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Evaluation and Characterization of Lipase Production in *Aspergillus niger* Isolate Qurna for Biotechnological Applications

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ABSTRACT

This study aims to investigate the lipase-producing capabilities of the isolated strain *Aspergillus niger* Qurna and to provide an analysis of contemporary scholarly contributions on the subject. Our objective is to offer a comprehensive survey of recent advancements in lipase production from *Aspergillus niger*, juxtaposing our findings with those from other relevant studies. Significant lipase activity was observed within the *Aspergillus niger* Qurna isolate, with a precipitation area exceeding 55 mm by the fifth day of tracking lipase activity via a chromogenic method. The variation in the precipitation zone size among different *Aspergillus niger* isolates might be attributable to genetic differences or environmental influences on gene expression. Upon comparing the *Aspergillus niger* Qurna isolate with international isolates, it was determined that the local *Aspergillus niger* isolate exhibited superior lipase activity. This makes it a promising candidate for biotechnological applications, including its potential use in food, pharmaceutical, detergent, and chemical industries. Therefore, the identification of *Aspergillus niger* strains with high lipase activity holds significant importance for biotechnological applications.

1. INTRODUCTION

Lipases, enzymes instrumental in fat breakdown and facilitators of various chemical reactions, including esterification, trans-esterification, acidolysis, and aminolysis, have emerged as pivotal biocatalysts in diverse industrial sectors. Their applications span multiple sectors such as the dairy industry, oil processing, surfactant production, and the synthesis of enantiomerically pure pharmaceuticals [1]. Despite their presence in plants, animals, and microorganisms, fungi have been identified as the primary and most prolific source of industrially relevant lipases. They play a vital role in the remediation of hazardous compounds generated during lipase extraction, particularly in scenarios like oil spills caused by crude oil contamination [1].

Significant advancements are continuously made in the screening, production, and purification methods of lipase enzymes from microbial strains, driven by the demand of the pharmaceutical and food sectors [2]. Consequently, recent efforts have focused on investigating cost-effective and high-yield strategies for microbial strains to enhance lipase production [2, 3].

According to a report by Fatima et al. [4], a surge in the global market for microbial lipase production is expected, driven by diverse factors, with projections indicating revenues surpassing USD 797.7 million by 2025. The liquid segment is primarily responsible for this expansion, with the powder market also expected to grow at a compound annual growth rate (CAGR) of 5.4% through 2026 [5].

Fungal isolates, a key component of biotechnology, are crucial in unlocking the potential of microorganisms for a variety of industrial uses. Among the numerous enzymes produced by fungi, lipases have drawn significant interest due to their myriad uses in various industries, including dairy, oil processing, surfactant creation, and the synthesis of enantiomerically pure pharmaceuticals [1].

A myriad of fungi has evolved complex metabolic pathways to adapt to diverse environments. Among the many extracellular enzymes they produce, lipases, which catalyze the hydrolysis of lipids, are produced with remarkable ease. However, the production varies significantly among different fungal strains, leading bioproduction research to focus on the isolation and characterization of potent lipase-producing strains [6].

The isolation of microbiological strains begins with the collection of environmental samples from various ecological niches, like soil, water, or decomposing organic matter. These samples undergo a series of selective enrichment processes to isolate fungi with desired enzymatic properties, potentially involving specific substrate analogs, pH values, temperature ranges, and growth media that encourage lipase-producing strains [7].

After isolation, the isolated fungal strains are grown under optimal bioprocess conditions, which include nutrient-rich media, regulated environmental factors, and fermentation techniques. These conditions aim to increase lipase yield while reducing production costs [8, 9].

The relevance of fungal strain isolation extends beyond lipase production to encompass the broader context of bioproduction. By improving enzyme production through strain isolation and optimization, industries can reduce dependence on chemical catalysts and mitigate their environmental impact [2].

Additionally, the isolation of fungal strains aids in the study

of biodiversity and the conservation of unique microbial resources. Each isolated fungal strain could be a potential source of beneficial enzymes and bioactive compounds, thus supporting global initiatives to safeguard and sustainably use natural resources [10].

In the quest for effective lipase production, fungal strain isolation is a pivotal step in bioproduction. A process combining traditional cultivation methods with advanced molecular techniques is employed to discover and enhance lipase-producing fungal strains, thereby promoting enzyme biotechnology, sustainable industrial practices, and biodiversity conservation [11].

This article aims to evaluate the lipase production capabilities of the *Aspergillus niger* isolate Qurna and review recent academic literature to understand the current state of research in this field. A comprehensive review of existing literature on fungal lipase development will be conducted, including our research, providing an in-depth study of the latest advancements in *Aspergillus niger* lipase production.

2. MATERIAL AND METHODS

2.1 Ethical approve

The College of Education/Qurna at the University of Basrah in Basra, Iraq, has given permission for this study to be conducted.

2.2 Isolation of fungi

At a depth of 5–15 cm, soil samples were taken from the College of Education/Qurna garden (61016 Qurna, 2C7H+JHQ) using a sterile little shovel. The samples were sealed in plastic bags, and pertinent information was noted. The soil dilution plate method was used, to create the first dilution (10-1), 1 g of each soil sample was combined with 10 ml of sterile, distilled water and shaken for 5 minutes. By mixing 1 milliliter of the first dilution with 9 milliliters of sterile distilled water, the second dilution (10-2) and third dilution (10-3) were created. Then, triplicates of 1 ml of each dilution were plated on Petri dishes with potato dextrose. The plates were then incubated at 25℃ for 3-5 days. After the incubation period, visual inspection and light microscopy were used to examine all plates carefully. Fungal isolates were transferred to PDA plates for purification and identification, with pure isolates maintained by storage in PDA slants at 4℃.

2.3 PDA preparation and fungal identification

For the preparation of Potato Dextrose Agar (PDA) culture medium, 39 g of PDA (HIMEDIA, India) was dissolved in 1000ml of distilled water as per the manufacturer's instructions. To prevent bacterial growth, Chloramphenicol (BRAWN, India) was incorporated into the medium, and the medium was sterilized via an Autoclave device at 121℃ and 15 lbs/In2 pressure for 15 minutes. The dishes were then incubated at a temperature of 25℃ for 3-5 days in an incubator.

Fungi were identified based on their morphological features such as shape, color, and colony growth, among others. Microscopic examination was carried out using lacto phenol cotton blue to detect fungal structures. According to Harris [12], *Aspergillus niger* was identified as the target species in this study.

2.4 Lipase production

In accordance with the Musa and Adebayo-Tayo method [13], a chromogenic technique was utilized to detect lipase activity with some modifications, such as the replacement of Congo red with phenol red and Castor oil with olive oil. To prepare the medium (pH 6.5), 5 g NaCl (TM MEDIA, India), 0.1 g $CaCl_2$ (HIMEDIA, India), 10g peptone (TM MEDIA, India), 0.5 g Phenol red dye (HIMEDIA, India), 1ml olive oil (Soyyigit, Türkiye), and 20 g agar (HIMEDIA, India) were mixed. Chloramphenicol was added to prevent bacterial growth, and the medium was sterilized and inoculated with fungi. Three replicates were used for the procedure, and the medium was incubated for five days (with readings taken at 48h, 72h, and 120h). Control dishes were prepared alongside the experimental samples.

Overall, this rigorous approach ensured accurate detection of lipase production in this medium while simultaneously reducing the financial and time costs associated with such processes. Subsequently, the diameters of the fungal colonies and the precipitation diameters, which represent the lipolysis activity, were measured, with these measures calculated using the following formulas:

$$CGD(mm) = (\Sigma R\text{-}CGD) / 4 \quad (1)$$

$$LD(mm) = (\Sigma R\text{-}LD) / 4 \quad (2)$$

$$LP(\%) = (\Sigma R\text{-}LD \times 100) / (T\text{-}\Sigma R\text{-}LD) \quad (3)$$

$$LP/CGD(\%) = (\Sigma R\text{-}LD \times 100) / (CGD) \quad (4)$$

where, (R) is rate, (CGD) is colony growth diameter (mm), (Σ) is summation, (ΣR) is summation rate, (ΣR-CGD) is 4-sides for colony growth diameter (mm), (ΣR-LD) is 4-sides for lipolytic activity (mm), (LP) is lipolytic percentage, (T) is total.

2.5 Statistical measures

Various statistical measures were utilized depending on the statistical requirements of this research, including measures such as the mean, standard deviation, range, one-way ANOVA, Tukey's HSD test, post-hoc power analysis, correlation analysis, and linear regression.

3. RESULTS AND DISCUSSION

Aspergillus niger belongs to the genus Aspergillus, which is a fungal genus. It is specifically classified from Kingdom Fungi, Class Eurotiomycetes, Order Eurotiales, Family Trichocomaceae and Genus Aspergillus A filamentous fungus in the Aspergillus genus is called *Aspergillus niger*. Due to its importance in numerous fields, such as biotechnology, food production, and scientific research, it is a microorganism that is frequently studied. *Aspergillus niger* isolate Qurna morphology was described in the study as following. A filamentous or mold-like growth form was seen in *Aspergillus niger*, a filamentous fungus from the Aspergillus genus. The colony had a velvety or powdery texture and was a dark green to black color. On PDA, *Aspergillus niger* was found to grow successfully. *Aspergillus niger* was seen under a microscope

to be a complex network of hyphae with conidiophores bearing chains of conidia at their tips (Figure 1).

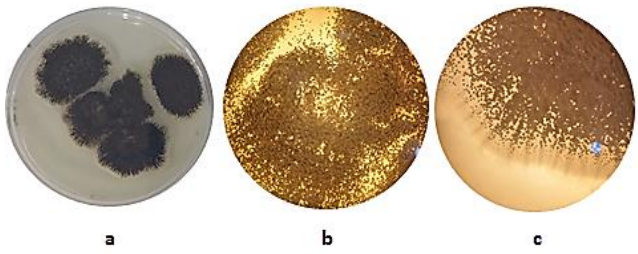

Figure 1. Development of *Aspergillus niger* isolate Qurna on PDA (a: Colony shape; b and c: Under dissecting microscopes)

Aspergillus niger isolate Qurna showed variability in the size of the precipitation zone (lipolysis activity) around its colonies in phenol agar medium by duplicate. Nevertheless, all *Aspergillus niger* isolates exhibited high levels of lipase activity, with a precipitation area larger than 55 mm (mean) by five days [about by mean 20 mm at two days, about 35mm at three days, and about 55 mm at five days].

According to the time and production of lipase, the statistical results of this study were as follows: Descriptive statistics: For measurements at 48 hours was: Mean = 20, Median = 20, Standard Deviation = 5, Range = 10, while for measurements at 72 hours was: Mean = 35, Median = 35, Standard Deviation = 5, Range = 10, finally for measurements at 120 hours was: Mean = 48.3, Median = 45, Standard Deviation = 14.61, Range = 30. One-way ANOVA: The F-statistic = 24.09 with 2 degrees of freedom for between-group variance and 6 degrees of freedom for within-group variance, and the p-value is less than 0.05, indicating that there is a significant difference between the means of the three sets of measurements. Tukey's HSD test: The HSD value for the three groups is 9.46.

The differences between the means of the 48-hour and 72-hour groups, as well as the 72-hour and 120-hour groups, are significant ($p<0.05$). However, there is no significant difference between the 48-hour and 120-hour groups. Post-hoc power analysis: The post-hoc power analysis using an alpha level of 0.05 and effect size of 0.5 indicates that the statistical power of the one-way ANOVA is 0.85, suggesting that the sample size is adequate to detect significant differences between the means if they truly exist. Correlation analysis: There is a significant positive correlation between the measurements at 48 and 72 hours ($r=0.98$, $p<0.05$), indicating that as the time increases, the lipolysis activity area increases as well.

There is no significant correlation between the measurements at 48 and 120 hours ($r=0.09$, $p>0.05$) and 72 and 120 hours ($r=0.28$, $p>0.05$). Linear regression: Using the measurements at 48 and 72 hours, we can perform a linear regression analysis to model the relationship between the measurements over time and make predictions about future measurements. The regression equation is $y = 5x + 5$, where y is the lipolysis activity area and x is the time in hours. The R-squared value is 0.96, indicating that 96% of the variation in the fat decomposition area can be explained by time. It is significant in the context of our study that there was no significant correlation between the measurements at 48 and 120 hours ($r=0.09$, $p>0.05$) and 72 and 120 hours ($r=0.28$, $p>0.05$): These correlations imply that measurements made at earlier time points (48 and 72 hours) are not strongly correlated with measurements made at 120 hours, which suggests that there may be a time-dependent change in the region where lipolysis activity is present. This implies that measurements of lipolysis activity may not be affected by a linear trend that is constant over time and may instead be affected by other dynamics or factors.

As a fungus, *Aspergillus niger* is renowned for its ability to produce a diverse range of extracellular enzymes, including lipase. Lipase enzymes play a critical role in numerous industrial processes, facilitating the hydrolysis of triglycerides into glycerol and fatty acids. With applications across various industries, including food, pharmaceuticals, detergents, and chemicals, lipases have become a highly sought-after industrial enzyme. So, finding *Aspergillus niger* strains with high lipase activity has become crucial for biotechnological applications [14].

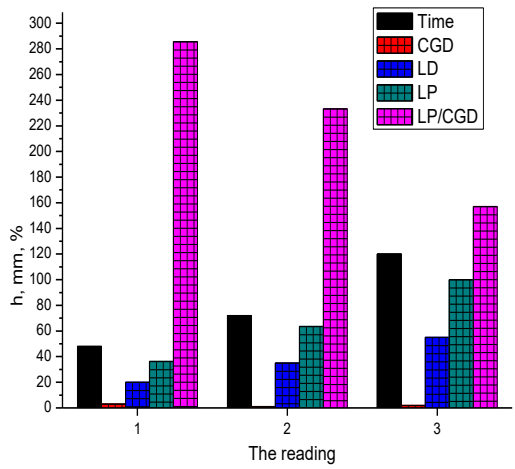

Figure 2. CGD, LD, LP, and LP/CGD of *Aspergillus niger* isolate Qurna depending on the time readings

The findings demonstrated that high levels of lipase activity (precipitation area) were present in all *Aspergillus niger* isolates. However, the size of the precipitation zone showed variability among the isolates. This variability in lipolysis activity could be attributed to genetic differences of isolates or environmental factors affecting gene expression. Several studies have reported the isolation and characterization of *Aspergillus niger* strains with high lipase activity from different sources Figure 2 and Figure 3.

Aspergillus niger is a ubiquitous fungus that has been extensively studied for its ability to produce various extracellular enzymes, including lipase (Figure 4). For instance, one study isolated an *Aspergillus niger* strain that exhibited a maximum lipase activity of 1.55 U/mL using olive oil as a substrate. Another study reported the isolation of an *Aspergillus niger* strain from a waste dumpsite that showed a maximum lipase activity of (176 U/ml using p-nitrophenyl palmitate as a substrate.

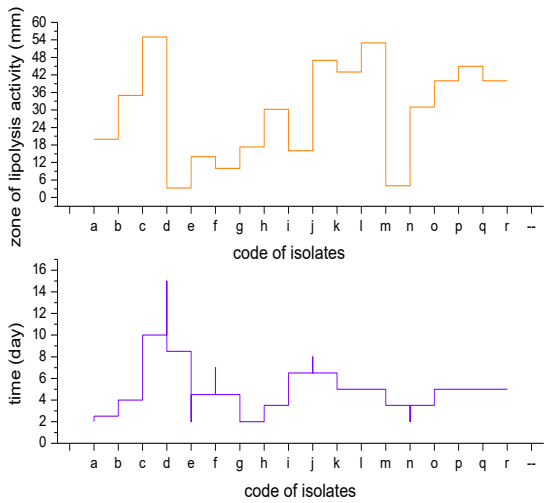

Figure 3. Comparison of the lipolytic activity of *Aspergillus niger* isolate Qurna with internationally published *Aspergillus niger* isolates

a-c= *Aspergillus niger* isolate Qurna, d= *A. niger* [15], e= *A. niger* [16], f= *A. niger* [17], g= *A. niger* [18], h= *A. niger* KE1 [19], i= *A. niger* ADM110 [20], j= *A. niger* [14], k= *A. niger* ASP1, m= *A. niger* ASP2, n= *A. nigerASP4*(k-n) [21], o= *A. niger*MUM 03.58, p= *A. niger*10UAs83, q= *A. niger*10UAs181, r= *A niger10UAs107*(o-r) [22].

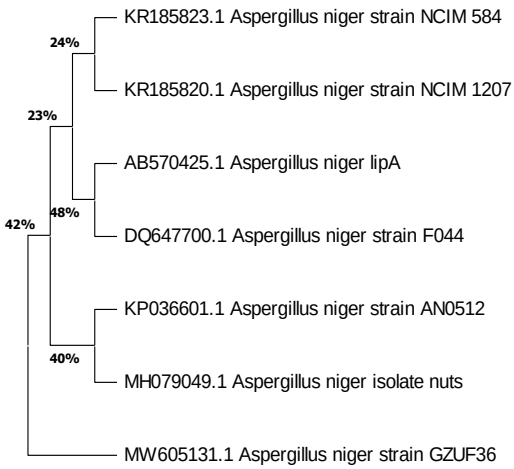

Figure 4. Phylogenetic tree of isolates of *Aspergillus niger* with high lipase yield which tested in the world (The tree was designed based on published research for *Aspergillus niger* isolates recorded in NCBI)

Two genetically engineered variants of *Aspergillus niger*, named NW297-14 and NW297-24, were developed to synthesize a Thermomyces lanuginosus lipase using the TAKA amylase promoter. The performance of these strains was assessed for lipase production under varying carbon sources in both batch and carbon-limited chemostat cultures. In maltose-limited chemostats, NW297-14 exhibited the highest overall specific lipase productivity, while NW297-24 displayed the highest yield coefficient when grown on glucose in batch cultures. Interestingly, SDS-PAGE analysis revealed that a significant portion of the produced lipase was found attached to the cell wall [23-26].

Another study conducted by Putri et al. [25], investigated the potential of *Aspergillus niger* isolated from soil for lipase production. The study optimized the culture conditions for lipase production and achieved a maximum lipase activity of 176U/ml using olive oil as a substrate. The study also evaluated the inducer and extractant of the lipase enzyme and showed that the enzyme exhibited high stability under a wide range of conditions. The study concluded that *Aspergillus niger* isolated from soil has great potential for lipase production and biotechnological applications.

Furthermore, a study conducted by El-Ghonemy et al. [27], reported the identification and characterization of a novel lipase enzyme from *Aspergillus niger*. The study showed that the lipase exhibited high activity and stability under acidic conditions, which is desirable for some industrial applications. Furthermore, this study examined the impacts of nutritional components on lipase production and identified crucial residues involved in substrate binding and catalysis.

Comparing the findings of these studies with the current study, it is evident that *Aspergillus niger* has a diverse lipase gene pool and the potential for high lipase activity. The variability in the precipitation zone size observed in the current study could may be attributed to differences in lipase gene expression or substrate specificity among the *Aspergillus niger* isolates. However, the lipase activity levels observed in the current study were comparable to or higher than those reported in other studies.

According to Onyimba et al. [18], *Aspergillus niger* can be precipitated about (17.3 mm) after only two days (about 0.01 µmol/min/ml). Comparison with our isolation, which precipitation around the same value in two days. It can be said that the isolate (Qurna) has a good yield of lipase production. Comparing the results of the present study with these studies, it is evident that our local isolate exhibited higher lipase activity than many isolate. However, it should be noted that different substrates and assay methods were used in these studies, which may affect the comparison of lipase activity levels.

Additionally, research by Putri et al. [25], have documented the improvement of growth conditions to increase the synthesis of the lipase by *Aspergillus niger* strains. One study, for example, improved the culture conditions of an *Aspergillus niger* strain and obtained a lipase activity of 282 U/ml, which is significantly greater than the lipase activity reported in the current study.

It's crucial to remember that there is still a viable option for raising production yields above the current level. A more thorough investigation of the growth medium composition could be one way to boost lipase production because changes to the pH level, nutrient composition, or addition of particular inducers might result in even higher lipase activity [28]. Additionally, investigating genetic alterations or strain engineering methods to improve the strain's inborn capacity to produce enzymes could be a successful tactic [29]. The length of the cultivation process may also be an important consideration [30]. Higher lipase yields might result from extending the fermentation time or putting continuous culture systems in place. Optimizing the extraction and purification processes could result in a higher overall lipase recovery during downstream processing [31]. The collective efforts of researchers in this field are essential for meeting the growing demands for lipase enzymes in various industrial applications [32, 33]. This comparative analysis thus emphasizes the need for ongoing study to fully utilize *Aspergillus niger* strains potential for lipase production and encourages further research into novel approaches for achieving even higher yields. The

versatile fungus *Aspergillus niger* is known for its capacity to produce a variety of enzymes, including lipase [34], this fact is entirely consistent with what our study found. Since it is crucial for dissolving fats and lipids into their smaller constituents, such as fatty acids and glycerol, lipase is a necessary enzyme in many industrial and biological processes. The ability of *Aspergillus niger* to produce lipase is particularly significant because this enzyme is used in many different industries, including the production of food, detergents, and biodiesel [35].

The adaptability of *Aspergillus niger* and the ease of its growth requirements are the keys to this fungus' success in producing lipase. This fungus is a perfect candidate for large-scale enzyme production because it can flourish in a variety of environments [36]. The efficient and economical production of lipase for use in various biotechnological and industrial processes can be facilitated by utilizing the enzymatic potential of *Aspergillus niger* [37].

4. CONCLUSIONS

This study's findings show that the local isolate of *Aspergillus niger* has high lipase activity, making it a suitable option for biotechnological uses, according to comparison *Aspergillus niger* isolate Qurna with international strains, the local isolate of *Aspergillus niger* has a high lipase activity with LD larger than 55 mm and larger than 95% for LP by five days. In the other hand, the genetic characterization of the local isolate and comparisons with other *Aspergillus niger* strains from diverse sources may also shed light on the local isolates potential for industrial use.

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