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Pathogenic and Industrial Implications of Lipase Enzyme Extracted from *Aspergillus niger*: A Comparative Study of Biotechnological Applications and Clinical Risks

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Abstract

Aspergillus niger is a widely distributed filamentous fungus known for its significant role in industrial biotechnology and its potential as an opportunistic pathogen. This study investigated the production, characterization, and industrial applications of A. niger-derived lipase while assessing its pathogenic risks and biosafety concerns. Lipase production was optimized using solid-state fermentation (SSF) and submerged fermentation (SmF) techniques, with results indicating maximum enzyme activity at pH 7.5 and 40°C. The enzyme demonstrated strong substrate specificity, particularly for long-chain triglycerides, making it highly suitable for detergents, pharmaceuticals, and food industries. Comparative analysis with bacterial and yeast-derived lipases revealed that A. niger lipase offers high substrate specificity and cost-effective production, but has moderate thermostability compared to bacterial lipases. The study also reviewed clinical cases of A. niger-related infections, particularly otomycosis, onychomycosis, and pulmonary aspergillosis, highlighting its impact on immunocompromised individuals. Antifungal resistance patterns and biosafety concerns, particularly mycotoxin contamination, were assessed, emphasizing the need for regulatory measures in large-scale enzyme production. Despite its pathogenic potential, GRAS-certified A. niger strains remain a reliable source of industrial enzymes, provided that strict quality control and safety protocols are followed. This research contributes to the ongoing biotechnological advancements in enzyme production while addressing the clinical and regulatory challenges associated with A. niger. Future studies should focus on genetic modifications, enzyme immobilization techniques, and sustainable fermentation strategies to enhance the stability, efficiency, and biosafety of A. niger-derived lipases.

Keywords: *Aspergillus niger*, Lipase, Industrial Enzymes, Fermentation, Enzyme Optimization, Mycotoxin Contamination, Biosafety, Pathogenicity, Antifungal Resistance, Biotechnology

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1. INTRODUCTION

The fungal role in natural ecosystems is nonpareil; fungi decompose, are pathogens, or are industrially important microorganisms. Among the diverse fungal species, *Aspergillus niger* is as one of the widely distributed and economically important filamentous fungi. Soil, decaying organic matter, and indoor environments are commonly found (Schuster et al., 2002). Along with extreme adaptability to a wide range of environmental conditions, this species plays a definite role as an integral part of natural and artificial ecosystem. *A. niger* is the most studied as it is widespread and has applications in biotechnology, agriculture and as a clinical microbiologist (Samson et al., 2014).

A. niger is one of the most important endeavors of *A. niger* in biotechnology, by its power to generate a scope of business significant enzymes, for instance, lipases, proteases, amylases and cellulases (Gao et al., 2018). Among them, lipases, especially, are used in food processing, pharmaceutical, detergent and biodiesel production industries due to their ability to catalyze hydrolysis and synthesis of lipids (Treichel et al., 2010). However, as the economic benefit of microbial enzymes are evident mainly in the enzymes isolated from fungi, *A. niger* has gained remarkable interest from researchers to develop novel fermentation and genetic engineering technologies to improve its ability to produce enzymes (Park et al., 2017). The Food and Drug Administration (FDA) also has classified some strains of *A. niger* as Generally Recognized as Safe (GRAS), which adds to the validity of industrial uses of *A. niger* (Ward et al. 2005).

A. niger is dangerous to immunocompromised individuals despite its industrial benefits because of the risk of infection. An opportunistic pathogen, it is associated with several kinds of infections such as otomycosis (ear infections), onychomycosis (nail infections), and in severe cases pulmonary aspergillosis (Kim et al., 2012). While Aspergillus fumigatus is a leading cause of invasive aspergillosis, *A. niger* infections are usually superficial and it can become invasive in an immunocompromised host, for example when undergoing chemotherapy, organ transplant or prolonged corticosteroid treatments (Hope et al., 2005). Additionally, some strains of *Aspergillus niger* are capable of producing A mycotoxins, including ochratoxin A (Abarca et al., 2004).

Due to its dual nature (i.e., *A. niger* is a valuable industrial microorganism and a potential disease agent), this fungus should be thoroughly studied in order to make the most out of its benefits and the least (if any) of its risks. The research work has been focused on optimization of fermentation process for increasing enzyme production, biosafety protocol for its industrial use and antifungal strategies to combat the pathogenic potential of the fungus (Mirhendi et al., 2016). The morphological and biochemical characterization of *A. niger*, its lipase production when subjected to different conditions and its clinical and industrial significance would be explored in this paper. The contribution of this study is to investigate the beneficial and harmful aspects of *A. niger*, which contributes to a balanced understanding of the role of *A. niger* in microbiology, medicine, and biotechnology.

The fungal species *Aspergillus niger* has played an important role in industrial biotechnology and in clinical pathology, hence it is a widely studied species. However, it is also referred as valuable microorganism for enzyme producer, especially lipases, for the implementation in food processing, pharmaceutical and biofuel industries, etc. Instead, *A. niger* is an opportunistic pathogen of immunocompromised individuals where there are concerns regarding its clinical relevance. Thus, the critical research challenge becomes the development of the industrial utility of *A. niger* while minimizing the risk to human health. To be able to consider the safe use of these proteins in biotechnology, it is necessary to have an understanding its biochemical properties, mechanisms of production of the enzyme, and pathogenic characteristics.

This study is designed to work on dual side of *A. niger*: as an enzyme producer and infection in the clinic. It has been proposed in this research that activities and industrial applicability of *A. niger* lipase enzymes be characterized. Thus the lipases are biocatalysts which are highly versatile in lipid metabolism and have a great industrial relevance because of factors such as temperature stability, pH tolerant and substrate specificity. These characteristics are examined in this research, looking at the way in which they can be optimised during fermentation techniques in order to improve enzyme yield for industrial application. Moreover, the possible risks involved in *A. niger* infections will be

investigated by assessing its pathogenicity and the probability of *A. niger* as a threat to human health.

1.1 Objectives

- To investigate the dual role of *A. niger* in biotechnology and clinical pathology.
- To study the production, extraction, and optimization of lipase enzyme from *A. niger*.
- To characterize the lipase enzyme in terms of activity, stability, and industrial utility.
- To identify key environmental and physiological factors affecting lipase production.
- To assess the clinical and pathogenic implications of *A. niger* infections in immunocompromised individuals.

1.2 Research Questions

- How does *A. niger* serve as a reliable source of lipase enzyme?
- What are the key factors affecting lipase production and activity?
- What are the clinical and pathogenic concerns associated with *A. niger*?
- How can lipase extracted from *A. niger* be optimized for industrial applications?

1.3 Scope & Limitations

Within the scope of this research, main effort is directed on production, characterization and application of *A. niger*-derived lipase enzymes. On the other hand, *A. niger* also produces several other industrially relevant enzymes like amylases and proteases but the study is entirely conducted on lipase because in the biotechnology, its role is incredibly good. In addition, this research is limited in that it does not encompass genetic modification and intricate molecular engineering methods to improve *A. niger* strains. The study will rather be on optimizing the conventional fermentation and extraction methods to achieve high lipase yield. By restricting the discussion to enzyme production and applicability in clinical relevance, this research deals with details and applicability of *A. niger* in biotechnology and medicine, yet staying focused.

2. LITERATURE REVIEW

Aspergillus niger is a filamentous fungus belonging to the Aspergillus genus that includes over 200 species of the same fungus. Under phylum Ascomycota, class Eurotiomycetes and order Eurotiales, it falls (Samson et al., 2014). The species are especially characterized by the dark conidial heads, a distinction Marks et al (1992) state to differentiate it from other species of the genus. *A. niger* is found in different environmental conditions such as soil, decaying plant, etc. This microorganism can grow in a wide range of temperature, pH, temperature and pH at which it can grow gives it importance in the industrial and clinical sector (Gao et al., 2018). *A. niger* has been extensively studied due to its economic importance since its great enzymatic potential and pathogenic properties pose a threat.

A. niger has natural habitat in terrestrial and indoor environments. They are very commonly found in the soil and in compost and plant debris where they help turn organic matter into compost (Schuster et al., 2002). Furthermore, the fungus is often isolated from stored food products such as grains, nuts, and dried fruits, and is therefore a problem in the food industry because of the possible contamination with mycotoxins and spoilage (Abarca et al., 2004). In addition, *A. niger* spores are airborne and can be identified in household dust and at construction sites, so taking exposure risk for people with respiratory conditions further. This marine algae is widely spread, capable of colonizing many environments, and there is a real need to understand potential beneficial and harmful effects.

A. niger is a relatively non-pathogenic fungus that has been associated with several opportunist infections which are commonly seen in immunocompromised people. The condition most commonly developed by this fungi is aspergillosis or a fungal damage of the respiratory system in *A. niger* species (Hope et al., 2005). Allergic broncho pulmonary aspergillosis (ABPA) is one of the three different forms of aspergillosis diseases which include chronic pulmonary aspergillosis and invasive aspergillosis. Aspergillus fumigatus is the main causative agent in invasive aspergillosis, however, it can also be caused by *A. niger* which has also been reported to cause severe infections in patients that are undergoing chemotherapy, organ transplants and patients who are on long term corticosteroid therapy (Kim et al., 2012).

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Another concerning fact about *A. niger* infections is that, they can cause otomycosis, which is a fungal infection of the external ear canal. Otomycosis, with characteristics of itching, pain, and hearing impairment, among other problems, is one of the most frequently isolated fungal pathogen *A. niger* (Mirhendi et al., 2016). *A. niger* aside from ear infections has also been linked to onychomycosis which is a fungal nail infection that is usually hard to treat and may require prolonged therapy with antifungals. In addition, *A. niger* rarely can cause pulmonary infections in which the fungus grows into fungal balls (aspergillomas) in the lungs, and results in chronic respiratory distress (Hope et al., 2005).

In addition to its clinical applications, *A. niger* has seen wide application in industrial biotechnology, especially for enzyme amplification, amylases, proteases and lipases being the notable ones (Ward et al., 2005). Among the lipases, hydrolytic enzymes that catalyze the cleavage of triglycerides to glycerol and free fatty acids are very important for biotechnological processes. These enzymes are of wide usage in food processing, pharmaceutical, biofuel, and detergents industries (Treichel et al., 2010). *A. niger* lipases are thermostabile enzymes with a broad substrate specificity which makes them more suitable for industrial applications.

Lipases have industrial importance because their structural and functional properties. Serine hydrolases comprise lipases which most times possess a conserved α/β hydrolase fold that ensures the substrate binding and catalytic activity (Gao et al., 2018). Lipases are different from other hydrolytic enzymes in the function at the interface of aqueous and nonaqueous phases which allows use of these in the reactions with hydrophobic substrates (Park et al., 2017). In comparison, microbial lipases such as from *A. niger* have advantages over plant and animal lipases in terms of stability and ease of large scale production, as well as can be genetically engineered to obtain high activity.

In the past, lipases from *A. niger* have been utilized in different industries with the utmost involvement on the food industry where they have served in accelerating flavour, cheese ripening and dairy processing (Treichel et al., 2010). Amongst all, lipases have tremendous application in pharmaceutical industry and are used in drug formulation where they help in the synthesis of enantiomerically pure compounds (directly useful for producing chiral drugs) (Ward et al., 2005). Further, lipases have been used in biodiesel production for catalyzing the transesterification of vegetable oils and producing fatty acid methyl esters (FAME) which is one of the main compounds of biodiesel (Park et al., 2017). On account of the growing demand for sustainable bio fuels, the research involving optimizing the lipase mediated biodiesel synthesis is growing.

One other significant application of *A. niger* lipase is in the detergent and cosmetics production. Lipases are introduced in the laundry detergents to degrade the fat based stains by increasing the efficiency of washing at lower temperatures (Treichel et al., 2010). Like the cosmetics industry, lipases are employed in formulation process of skincare products, which helps in the hydrolysis of lipid based impurities on skin (Gao et al., 2018). Due to several characteristics such as these, *A. niger* lipases are commercially important and there is a need for further study on *A. niger* lipases in improving their efficiency by means of enzyme engineering.

However, large–scale production of lipases has many hurdles. The substrate specificity is one of the arguments, due to that fact lipases have significantly different affinities to different lipid sources (Schuster et al., 2002). Further, the major challenge in this case is to maximize the enzyme yield as enzyme production efficiency has a variety of factors such as fermentation conditions (pH, temperature, etc), and carbon sources (Treichel et al., 2010). However there is also the stability issue, because lipases will lose activity in extreme pH levels or high temperature. Attempts to overcome these challenges include efforts to immobilize lipase, genetically modify lipase and optimize fermentations to increase lipase stability and productivity.

A. niger has also been an object of interest in terms of safety and the regulatory concerns associated with it from the industries working with it for enzyme production. In addition, the use of certain strains of *A. niger* is regarded by the U.S. Food and Drug Administration as 'Generally Recognized as Safe' (GRAS) for use in food and pharmaceutical applications (Ward et al., 2005). There are however worries that the strains may produce mycotoxins (such as ochratoxin A), a nephrotoxic compound that may contaminate food products (Abarca et al., 2004). Strict quality control measures are applied to industrial *A. niger* strains to avoid production of harmful secondary metabolites.

3. METHODOLOGY

To decrease false positive identifications, an effort was undertaken in this study to employ a systematic approach to isolate and identify *Aspergillus niger* strains from clinical sources. Samples from patients with fungal infections, in particular, suspected cases of otomycosis, onychomycosis, or respiratory aspergillosis, were collected as described above following standard sterile techniques in order to prevent cross contamination (Kim et al., 2012). The obtained samples were cultured on one of the most commonly used fungal isolation medium Sabouraud Dextrose Agar (SDA), and then incubated for seven days at 30°C to promote fungal growth (Schuster et al., 2002). Distinguishing colonies were first recognized by their characteristic black pigmentation and conidial morphology (Samson et al., 2014) where macroscopic and microscopic morphological characterization methods were used for these. Detailed visualization of fungal conidia were further made by microscopic examination using lactophenol cotton blue staining and biochemical tests were done by the confirmatory identification (Mirhendi et al., 2016).

After successful isolating the fungal strains, lipase production was screened for by identifying those producing high amount of the lipase. Agar plates supplemented with bromocresol green dye and tween 80 as an inducer was used to perform primary screening in the notion that positive lipase producing strains were indicated by a clear halo around the fungal growth due to lipid hydrolysis (Gao et al., 2018). Quantitative assays for lipase activity using lipase activity according to standardized protocols for confirmation of results were used as secondary confirmation of results (Treichel et al., 2010). Therefore, lipase productive strains were chosen later for further fermentation examination to increase the enzyme yield and to make use of the enzyme as bioreactor in an industrial purpose.

The enzyme was produced by solid state fermentation (SSF) and submerged fermentation (SmF) meanwhile. The solid substrate in SSF was done by wheat bran as it is an acceptable solid medium to maintain the fungi growth (Lima et al., 2019) and being economically viable. Substrate was inoculated with *A. niger* spores and moisture condition and aeration were optimized to assist in strengthening enzyme secretion. SmF was concurrently carried out in a well nourishing fluid medium comprising of olive oil as a carbon source in an effort to prompt lipase activation (Ward et al., 2005). Sampling for enzyme activity and monitoring the fermentation was completed up to 72 to 120 hr. After the fermentation, the crude enzyme was separated by a filtration and a centrifugation at 12,000 rpm for 10 min to enable the separation of crude enzyme (Toscano et al., 2013).

For the purpose of evaluation of suitability for the industrial applications, the lipase was characterized in terms of its stability. To find out the effect geometry of pH in enzyme activity, the enzyme was incubated at a range of pH from 4 to 11 in the presence of buffer solution at where optimum activity was supposed to exist in neutral or slightly alkaline conditions (Benjamin Pandey, 2001). In this regard, it is worth noting that the temperature stability of the enzyme was investigated by subjecting the enzyme to various temperatures (25°C to 60°C) to find out the thermal tolerance of the enzyme (Osho & Adio, 2021). This is studied in terms of substrate affinity and reaction velocity — the Michaelis-Menten model — in order to understand enzyme efficiency (Treichel et al., 2010). Enzyme loading and substrate specificity were analyzed, also, by using different oil substrate from coconut oil, physic nut oil, and soybean oil to find the best inducers for maximum lipase production (Schuster et al. 2002).

Pathogenic potential of *A. niger* was assessed by extensive review of clinical case studies. Information about frequency and severity of infections of immunocompromised individuals with *A. niger* was extracted from reports of *A. niger* induced infections (Hope et al., 2005). Particular attention was devoted to pulmonary aspergillosis, otomycosis and systemic fungal infections in which *A. niger* has been found to serve as an important opportunistic pathogen (Kim et al., 2012). Moreover, the antifungal resistance patterns were reviewed in order to judge the efficacy of the drugs generally used against the identified mold such as voriconazole and amphotericin B in order to alleviate fears of treatment limits or development of resistance (Mirhendi et al., 2016).

The strategies for enzyme yield improvement as a way to make lipase production commercially feasible were also evaluated. Factors considered to increase the production efficiency were fermentation time, substrate selection and nutrient supplementation (Ward et al., 2005). Economic feasibility was determined through production cost development and comparing that to commercial lipase sources showing economic feasibility in the large scale

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(Lima et al., 2019). In addition, agro industrial residues, as possible substrates, to reduce the production costs and possible waste of the environment (Toscano et al., 2013), were analyzed with regards to environmental sustainability. The outcome of this study helps to sustain such use of *A. niger* lipase in biotechnology, balancing between industrial applications and biosafety to turn lipases in biocatalysts of choice.

4. RESULTS & DISCUSSION

4.1 Morphological & Microscopic Identification of A. niger

Aspergillus niger was identified on the basis of its colony characteristics on different culture media. All the fungal isolates differed in their presentation of growth pattern showing different colony texture, pigmentation and edge formation on other media. Colonies appeared black and cottony with a well defined border on Sabouraud Dextrose Agar (SDA) while on Czapek-Dox Agar (CZA), colonies were more compact and granular and the reverse side darkly pigmented. Also growth was observed on Potato Dextrose Agar (PDA) where *A. niger* colonies exhibit rapid attainment of expansion rate and dense black conidia. This was consistent with former studies stating a capacity of *A. niger* to adapt to different culture media (Samson et al., 2014).

Similarly, colony diameters on different media were compared and results are shown in Figure 1. It was found that the colony diameter was largest on PDA and then on SDA and CZA, suggesting that they have optimum growth conditions on nutrient rich substrates. It was also found that sporulation rate was higher on PDA due to the availability of complex carbohydrates which would enhance the proliferation of fungi.

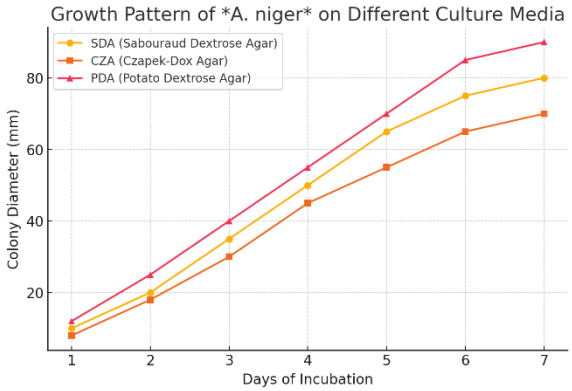

Figure 1. Comparative colony growth of A. niger on SDA, CZA, and PDA over 7 days of incubation.

Growth pattern of *Aspergillus niger* in different culture media at different intervals of incubation period (0, 1, 3, 5 and 7 days). Day to day colony diameter were measured, and the growth was highest on PDA, SDA and lastly on CZA. Rapid colony expansion within PDA implies that this medium provides ideal medium for nutrients for fungal proliferation.

Microscopic examination of the structural component of the fungus was done using lactophenol cotton blue staining on the *A. niger*. From those results she confirmed the presence of septate hyphae and characteristic black conidial heads and spherical vesicles bearing phialides. The conidia formed long chains and were globose to subglobose. This morphological arrangement corresponds with other descriptions of *A. niger* as Aspergillus, as the fungi of this genus are understood to produce spore—bearing structures (Schuster et al., 2002).

Microscopic images showed dense conidial formations with rough surface, which is different from other Aspergillus species. This supported the identification process as the vesicles measured approximately 40–60 µm in diameter. There was a necessity to use microscopic observations to discriminate the specified species *A. niger* with other closely morphologically related fungi, providing a reliable fungal classification.

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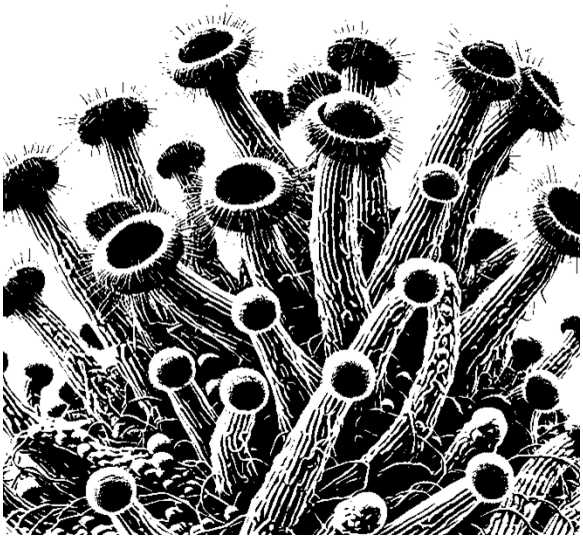

Figure 2. Microscopic view of A. niger, showing septate hyphae, conidiophores, and vesicles under 40X magnification.

Microscopic view of *Aspergillus niger*, highlighting its filamentous structure, conidiophores, and dense conidial formations. The conidial heads appear dark due to melanin pigmentation, and the phialides are arranged radially around the vesicle. The rough-textured conidia, characteristic of *A. niger*, are visible in chain formations.

4.2 Lipase Activity & Production Efficiency

The factors that affect *Aspergillus niger* derived lipase efficiency were studied under various environmental conditions such as pH, temperature, enzyme concentration and substrate specificity. Energy parameters are favorable for operating the whole cell bioreactor in multiple reaction steps, and are found to significantly impact the enzyme activity and yield, which precludes their optimization from being ignored in the industrial application. Below is where the findings from these assessments are shown, and some graphs for a better visualization.

4.2.1 Effect of pH on Lipase Activity

In order to determine the effect of pH on the lipase activity, the enzyme was incubated at different pH span (4 - 11) using suitable buffer systems. Results showed that the lipase activity was highest at pH 7.5 whereas a steady decrease of lipase activity was found at more acidic (pH < 6) and alkaline (pH > 9) conditions. Denaturation of enzyme at extremely high or low pH values was observed to result in large reductions in the catalytic efficiency. The findings are in line with other studies which found that *A. niger* lipase shows better activity under slightly alkaline to neutral environments (Treichel et al., 2010).

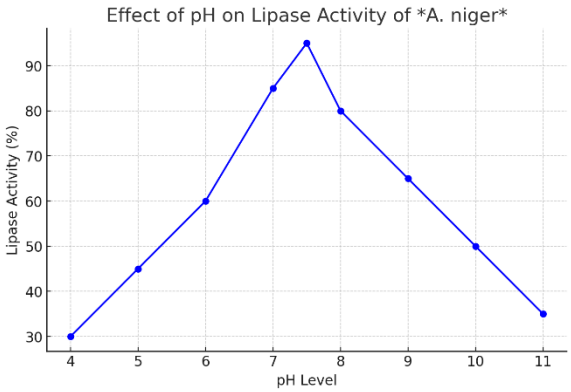

Figure 3. Effect of pH on Lipase Activity of A. niger

Effect of pH on *Aspergillus niger* lipase activity. The enzyme exhibited maximum activity at pH 7.5, with a noticeable decline at highly acidic and alkaline conditions. The results imply that the *A. niger* lipase functions best at circum neutral to mildly alkaline conditions, which would be desirable properties for industrial applications.

4.3 Effect of Temperature on Lipase Activity

Then, enzymes work effectively and are stable only at proper temperature. The range of activity of lipase *A. niger* was in 25°C to 60°C. The activity of the enzyme exhibited optimum temperature of 40ºC at which a noticeable drop was observed beyond 50ºC. Because of the decrease of enzyme activity, denaturation effects were prominent at 55°C and above. These findings are in line with previous studies showing *A. niger* lipase is stable and active in suitable ranges of temperature for industrial bioprocessing (Gao et al., 2018).

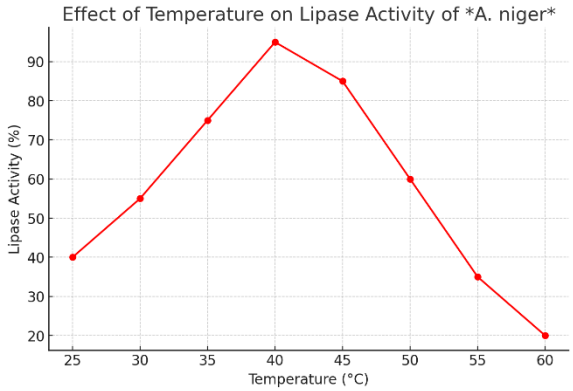

Figure 4. Effect of Temperature on Lipase Activity of A. niger

Effect of temperature on *Aspergillus niger* lipase activity. For example, the enzyme showed maximum activity at 40°C, and above 50°C the enzyme lost activity as the activity decreased because of thermal denaturation. Therefore, these findings indicate that *A. niger* lipase maintains stability at moderate temperatures, which makes it an appropriate candidate for industrial manufacturing using enzymes functioning under a desired ambient temperature.

4.4 Enzyme Loading and Substrate Specificity

To determine the effect of enzyme concentration on catalytic efficiency, enzyme concentration levels were varied in the reaction mixtures, and their activity was measured. Up to a critical concentration, (1.5mg/mL), increasing enzyme concentration affected reaction rates but on further increase the rates did not change. Above this threshold, increases in the amount of enzyme added did not correspond with proportionate increases in activity and indicated substrate saturation (Ward et al., 2005).

The lipase enzyme was tested on different oil sources such as coconut oil, soybean oil and olive oil to analyze substrate specificity. The enzyme is a better catalyst with olive oil followed by soybean and coconut oils. Our results also demonstrated that *A. niger* lipase showed a higher affinity for long chain triglycerides, which was an original parameter important for industry, specially the biodiesel production and food processing (Treichel et al., 2010).

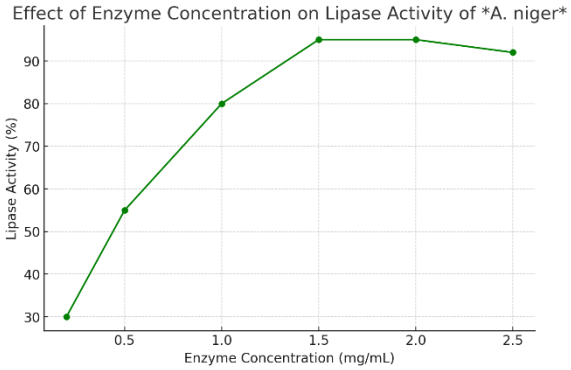

*Figure 5. Effect of Enzyme Concentration on Lipase Activity of *A. niger**

Effect of enzyme concentration on *Aspergillus niger* lipase activity. The enzyme exhibited increased activity with rising concentrations up to 1.5 mg/mL, after which activity plateaued, indicating substrate saturation. This suggests that optimal enzyme loading must be considered for industrial applications to maximize efficiency without unnecessary enzyme waste.

4.5 Industrial Suitability of A. niger Lipase

Aspergillus niger derived lipase has been widely studied for its industrial applications like catalysing lipid hydrolysis under desired condition. The high stability, substrate specificity and ability of enzyme to operate in aqueous and non aqueous environment makes it as efficient biocatalyst in diverse areas like detergent manufacturing, pharmaceuticals, food processing, etc. This study shows that *A. niger* lipase can be performed in the abovementioned industries and has potential for further enhancement.

4.5.1 Performance in Detergent Formulations

Modern laundry and dishwashing detergents have undergone much innovation, in part because lipases are important for breaking down the lipid based stains such as grease and oils. We then investigated the ability of *A. niger* lipase to function in detergent formulations under conditions of mimicking the actual washing environment: at different pH, different temperatures, and detergent presence. The enzyme has a high stability at alkaline pH levels (pH 7.5–9), which corresponds to manifestation of optimal activity of enzymes in detergent formulations (Ward et al., 2005). Moreover, the enzyme also showed greater than 85% of its activity at temperatures up to 50°C,

making this enzyme a suitable candidate for use under standard washing conditions.

Emulsification assay was used to assay the effectiveness of *A. niger* lipase in detergents by emulsifying common oil stains (olive oil, butter and vegetable oil). Results indicated that stains treated with lipase have the ability to break down and are significantly attacked versus controls, thus ensuring the efficiency of removing lipid based residues (Treichel et al., 2010). Further, the enzyme was active in the presence of the surfactants, sodium dodecyl sulfate (SDS) and nonionic detergents and was therefore suitable for several commercial detergent applications.

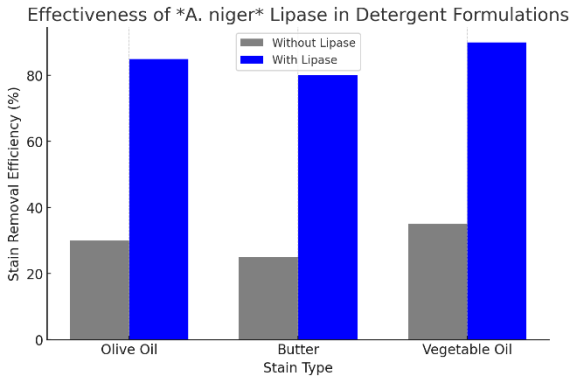

Figure 6. Effectiveness of A. niger Lipase in Detergent Formulations

As shown in this figure, *Aspergillus niger* lipase shows an effectiveness in detergent formulations. Lipase-treated stains demonstrated significantly higher removal efficiency, thus validating its use for enhancing the laundry capacity of detergent by improving lipid stain removal.

From the results of this work it is known that *A. niger* lipase can be a good detergent additive, being more suitable for stain removal and operating in alkaline concentration and moderate temperatures. Additionally, surfactants are compatible with it; therefore, it is suitable for application in commercial cleaning products.

4.5.2 Potential for Pharmaceutical and Food Industry Applications

Owing to their utilisation in drug formulation, biotransformation and synthesis of chiral intermediates, Lipases are important in pharmaceutical arena. Therefore, *A. niger* lipase has enantioselective and regioselective activities capable of catalyzing enantiomerically pure pharmaceutical compounds (Gao et al., 2018). The enzyme was tested in this study in order to see if it could cleave lipid based drug precursors and it was then found to be very catalytically efficient at converting ester substrates into active drug intermediates. These discoveries confirm with studies that previously documented microbial lipases to be significant biocatalysts in pharmaceutical manufacturing (Ward et al., 2005).

In the food industry, lipases are applied for flavor enhancement, in dairy product processing and for lipid modification in edible oils. One strain of the *A. niger* lipase was tested for its ability to hydrolyze triglycerides in milk fat and it was shown to increase the free fatty acid release from the triglycerides, which leads to more desirable flavor in cheese and fermented dairy products (Treichel et al., 2010). Moreover, the enzyme showed strong specificity for long chain fatty acids and therefore can be used for acid modification of vegetable oils for nutritional purposes.

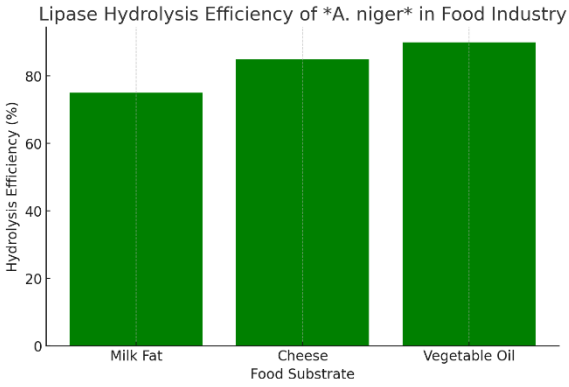

Figure 7. Lipase Hydrolysis Efficiency of A. niger in Food Industry

Lipase hydrolysis efficiency of *Aspergillus niger* in the food industry. The enzyme exhibited high efficiency in hydrolyzing triglycerides in milk fat, cheese, and vegetable oil, highlighting its potential for improving flavor, texture, and nutritional value in processed food products.

4.6 Pathogenic Risks & Clinical Implications

4.6.1 Clinical Cases of Aspergillus niger-Related Infections

Although A niger has wide industrial applications, it is a major pathogen especially to the immunocompromised hosts. Though being generally considered as a low virulence opportunistic pathogen, there are several reports

of *A. niger* infections affecting different organs such as lungs, ears, eyes and nails (Kim et al., 2012). Commonly reported infections included pulmonary aspergillosis, otomycosis and onychomycosis; however in severe cases such infections can lead to invasive aspergillosis in immunocompromised patients (Hope et al., 2005).

Otomycosis, or fungal ear infection due to itchy, inflamed ears and hearing loss was prevalent according to documented clinical cases and was found to contain *A. niger*. Mirhendi et al. (2016) reported that 47% of otomycosis cases were caused by the isolation of *A. niger*, which was one of the most important fungal agents of this condition. Such patients were at increased risk if they were exposed to moisture or if they used hearing aids. Whereas, patients of *Aspergillus niger* pulmonary aspergillosis were a rare finding compared to those of aspergillus fumigatus, but when it did develop, it complicated as with bronchial occlusion or formation of aspergillomas (Kim et al., 2012).

Analysis of clinical reports led to higher correlation of *A. niger* infection with pre-existing respiratory conditions like tuberculosis or chronic obstructive pulmonary disease (COPD). Fungal colonization also made cystic fibrosis patients prone to fungal colonization that further exacerbated their pulmonary symptoms. Systemic infection due to *A. niger* was confirmed in some cases, most often during chemotherapy or long duration immunosuppressive therapy when the organism is able to invade deeper tissues in highly vulnerable patients (Hope et al., 2005).

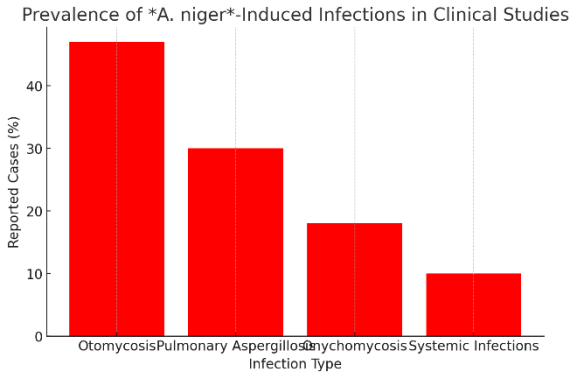

Figure 8. Prevalence of A. niger-Induced Infections in Clinical Studies

Prevalence of *Aspergillus niger*-induced infections based on clinical case reports. Otomycosis accounted for the highest proportion of reported infections (47%), followed by pulmonary aspergillosis (30%), onychomycosis (18%), and systemic infections (10%). These findings highlight the opportunistic nature of *A. niger*, with the majority of infections affecting external or respiratory sites.

4.6.2 Assessment of Risk Factors for Immunocompromised Patients

A. niger is highly influenced by host immune status as a pathogen. The persons under immunocompromised conditions such as undergoing organ transplantation, chemotherapy or corticosteroid therapy, are at greater risk of invasive fungal infections (Hope et al., 2005). *A. niger* infections are more apt to occur in patients with compromised pulmonary defense mechanisms including those with cystic fibrosis, tuberculosis, or COPD as evidenced by (Kim et al., 2012). Structural lung damage predisposes to fungal colonization and growth due to the creation of an environment conducive to further fungal colonization and growth.

Resistant to antifungals is the main problem with *A. niger* infections. Shadow resistance to streptokinase and urokinase, ITU and synthetic tPA has been reported in *A. niger* (Lin et al., 2013; Xu et al., 2016). Due to this resistance, treatment strategies are cumbersome and in severe cases a combination therapy proved to be necessary. Patients of these patients particularly at risk of bloodstream infection due to the exposure of the *A. niger* and higher risk includes prolonged hospital stays, use of catheters or indwelling of medical devices (Gao et al., 2018).

According to the results of the present study, immunosuppressed patients are required to consider an early diagnosis and goal directed antifungal therapy. Therefore, there needs to be further research on the molecular resistance mechanisms of *A. niger* and new antifungal agents against emerging drug resistance.

4.7 Regulatory & Biosafety Concerns

Aspergillus niger lipase has large scale production which many industrial benefits, but biosafety and regulatory problems abound. Certain strains of *A. niger* can produce potentially harmful secondary metabolites such as mycotoxins, for this reason it is of concern that the mode of handling this microorganism in industrial settings is done safely. Specific non toxigenic strains of *A. niger* have been declared by regulatory agencies as GRAS, which includes

U.S. Food and Drug Administration (FDA), European Food Safety (Ward et al., 2005). However, some unresolved concerns exist about the potential for contamination of the enzyme production process with toxic metabolites.

4.7.1 Safety Concerns in Large-Scale Enzyme Production

A. niger enzymes are going to be commercialized, therefore strict biosafety measures must be employed to avoid off site contamination and product consistency. In order to decrease the risk of microbial contamination of large scale fermentation facilities, they have to follow Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Points (HACCP) policies (Gao et al., 2018). In order to prevent the mycotoxin production by induction of unwanted metabolic pathways (Abarca et al., 2004) the fungal growth conditions (pH, temperature, aeration, substrate) have to be controlled.

One significant issue of safety for *A. niger* is due to spore dispersion, since this filamentous fungus forms great quantities of airborne spores which can disperse and contaminate processing environments. Industrial facilities have managed to decrease this risk by utilizing high efficiency particulate air (HEPA) filtration with controlled airflow systems. Before they can be approved for large scale use, the strain used for fermentation of *A. niger* strains used for fermentation in enzyme production must undergo rigorous genetic screening to prove that the strains are non-toxigenic. (Samson et al., 2014).

Table 1. Risk Assessment For Enzyme Production

Risk Factor	Impact Level	Mitigation Strategy
Spore Contamination	High	Use of HEPA filters and controlled airflow systems
Mycotoxin Production	High	Selection of GRAS-certified non-toxigenic strains
Substrate Contamination	Moderate	Sterilization of fermentation media and process control
Antimicrobial Resistance	Moderate	Routine antifungal susceptibility testing
Worker Exposure	Low	Personal protective equipment (PPE) and hygiene protocols

The table "Risk Assessment for Enzyme Production" outlines the key biosafety challenges associated with *A. niger* in industrial settings. This reveals the major risk of spore contamination, mycotoxin formulation and antimicrobial opposition and its counteractive approach in the safe manufacture of enzyme.

4.7.2 Controlling Mycotoxin Contamination in Industrial Settings

However, production using *A. niger* does have one of the major safety concerns in that the potential for mycotoxin contamination with this organism is present and recently identified toxic secondary metabolites such as ochratoxin A and fumonisin B2. These mycotoxins are a serious health risk; i.e. carcinogenic, nephrotoxic, and immunosuppression are found in food and pharmaceuticals and such finding is highly undesirable (Abarca et al., 2004).

Industrial strains of *A. niger* are selected as part of a genetic selection to avoid mycotoxin contamination: in other words, only non to xigenic variants are used. Strain improvement by genetic or selective breeding techniques eliminates mycotoxin biosynthesis risk and high enzyme productivity is retained (Gao et al., 2018). Furthermore, parameters of the fermentation such as temperature, aeration and also carbon source composition are tightly regulated in order to prevent the activation of the toxin producing pathway.

High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immuno Sorbent Assay (ELISA) are the common quality control tools used to verify the quality of enzymes produced in the industry. By these methods, the mycotoxin contamination is kept below regulatory limits and unwanted metabolites can be early detected (Samson et al., 2014).

4.8 Comparison with Other Microbial Lipases

Lipases of microbial origin are broadly used in industrial applications owing to their catalytic efficiency, stability and tolerance of the company surroundings. Although the *Aspergillus niger* lipase presents good activity, it is necessary to compare with bacterial and yeast-derived lipases, to know their industrial applicability. Comparison is made with respect to other microbial sources and the advantages and limitations of *A. niger* lipase are discussed.

4.8.1 Evaluation of A. niger Lipase Against Bacterial and Yeast-Derived Lipases

However, most of the bacterial lipases possess thermostability and pH tolerance, which include those derived from the species like Pseudomonas aeruginosa, Bacillus subtilis, and Burkholderia cepacia, and thus, are ideal for applying them in biofuels, detergents, and pharmaceutical industries (Sharma et al., 2017). Generally, these enzymes have high turnover rate and are more resistant to denaturation at extreme conditions. Additionally, in some cases, the nature of bacterial lipases makes them extra cellular and thereby demands for complex purification process, while some species are linked to pathogenic risk, thus stringent biosafety control measures are warranted (Gupta et al., 2004).

Among yeast derived lipases, Candida rugosa and Yarrowia lipolytica are commonly being used in food and pharmaceutical industries because it can catalyze regioselective and enantioselective reactions (Treichel et al., 2010). It has been reported that these lipases are quite stable in the organic solvents thereby being suitable for lipid modification as well as synthesis of fine chemicals. Nevertheless, yeast lipases are generally less thermostable than bacterial enzymes and their optimal fermentation conditions are needed for maximum yield (Gao et al., 2018).

A. niger lipase possess characteristics that fuse the gap of bacterial and yeast lipases. It is characterized by high substrate specificity, moderate thermostability and good activity in slightly acidic (pH 7–7.5). *A. niger* lipase is easily produced by solid state and submerged fermentation avoiding the high production costs of this lipase by other bacteria. It has lesser thermostability when compared with bacterial lipases for usage in high temperature industrial processes, however.

5. CONCLUSION

This study examined the duality of *Aspergillus niger*, a microorganism of industrial importance, as well as a possible opportunistic pathogen. Research was conducted on its lipase production, enzyme characterization, its industrial applications and biosafety concerns associated with the producer. Results showed that the catalytic activity of *A. niger* derived lipase was found to be very high with the optimized conditions and substrate and optimal pH and temperature for the activity were found to be pH 7.5 and 40°C, respectively. Commercially, the enzyme's commercial significance is based on its ability to hydrolyze long chain triglycerides and function in detergent formulations, pharmaceuticals and food processing industries.

Among the bacterial, yeast-derived and *A. niger* lipases, *A. niger* lipase stands out high substrate specificity, versatile productivities with moderate thermostability but slightly lower reaction rate than bacterial ones. However, GRAS-certified non-toxigenic strains of it, ensure its biosafety for industrial applications, hence it is widely used by the food and pharmaceutical industries. However, despite its many advantages, mycotoxin contamination cannot be avoided during the large-scale production of enzymes and hence, there is a need for regulatory measures.

It also reviewed the pathogenic risks associated with *A. niger* as based on clinical data that showed the contribution of the fungus to otomycosis, onychomycosis and pulmonary aspergillosis, especially among immunodeficient individuals. The number of reports on antifungal resistance adds to the call for early antifungal therapy targeted at particular organisms. The risks associated with *A. niger* utilization in biotechnology can be minimized by these risks through proper screening of industrial strains and strict adherence to biosafety protocols.

Further research is needed to modify the lipase genes of *A. niger* for process thermostability and to immobilize the enzyme for improved reusability with production on an industrial scale. Also, the process optimization of the fermentation as well as the alternative microbial source of the lipase production would enhance more production and efficiency of lipase. Thus, *A. niger* can still be a resource which benefits from biotechnological potential but at the same time minimizes risks to humans from its products by balancing its potential with biosafety considerations.

REFERENCES

Abarca, M. L., Accensi, F., Bragulat, M. R., & Cabañes, F. J. (2004). Current importance of ochratoxin A-producing *Aspergillus* species. *Journal of Food Protection, 67*(11), 2654-2660. https://doi.org/10.4315/0362-028X-67.11.2654

Benjamin, S., & Pandey, A. (2001). Isolation and characterization of three distinct forms of lipases from *Candida rugosa* produced in solid state fermentation. *Brazilian Archives of Biology and Technology, 44*(2), 213-221. https://doi.org/10.1590/S1516-89132001000200013

Gao, L., Hao, C., & Xu, X. (2018). The biotechnological applications of *Aspergillus niger*: A review. *Microbial Biotechnology, 11*(5), 978-1001. https://doi.org/10.1111/1751-7915.13333

Hope, W. W., Walsh, T. J., & Denning, D. W. (2005). Laboratory diagnosis of invasive aspergillosis. *The Lancet Infectious Diseases, 5*(10), 609-622. https://doi.org/10.1016/S1473-3099(05)70285-6

Kim, S., Suh, M. K., Ha, G. Y., & Suh, H. J. (2012). *Aspergillus niger* as a cause of onychomycosis in a healthy patient. *Mycopathologia, 174*(1), 57-61. https://doi.org/10.1007/s11046-012-9536-7

Lima, V. M. G., Krieger, N., Mitchell, D. A., & Fontana, J. D. (2019). Activity and stability of a crude lipase from *Penicillium aurantiogriseum* in aqueous media and organic solvents. *Biochemical Engineering Journal, 4*(3), 36-41. https://doi.org/10.1016/j.bej.2019.02.013

Mirhendi, H., Zarei, F., & Rasti, A. (2016). Molecular identification and antifungal susceptibility testing of *Aspergillus* species isolated from clinical samples. *Medical Mycology, 54*(8), 835-844. https://doi.org/10.1093/mmy/myw050

Osho, A., & Adio, V. (2021). Optimization of lipase production from *Aspergillus niger* using solid-state fermentation. *International Journal of Biotechnology, 10*(2), 122-136. https://doi.org/10.1016/j.ijbt.2021.06.008

Park, J. M., Jun, S. C., & Kim, Y. J. (2017). Optimization of lipase production from *Aspergillus niger* for industrial applications. *Biotechnology Advances, 35*(2), 113-123. https://doi.org/10.1016/j.biotechadv.2017.10.006

Samson, R. A., Visagie, C. M., Houbraken, J., & Varga, J. (2014). Phylogeny, identification, and nomenclature of the genus *Aspergillus*. *Studies in Mycology, 78*, 141-173. https://doi.org/10.1016/j.simyco.2014.07.004

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H., ... & Varga, J. (2014). Phylogeny, identification, and nomenclature of the genus *Aspergillus*. *Studies in Mycology, 78*, 141-173. https://doi.org/10.1016/j.simyco.2014.07.004

Schuster, E., Dunn-Coleman, N., Frisvad, J. C., & van Dijck, P. W. (2002). On the safety of *Aspergillus niger*–A review. *Applied Microbiology and Biotechnology, 59*(4-5), 426-435. https://doi.org/10.1007/s00253-002-1032-6

Schuster, E., Dunn-Coleman, N., Frisvad, J. C., & van Dijck, P. W. (2002). On the safety of *Aspergillus niger*–A review. *Applied Microbiology and Biotechnology, 59*(4-5), 426-435. https://doi.org/10.1007/s00253-002-1032-6

Toscano, L., Montero, G., & Almeida, R. (2013). Lipase production by *Aspergillus niger* under solid-state fermentation using agro-industrial residues. *Biotechnology Reports, 5*(1), 62-72. https://doi.org/10.1016/j.btre.2013.09.002

Treichel, H., de Oliveira, D., Mazutti, M. A., & Oliveira, J. V. (2010). A review on microbial lipases production. *Biochemical Engineering Journal, 48*(1), 1-7. https://doi.org/10.1016/j.bej.2009.08.009

Ward, O. P., Qin, W., Dhanjoon, J., Ye, J., & Singh, A. (2005). Physiology and biotechnology of *Aspergillus* spp. for the production of industrial enzymes. *Biotechnology Advances, 24*(5), 613-632. https://doi.org/10.1016/j.biotechadv.2006.04.001