyme activity by altering the ionization of functional groups and enzyme structure conformation (Fetyan et al., 2023).
Several yeast species, including S. flava, C. rugosa, C. curvata, Y. lipolytica, and G. pullulans, exhibit **congruent ideal pH values** centered around **8.0** for lipase activity (Thapa et al., 2019). In contrast, Candida guilliermondii lipase performed best at a pH of 6.5. The lipases produced by H. wangnamkhiaoensis and Y. deformans exhibit pH-sensitive behavior, achieving peak stability at pH 8.0 and maintaining at least 40% relative activity between pH 7.5 and 9.0. In contrast, various microbial lipases such as those from C. rugosa, G. candidum ATCC34614, Y. lipolytica, and Trichosporon species—demonstrate broad stability across multiple pH levels. Generally, most microbial and fungal lipases remain stable within a wide window of pH 2 to 10.5 (Fetyan et al., 2023).

Impact of various substrate concentrations on lipase activity
Enzyme kinetics of lipase from A. flavus RRF2- MH279408 were determined by measuring K_m and V_{max} values using Michaelis-Menten plots with varying p-NPP substrate concentrations at optimal pH and temperature. The K_m and V_{max} values were calculated using Line Weaver and Eadie-Hofstee plots. The K_m and V_{max} values for the lipase were found to be 0.4 mM and 3.125 µM/min/mg, respectively (Fig. 12).
Earlier studies stated that the K_m and V_{max} values for a lipase derived from Bacillus sp. J33 were 2.5 mM and 0.4 M/mL/min, respectively, with pNPL as the substrate. For lipase from Bacillus Stearothermophilus, the K_m and V_{max} values were 0.33 mM and 188 µM/min/mg, respectively .(Fetyan et al., 2023). The purified lipase enzyme derived from A. flavus exhibited a Vmax of 85 U/mg and a Km of 0.8 mg/mL. A low K_m value indicates high affinity. K_m values for enzymes vary widely, but for the most industrially relevant enzymes, K_m typically falls within the range of 10^{-1} to 10^{-5} M. In this study, the K_m and V_{max} values were 0.7 mg/mL and $0.97x10^{-3}$ U/min, respectively (Romo-Silva et al., 2024). Furthermore, the lower Km values of Aspergillus flavus lipases indicate a strong affinity for pNPP. The K_m value of Y. deformans lipase was found to be lower, similar to, and greater than that of lipases from Y. lipolytica (0.234 mM), G. pullulans (0.368 mM), and Candida antarctica (0.675 mM) (Fetyan et al., 2023). This reveals that Y. deformans lipase has a greater, similar, and lower substrate affinity than Aspergillus flavus lipases.

Impact of Temperature on Lipase Activity and Stability
The activity and use of lipases in industrial processes are significantly influenced by temperature (Fetyan et al., 2023). Aspergillus flavus lipase activity dramatically increased with temperature, peaking at 30°C (Table 1). This outcome is explained by the fact that a rise in temperature increases the kinetic energy of the molecules of the enzyme and substrate, making them move more quickly and resulting in successful impacts between the substrate and the lipases' active sites. Nevertheless, Aspergillus flavus lipases were less active at temperatures over 30°C (Table 1). This study represents the first report on the isolation, OFAT-based optimization, and partial purification of a lipase from A. flavus obtained from [Rijal Almaa, Saudi Arabia].
The denaturation of lipases, which causes irreversible modifications to the molecular structure, a change in the conformation of the active site, and a decrease in substrate-enzyme interaction, may be the cause of this decrease (Wadia and Jain 2017). The optimal temperatures for the lipases from T. asteroides, C. curvata, and Kurtzmanomyces sp. I-11 are 50°C, 60°C, and 75°C, respectively, whereas the majority of yeast lipases show maximal activity at temperatures between 28°C and 40°C (Fetyan et al., 2023).

Although the lipase from Penicillium wortmanii is classified as a moderately thermostable enzyme, it preserves only 55% of its original activity following one hour at 50°C (Wadia and Jain 2017). Comparable thermostability has been observed for the lipase of the halophilic archaeon Natronococcus sp., which retains more than 90% of its initial activity under the same conditions (Thegarathah et al., 2024).

Effect of metal ions and enzyme inhibitors on Lipase activity

Metal ions play a crucial role in the binding between the enzyme and the substrate, as they come into contact with both and hold them together at the enzyme's active site. The results of metal ions on lipase activity were presented in Table 1, showing that the lipase activity ranged from 2.73 to 2.75 U/ml. Additionally, several authors have reported the involvement of calcium ions in altering the position specificity of the active site during the binding process (Thegarathah et al., 2024). However, the results indicated a decrease in relative activity when using $Cu^{2+,}$ Fe^{2+}, Hg^{2+}, and EDTA. This inhibitory effect of metal ions may be attributed to changes in the solubility and behavior of ionized fatty acids at interfaces, or alterations in the catalytic properties of the enzyme itself (Golani and Hajela 2024). A similar inhibitory effect of Cu^{2+}, Fe^{2+}, and Zn^{2+} metal ions on lipase from B. methylotrophicus PS3 was also reported by (Romo-Silva et al., 2024).

CONCLUSIONS

Lipase is a highly valuable industrial enzyme with a wide range of applications. Due to the diverse uses of lipases in various industries, it is important to investigate their features as lipases from diverse sources may exhibit varying properties. This study aimed to partially characterize lipases obtained from Rijal Almaa, Saudi Arabia, produced by Aspergillus flavus (MH279408.1), with a molecular weight of 44 KDa. The enzyme demonstrated stability at 20°C, pH 5, and in various vegetable oils. Since these microorganisms are considered safe for food, brewing, and pharmaceutical use, additional research is required to optimize fermentation for potential commercial scale-up across various industries.

Data Availability Statement:

The datasets from the study are available upon request from the corresponding author.

Author contributions

The author searched the literature, vetted and structured the data, created the illustrations, and revised and approved the final version.

Acknowledgments:

Conflicts of Interest:

The authors have no conflicts of interest.

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