SCREENING, PRODUCTION AND CHARACTERIZATION OF LIPASE FROM *Trichoderma reesei*

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DECLARATION

I, ALABI ADURAGBEMI OLUWAFUNKE, hereby declare that this project report written under the supervision of Dr. Adebami G. E. is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and a list of references provided.

Alabi Aduragbemi _____

17010101010 Signature / Date

CERTIFICATION

This is to certify that this research project titled "**SCREENING, PRODUCTION AND CHARACTERIZATION OF LIPASE FROM *Trichoderma reesei*"** was carried out by **ALABI, ADURAGBEMI OLUWAFUNKE** with matriculation number **17010101010** as a requirement for the award of Bachelor of Science (B.Sc.) degree in Microbiology, Department of Biological sciences, Mountain Top University, Ogun state, Nigeria and is approved for its contribution to knowledge and literary presentation.

Dr. Adebami G.E. _____

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(Head of Department) Signature / Date

DEDICATION

I dedicate this work to God Almighty; whose love is everlasting and his mercy endures forever.

ACKNOWLEDGEMENT

My sincere and utmost appreciation goes to God, my Lord and Redeemer, who in His infinite mercies has given me the wisdom, knowledge, assistance, support and protection to successfully complete this project.

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ABSTRACT

Lipases (triacylglycerol acyl hydrolases, E.C. 3.1.1.3), are a family of enzymes that catalyze the conversion of triacylglycerol to glycerol and fatty acids. They are ubiquitous and are produced by a variety of plants, animals, and microbes. Lipase plays an important role as commercial biocatalysts. In this study, fungal isolates from domestic wastewater polluted soil were screened on solid agar for lipase production. The best isolate was identified using morphological and biochemical characterizations. Effects of physico-chemical parameters such as carbon and nitrogen sources, temperature, and pH on lipase production were investigated. A total of 12 fungal strains were isolated and screened for lipase production. Isolate APD1 produced the highest lipase activity and was identified as *Trichoderma reesei*. Carbon sources, nitrogen sources, incubation temperature and initial pH had significant effects ($p<0.05$) on lipase production. Glucose (75.95^{a} U/mL), peptone (76.16^{a} U/mL), 30^{o}C (59.41^{a} U/mL) and pH 6.0 (70.64^{a} U/mL) supported the optimum lipase production by the selected isolate. *Trichoderma reesei* APD1 isolated from wastewater polluted soil sample was a good lipase producer which can be harnessed for industrial production.

Key words: fungi, *Trichoderma reesei,* lipase, carbon sources, nitrogen sources

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Lipase (EC 3.1.1.3) are enzymes that speed up the breakdown of triacylglycerol into glycerol and free fatty acids (Sharma and Kanwar, 2014). They are biocatalysts used in industries that catalyze a range of new processes in both aqueous and non-aqueous environments (Kumar *et al.,* 2016). Due to its large potential in manufacturing applications such as food additives, fine chemicals, waste water treatment, cosmetics, pharmaceutical, leather, and medicine, there is an increasing demand for lipases (Kumar and Ray, 2014). Interest in microbial lipase production has also increased in the last decade (Mehta *et al.,* 2017). They're also recognized for their outstanding capacity to execute a wide range of chemo-, regio-, and enantioselective transformations (Kumar *et al.,* 2016). Due to the flexibility of lipase for synthesis and hydrolysis, in addition to their enantio-selective, chemo-selective, or regio-selective catalysis, their impact on enzyme biotechnology has grown (Helal *et al.,* 2017).

Lipase are abundant in nature, and may be found in a variety of plants, animals, and microbes (Thakur, 2012). Microbial lipases have piqued industrial interest due to their ability to remain active in extremes of temperature, pH, and organic solvents, as well as their chemo-, regional, and enantioselectivity (Chandra *et al.,* 2020). Lipases may catalyze a number of chemical processes, including esterification, trans-esterification, acidolysis, and aminolysis, in addition to triglyceride hydrolysis (Joseph *et al.,* 2008).

Fungal lipases have been widely employed due to the specificity of their substrates and their resistance to a variety of chemical and physical conditions (Mehta *et al.,* 2017). Oil-contaminated soils, vegetable oil waste, dairy industries, seeds, and deteriorated food sectors are all places where fungi capable of generating lipases can be found (Ko *et al.,* 2005). Fungal enzymes are naturally extracellular and easy to extract, which reduces costs and makes them a better source than bacteria (Mehta *et al.,* 2017). Fungal species that produce lipases are *Candida antarctica, Candida rugosa, T. lanuginosus, Rhizomucor miehei, Geotrichum sp., Mucor sp.* and *Trichoderma sp.* (Chandra *et al.,* 2020).

1.2 Statement of Problem

Due to its large potential in manufacturing applications such as food additives, fine chemicals, waste water treatment, cosmetics, pharmaceutical, leather, and medicine, as well as the quest for sustainable production to meet the increasing demand for lipase, interest in microbial lipase production has increased in the last decade but not without challenges. The cost of lipase production has been the major stumbling, most especially from the plant and animal sources. There is a need for an alternative source through microbial origin.

1.3 Justification

The increasing rate of industrialization and demand for lipases necessitate alternative and cheaper sources of production. Lipase can catalyze esterification, interesterification, and transesterification processes in non-aqueous environments. They are the enzymes of choice for prospective uses in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper sectors due to their flexibility. Fungal lipases are extracellular in nature and may be readily removed, which reduces expenses and makes this source preferable to bacteria. Most importantly, strain improvement can be carried out on microorganisms to improve their productions.

1.4 Aim and Objectives of the Study

The aim of this study is to produce lipase from fungi isolate. The objectives of the study include:

i. To isolate fungi from wastewater contaminated soil samples.
ii. To screen the isolates for lipase production, and select the best lipase producer.
iii. To identify the selected isolates using morphological and biochemical characterizations.
iv. To investigate the effects of physio-chemical parameters on the selected isolate

CHAPTER TWO

LITERATURE REVIEW

2.1 Fungi

Fungi are a varied and widely distributed collection of organisms that belong to the kingdom Fungi (Carris *et al.,* 2012), which Whittaker originally classified as the fifth kingdom (Whittaker, 1959). Mycologists have defined fungi as eukaryotic, nucleated, spore-bearing, achlorophyllous organisms that generally reproduce sexually and asexually and whose usually filamentous, branched somatic structures are typically surrounded by cell walls containing cellulose or chitin or both, despite difficulties in defining the group's boundaries (Alexopoulos *et al.,* 1962; Leera *et al.,* 2019).

However, they lack photosynthetic pigments and are chemo-organotrophs (Carris *et al.,* 2012). The majority of them grow aerobically, getting their energy from the oxidation of organic molecules (Buckley, 2008). In ecological systems, fungi are the primary decomposers (Kathleen *et al.,* 2015). The study of fungus is known as mycology (Alexopoulos *et al.,* 1962).

They are ubiquitous (Alsohaili and Bani-Hasan, 2018). Fungi are found in almost every ecosystem on the planet, including marine environments ranging from high alpine lakes to the deep ocean (Grossart *et al.,* 2019). Fungi are one of the most complex groups of organisms on the planet, and they play a vital role in soil carbon cycling, plant development, and disease (Tedersoo *et al.,* 2014). Fungi play a vital part in the breakdown of organic materials as well as the cycling and exchange of nutrients. At least 100,000 fungus species have been identified (Brandt and Warnock, 2015). However, the number of unknown species has been estimated to be between one million and ten million, with between 1,000 and 1,500 new species being reported each year (Buckley, 2008; Guarro *et al.,* 1999). Only around 500 fungus species have been discovered as being related with human or animal illnesses, and only about 50 are capable of infecting otherwise healthy people (Brandt and Warnock, 2015).

Fungi are also important in industry (Azizan *et al.,* 2016). In the wine and brewing industries, yeasts, a kind of fungus, are employed in baking and the fermentation of carbohydrates into carbon dioxide and ethanol (Walker and Stewart, 2016). Another fungal colony that provides nourishment is mushrooms (Valverde *et al.,* 2015). Other fungi are crucial in the manufacture of cheese and antibiotics.

Fungi may be dangerous, resulting in significant financial losses (Lucca, 2007). Pathogenic fungi cause many plant diseases (wheat rust, corn smut, potato blight, root rot, and stem rot), some human diseases (ringworm, athlete's foot, and histoplasmosis), and some animal diseases (dog and cat mange) (Almeida *et al.,* 2019). Wood, clothing, and food deterioration and decay are caused by fungus that grow in wet, damp climates.

2.1.1 General Characteristics of Fungi

Fungi are a separate group of eukaryotic organisms that differ from other groups like plants and animals in a number of ways (Brandt and Warnock, 2019). A stiff cell wall surrounds fungal cells, which is made up of chitin, glucan, chitosan, mannan, and glycoproteins in various combinations (Leera *et al.,* 2019). These characteristics contrast with animals, who have no cell walls, and plants, which contain cellulose as the primary component of their cell walls (Brandt and Warnock, 2019). Fungal cells, like other eukaryotes, contain a genuine nucleus with a membrane around it, and cell division is followed by meiosis or mitosis (Bueno and Silva, 2014).

Fungi can be either multicellular or unicellular in nature (Naranjo-Ortiz and Gabaldon, 2019). A chain of multinucleate, tubular, filament-like cells is the fundamental structural unit of multicellular creatures (termed a hypha). Most multicellular fungi's vegetative stage is characterized by a mass of branching hyphae known as a mycelium or thallus (Brandt and Warnock, 2019). Each individual hypha has a stiff cell wall and grows in length as a result of apical expansion with mitotic cell division (Bueno and Silva, 2014). The hyphae of more primitive fungus remain aseptate (without cross walls). The development of more or less frequent cross-walls, known as septa, divides hyphae into sections or cells in more mature groupings. Septate hyphae are those that are divided into two halves. Molds are tiny multicellular fungi that live in the form of microscopic mycelium (Brandt and Warnock, 2019; Bueno and Silva, 2014).

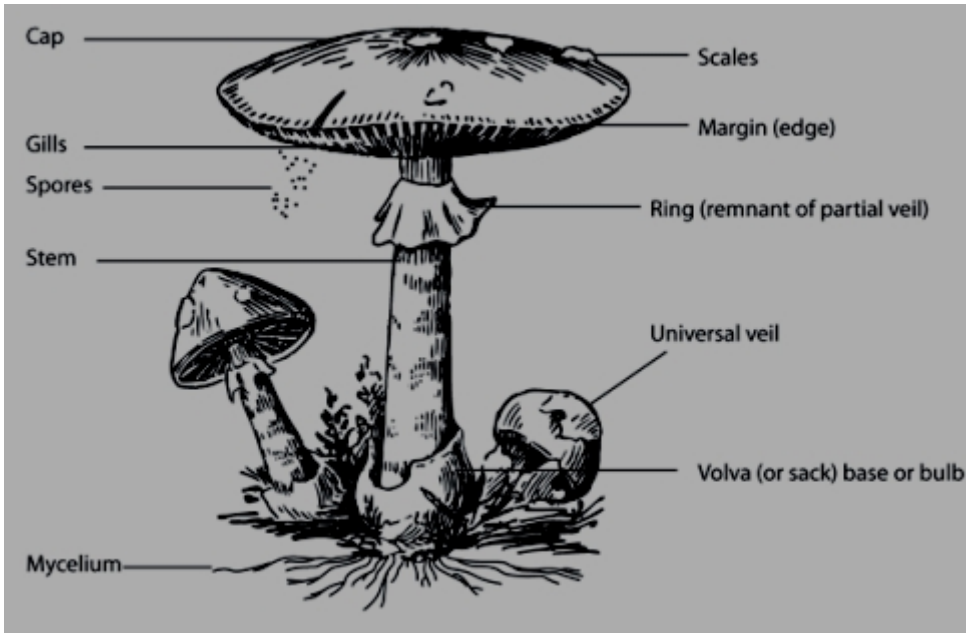

Figure 2.1: Structural diagram of a mushroom (Kamalakannan *et al.,* 2020)

2.1.2 Fungal Cell Structure

A rigid cell wall surrounds all morphological forms of fungi (yeast and hyphae) (Samantha, 2015). Chitinous fibrils make up the majority of the cell wall, which is made up of polysaccharides, proteins (acid phosphatase, protease and -amylase), lipids, and inorganic ions (magnesium, calcium and phosphorus) (Gow *et al.,* 2016; Samantha, 2015). Chitin is a N acetyl-D-glucosamine (1, 4) linked polymer (GlcNAc). Chitin synthetase, which is found in the chitosome (cell organelle), produces it (Samantha, 2015). The wall's flexibility (extensibility) determines the degree of cross-linking, but the pore size (permeability) is a feature of the wall matrix. The inner layer of the wall is made up of the scaffold, whereas the outer layer is mostly made up of the matrix (Nobel *et al.,* 2001).

Table 2.1 The chemical composition of cell walls of selected groups of fungi (dry weight of total cell wall fraction, in per cent) (Webster and Weber, 2007).

Group	Example	Chitin	Cellulose	Glucans	Protein	Lipid
Oomycota	*Phytophthora*	0	25	65	4	2
Chytridiomycota	*Allomyces*	58	0	16	10	-
Zygomycota	*Mucor*	9	0	44	6	8
Ascomycota	*Saccharomyces*	1	0	60	13	8
Basidiomycota	*Fusarium*	39	0	29	7	6
	Schizophylum	5	0	81	2	-
	Coprinus	33	0	50	10	-

2.1.3 Fungal Nutrition and Growth

Fungi are heterotrophic, meaning they don't produce chlorophyll and rely on preformed organic carbon molecules for nourishment (Brandt and Warnock, 2019). Water (69-90 percent), carbohydrates, proteins, and lipids make up the majority of fungal organisms (Table 2.2). Fungi live and get their nourishment from a food source or substrate by producing enzymes into the outer substrate and absorbing the nutrients released via their cell walls (Brandt and Warnock, 2019).

Table 2.2: Proximal compositions of fungi (Bueno and Silva, 2014)

Class of compound	Dry weight (%)
Carbohydrates	16-85
Lipids	0.2-87
Proteins	14-44
RNA	1-10
DNA	0.15-0.3
Ash	1-29

Fungi are chemoorganoheterotrophic organisms (Leera *et al.,* 2019). Organic compounds are utilized as an electron and carbon source, whereas chemical compounds are used as a source of energy. They get their nourishment by absorbing it from the environment (saprophyte) or the host (osmotrophic) (parasite). At 20–30°C, most saprophytic molds grow aerobically in artificial culture media (Kuhn and Ghonnoum, 2003).

At 37°C, pathogenic yeasts and dimorphic fungi's yeast phase prefer to develop (Boyce and Andrianopoulos, 2015). For optimal fungal development, high humidity, an acidic pH (3.8–5.6), a high sugar content (4–5%), carbon, phosphorus, sulphur, and traces of potassium, magnesium, iron, and calcium are necessary (Adinarayana *et al.,* 2003). A nitrogen supply is provided by peptone in the media and keratin in the skin. Amino acids for protein synthesis, purines and pyrimidines for nucleic acids, glucosamine for chitin, and different vitamins all require nitrogen (Samanta, 2015).

The majority of fungi utilize nitrogen as nitrate, which is then converted to nitrite and ammonia. None of them can fix nitrogen directly (Carris *et al.,* 2012). Fungi grow at a slower pace than bacteria, and bacteria can quickly infect the media. To avoid bacterial and saprophytic fungus contamination, antibiotics (e.g. chloramphenicol) and antifungals (e.g. cycloheximide) are added to the medium (Carris *et al.,* 2012). Certain pathogenic fungi and yeasts, such as *Aspergillus fumigatus, Candida albicans,* and *Cryptococcus niger*, are inhibited by cycloheximide (Vandeputte *et al.,* 2012).

2.1.4 Fungal Reproduction

Fungi reproduce in two ways: asexually and sexually or parasexually (Carris *et al.,* 2012). Asexual reproduction, also known as somatic or vegetative reproduction, occurs without the union of nuclei, sex cells, or sex organs. The fusing of two nuclei is the hallmark of sexual reproduction (Lee *et al.,* 2010). The majority of fungus reproduce by spores and have a thallus, which is made up of tiny tubular cells called hyphae (Carris *et al.,* 2012). In general, these spores have a diameter of 40 to 800 m and may contain hundreds or thousands of nuclei (Carris *et al.,* 2012). Spores are a type of survival or dispersion unit that can germinate and create a new hypha with one or more cells. Individual spores or clusters of spores can be generated. Fungal spores, unlike plant seeds, lack an embryo, but they do contain the necessary nutritional stores for germination (Bareke, 2018). Many fungus produce many types of spore as part of their life cycles. Mitospores are created through an asexual process

called mitosis, and meiospores are formed by a sexual process called meiosis (Carris *et al.,* 2012).

2.1.5 Fungi's Shared Features with other Organisms

Fungal cells, like other eukaryotes, have membrane-bound nuclei and coding sections called exons on their chromosomes, which include DNA with non-coding portions called introns (Ivaschenko *et al.,* 2009). Sugar alcohols, disaccharides, and polysaccharides are among the soluble carbohydrates and storage compounds found in them (Deacon, 2005). Fungal cells also include mitochondria and an internal membrane structure that is inherently complex, such as the endoplasmic reticulum and the Golgi apparatus (Mullock and Luzio, 2013).

Fungi, like mammals, are heterotrophs, meaning they utilise complex organic molecules as a carbon source rather than fixing carbon dioxide from the atmosphere, as do certain bacteria and most plants (Ren and Yuan, 2015). Furthermore, fungi are unable to take nitrogen from the air (Brandt and Warnock, 2019). They, like animals, must obtain it from their diet. Unlike other mammals, which take food and then digest it inside in specialized organs, fungus do things backwards: digestion comes before ingestion (Brandt and Warnock, 2019). Exoenzymes are first transported from the hyphae to the environment, where nutrients are stored (Brandt and Warnock, 2019). Polysaccharides are retained in plant cells by glycogen, a branched polymer, rather than amylopectin, a less densely branched carbohydrate, and amylose, a linear polysaccharide.

2.1.6 Fungi Diversity

Fungi may be found and thrive in a broad range of environments, including severe environments like deserts and regions with high levels of salt or ionizing radiation, as well as deep marine deposits (Grossart *et al.,* 2019). Only 7% of the total estimated fungal species on Earth have been found, despite the fact that they conduct some of life's most important core activities and have some of the highest biotechnological capabilities (Tang *et al.,* 2006).

About 120,000 species of fungus have been recognized by taxonomists, but the fungal kingdom's worldwide biodiversity is unknown. According to a 2017 estimate, there may be between 2.2 and 3.8 million species (Hawksworth and Lucking, 2017). Various techniques and criteria in mycology have usually distinguished organisms. Fungal taxonomy has historically been classified based on physical features such as spores or fruit structures.

It's possible to discern between organisms' biochemical and physiological features, such as their capacity to metabolize specific biochemicals and their reaction to chemical analysis.

The biological species definition distinguishes between species based on their ability to reproduce (Tang *et al.,* 2006). The introduction of molecular technologies to investigate variety, like as DNA sequencing and phylogenetic analysis, has greatly increased the precision and robustness of estimates of genetic diversity within various taxonomic groupings (Li *et al.,* 2018).

2.2 Fungal Classification

According to Ainsworth (1966), the kingdom is divided into two divisions namely: Division Myxomycota and Division Eumycota (Gupta *et al.,* 2012; Katoch and Kapoor, 2014):

2.2.1 Division Myxomycota (False fungi)

They're a unique species with no cell walls (Clark and Haskins, 2015). Have a plasmodium, which is a mass of naked, multinucleate protoplasm that feeds by eating particulate matter and moves like an amoeboid, or a pseudoplasmodium, which is an aggregation of distinct amoeboid cells that feeds by ingesting particulate matter and moves like an amoeboid (Helmenstine, 2018). They're also known as 'Slime moulds' because of their slimy nature. Acrasiomycetes, Hydromyxomycetes, Myxomycetes, and Plasmodiophoromycetes are the four classes that make up this fungus.

2.2.2 Division Eumycota (True fungi)

Ascomycotina, Basidiomycotina, Deuteromycotina, Mastigomycotina, and Zygomycotina are the five subclassifications (Helmenstine, 2018).

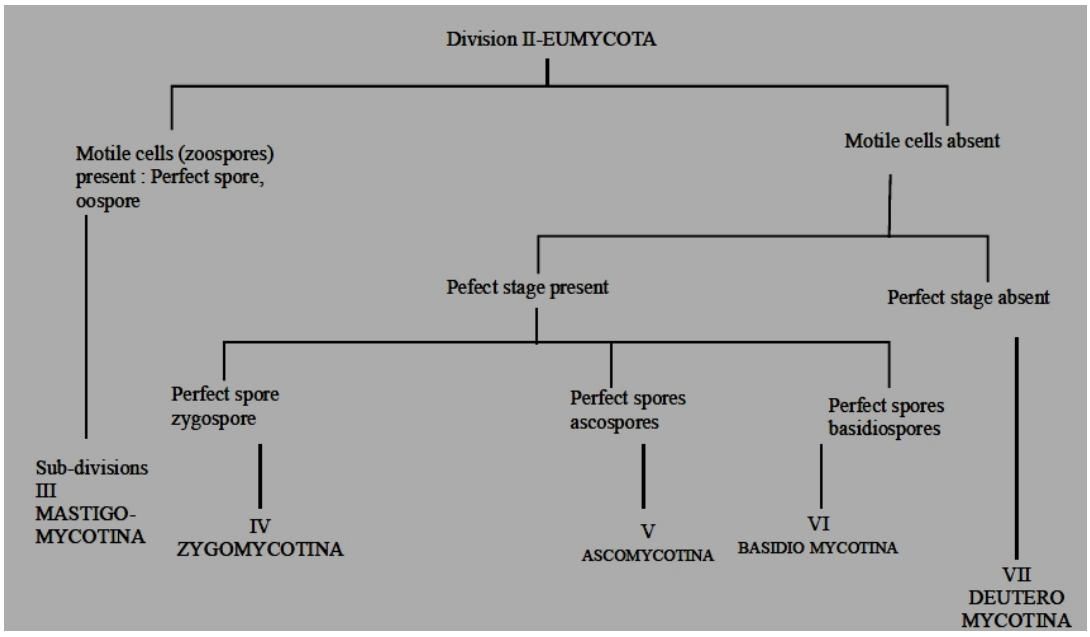

Figure 2.2: Classification of Eumycota (Brandt and Warnock 2019).

2.3 Economic Importance of Fungi

Fungi are important for their ecological functions (Blackwell *et al.,* 2012). Fungi are also used in the production of antibiotics and organic acids, as well as in food processing and flavoring (baker's and brewer's yeasts, *Penicillium* in cheese-making). Fungi are also known to generate secondary metabolites, such as enzymes utilized in in situ lipid metabolism and a variety of industrial uses (Mehta *et al.,* 2017).

2.3.1 Role of Fungi in Agriculture

Fungi breakdown cellulose, lignins, gums, and other organic complex compounds, making them excellent decomposers of organic waste. They are also in charge of the production of plant hormones (Table 2.3). Filamentous soil fungi's major activities include organic matter breakdown and soil aggregation. In addition to this feature, bound *Alternaria, Cladosporium, genus Aspergillus, Dematium, Gliocladium,* and *Humicola* species generate organic compounds in the soil and can thus be utilized to maintain soil organic matter (Yuvaraj and Ramasamy, 2020). To boost agricultural output, plant growth regulators and artificial fertilizers have been employed.

Table 2.3: Agricultural application of fungi (Yuvaraj and Ramasamy, 2020)

Fungi Species	**Product**	**Application**
Fusarium moniliforme	Gibberellins	Plant growth hormone
Fusarium graminearum	Zearalenone	Growth promoter in cattle
Phytophtora palmivora	DeVine	Control of milkweed vine
Colletotrichum sp.	Collego	Control of northern joint vetch
Chondrostereum purpureum	Chontral	Control of hardwoods
Phanerochaete gigantea	Rotstop	Control of butt rot of conifers

The major negative impact of fungus on commercial agriculture is disease. They cause a variety of illnesses in plants and animals. The most common and important fungal crop diseases are damping off disease, which affects seedlings of almost all types of crops, such as tomatoes, beans, spinach, and tobacco, downy mildews of grapes, ergot disease of rye, which causes the formation of poisonous sclerotia in the rye kernel, and brown rot of stone fruits

(Yuvaraj and Ramasamy, 2020). Sugar cane red rot disease impacts fruits such as cherries, peaches, apricots, and plums.

2.3.2 Role of Fungi in Medicine

Fungi are vital in many aspects of human existence, including as medicine, food, and agriculture (Beg *et al.,* 2004). Fungus that employs biotechnology to create or may be encouraged to produce medically important compounds are known as medicinal fungi (Mehta *et al.,* 2017). Antibiotics, anti-cancer medicines, cholesterol inhibitors, psychiatric pharmaceuticals, immunosuppressants, and even fungicides are among the medicinally active chemicals that have been discovered (Kidd *et al.,* 2016).

Several fungal secondary metabolites are commercially important (Jadon *et al.,* 2020), and these organisms are well recognized for naturally producing antibiotics that kill or hinder bacteria's development (Dumancas *et al.,* 2014). Sir Alexander Fleming discovered the significance of fungi in antibiotic synthesis in 1929. (Alexopoulos *et al.,* 1996). Penicillin was derived from *Penicillium notatum*. It was the first commonly used antibiotic. Penicillin is a natural antibiotic that kills bacteria (Dumancas *et al.,* 2014). It destroys bacteria, especially those that are gram positive.

Streptomycin is made from the bacteria *Streptomyces griseus* (Westhoff *et al.,* 2020). It is quite useful in medicine. It kills numerous organisms that are resistant to penicillin, especially gram-negative bacteria. Antibiotics have been isolated from Aspergillus cells as well (Al-Fakih and Almaqtri, 2019). Mushrooms including *Antrodia camphorata, Ganoderma spp., Hericium erinaceus, Lignosus rhinocerotis,* and *Pleurotus giganteus* have a long history of usage in peripheral nervous system enhancement (Phan *et al.,* 2015). Nerve growth factors play a crucial role in the adult brain's survival, maintenance, and regeneration of particular neuronal populations (Petruska and Mendell, 2009).

There are several roles that fungi have played and continue to play in the medical profession (Abdel-Razek *et al.,* 2020). Fungi are utilized in medicine to treat a variety of illnesses, including cancer (Patel and Goyal, 2012), bacterial infections, diabetes, and others. According to studies, a species of Ganoderma (*Ganoderma applanatum*) includes anti-tumor and anti-fibrotic chemicals (Hyde *et al.,* 2019).

Fungi have many advantages, but they can have drawbacks in medicine. Aspergillosis is a disease caused by the fungus *Aspergillus flavus* (Bazaz and Denning 2019). It affects patients who have lung illnesses and a weakened immune system (Bazaz and Denning 2019). Mucormycosis is caused by fungus in the order Mucorales, and it affects those who have a weak immune system (Ibrahim *et al.,* 2012). Skin diseases like ringworm have been linked to several fungus species (White *et al.,* 2014). Neoformans infection is caused by *Cryptococcus neoformans*, a bacterium that infects the brain and causes meningitis (King, 2018). Fungi are also responsible for a variety of other diseases, including athlete's foot, fungal nail infections, and fungal eye infections (White *et al.,* 2014).

2.3.3 Role of Fungi in Petroleum Industries

The oil industry, sometimes referred to as the petroleum industry, encompasses the extraction, processing, refining, transportation (typically via pipelines and oil tankers), and distribution of petroleum products around the world (Demain *et al.,* 2004). Bioremediation is the application of biotechnology to the environment (Leera *et al.,* 2019), and it is described as the employment of biological agents or organisms to biodegrade contaminants in the environment (Elshafie, 2007*). Graphium, Fusarium, Penicillium, Paecilomyces, Acremoniun, Mortierella, Giiocladium, Trichoderma,* and *Sphaeropsidales* are among the major families of soil fungus capable of utilizing petroleum hydrocarbons (Leera *et al.,* 2019). The petroleum industry makes use of a variety of fungal species (Table 2.4).

Table 2.4: Petroleum hydrocarbon degradation by different species of filamentous fungi (Leera *et al.,* 2019).

Fungi	**Compound**
Trichoderma reesei	Naphthalene
Aspergillus spp.	Crude oil
Cunninghamella elegans	Phenanthrene
Aspergillus niger	n-hexadecane
Cunninghamella elegans	Pyrene
Aspergillus ochraceus	Benzo[a] pyrene
Penicillium spp.	Crude oil

2.3.4 Role of Fungi in Industry

Fungi have a wide range of industrial uses (Hyde *et al.,* 2019). Many fungi have been commercially and industrially utilized because they are helpful to humans (Leera *et al.,* 2019). Fungi, in fact, are the foundation of a number of significant enterprises. There are a number of industrial procedures that take specific fungi's biochemical activity into account (Azizan *et al.,* 2016). Fungi have the following functions in agriculture-based markets:

2.3.4.1 Alcoholic Fermentation

In the fermentation business, fungi are commonly employed to process ethanol, organic acids, antibiotics, and enzymes such as fungal lipase, cellulase, gluconase, and glycosidase (Hyde *et al.,* 2019; Leera *et al.,* 2019). In the manufacture of ethanol, *S. cerevisae and Monilia sp.* are utilized. The fermentation process is used to break down carbohydrates in yeast (*Saccharomyces cerevisae*) to produce alcohol and carbon dioxide (Maicas, 2020). Because fungi are sensitive to high alcohol concentrations, up to 50% of the sugar can be turned to alcohol, although this seldom exceeds 15%. Beer, wine, and sake are the three primary products of this procedure (Leera *et al.,* 2019).

2.3.4.2 Organic Acids

An organic acid is a substance that is both organic and acidic (Kulshrestha *et al.,* 2012). Fungi, particularly aspergilla, are well recognized for their ability to generate excessive amounts of a variety of organic acids (Liaud *et al.,* 2014). Essential organic acids such as oxalic acid, citric acid, gluconic acid, gallic acid, fumaric acid (Table 2.5), and others are economically generated as a result of mold biochemical activity (Hyde *et al.,* 2019).

Table 2.5: Various organic acids and their corresponding producing microorganisms. (Hyde *et al.,* 2019).

Organic acid produced	**Fungi**
Gluconic acid	*Penicillium* and *Aspergillus*
Gallic acid	*Aspergillus gallomyces*
Oxalic acid	*Aspergillus niger*
Ethanol	*Saccharomyces cerevisae*
Citric acid	*Aspergillus niger*

2.3.5 Role of Fungi in Enzyme Production

The genera *Rhizopus sp., Aspergillus sp., Penicillium sp., Geotrichum sp., Mucor sp.,* and *Rhizomucor sp.* are the most economically significant enzyme-producing fungus (Rodrigues *et al.,* 2016). Filamentous fungi are arguably the most favoured source of commercial enzymes due to their high ability for extracellular protein synthesis (Jun *et al.,* 2011). Exoenzymes are produced within the cell and then released outside the cell, where they break down large macromolecules into smaller components for growth and absorption by the cell (Kamalanathan *et al.,* 2020). *Trichoderma reesei*, for example, is known to secrete a significant quantity of cellulases (Keshavarz and Khalesi, 2015). *Saccharomyces cerevisae* is used to make invertase (Qureshi *et al.,* 2017). Sucrose is hydrolyzed into glucose and fructose. Fungal lipases have gotten a lot of interest because of their potential use in food processing, medicines, cosmetics, detergents, and the leather industry (Adinarayana, *et al.,* 2003).

Fungal enzymes have attracted attention for a variety of applications because fungus can grow on low-cost materials and release vast amounts of enzymes into the culture medium, making downstream processing easier (Anitha and Palanivelu, 2013). Amylases, cellulases, lipases, phytases, proteases, and xylanases are among the commercially available fungal enzymes (Lakshmi *et al.,* 2014; Singh *et al.,* 2016). The utilization of enzymatic reactions instead of organic solvents or chemical reactions is well appreciated, and there is a broad interest in the beneficial environmental impacts of industrial processes (Chapman *et al.,* 2018) Table 2.6 lists several important fungal enzymes and enzyme sources that are employed in a variety of applications.

Table 2.6: Examples of enzyme sources and applications of fungi (Hyde *et al.,* 2019).

Enzymes	Applications	Fungal Sources	References
Amylases	Starch hydrolysis in starch manufacturing industries	*Penicillium fellutanum*	Sahoo *et al.,* 2014
		Aspergillus fumigatus	Ko *et al.,* 2005
		Aspergillus niger	Ko *et al.,* 2005
		Rhizopus stolonifer	Saleem and Ebrahim, 2014
Cellulases	Animal feed industry	*Aspergillus niger*	Ko *et al.,* 2005
		Lentinula edodes	Pachauri *et al.,* 2017
Keratinases	Biomass conversion into biofuels	*Aspergillus oryzae*	Anitha and Panivelu, 2013
Laccase	Biopulping biobleaching deinking in pulp and paper industry.	*Agaricus subrufescens*	Ergun and Urek, 2017
Lipase	Degradation of fat in wastewater treatment; animal feed industry; pulp paper industry; detergent industry; food processing; textile industry; pharmaceutical industry	*Aspergillus niger*	Falony *et al.,* 2006
		Fusarium solani	Facchini *et al.,* 2015
		Penicillium sp	Ulker *et al.,* 2011
		Trichoderma sp	Vallado *et al.,* 2011
		Mucor sp	Falony *et al.,* 2006
		Pseudomona aeruginosa	

2.4 Lipase

Triacylglycerol acyl hydrolases (E.C. 3.1.1.3) are a family of enzymes that catalyze the conversion of triacylglycerol to glycerol and fatty acids (Kumar and Ray, 2014). They are found all throughout the world and are produced by a variety of plants, animals, and microbes (Thakur, 2012). Lipases have become one of the most popular biocatalysts due to their recent and diverse uses in oleochemistry, chemical synthesis, detergent formulation, and nutrition (Gunasekaran and Das, 2005; Saxena *et al.,* 2003). Lipases specialize in fat hydrolysis into fatty acids and glycerol at the water-lipid interface, as well as reversing the process in non-aqueous environments (Saxena *et al.,* 2003). They catalyze the hydrolysis of triglycerides as

well as the production of glycerol and long-chain fatty acid esters. Alcoholysis, acidolysis, esterification, and aminolysis are all biocatalyzed by them (Gunasekaran and Das, 2005).

2.4.1 Properties of Lipase

The number of lipases accessible has grown since the 1980s (Arife *et al.,* 2016). They have been used as commercial biocatalysts (Chapman *et al.,* 2018) due to properties such as biodegradability, high specificity (Amoah *et al.,* 2016), high catalytic efficiency (Amoah *et al.,* 2016b), temperature (Liu *et al.,* 2015), pH dependency, activity in organic solvents (Singh *et al.,* 2016), and non-toxic nature. The capacity of lipase to transesterify mono-, di-, and tri-glycerides as well as free fatty acids is the most desirable characteristic (Sharma *et al.,* 2016b).

Furthermore, lipases may respond at mild pH and temperature conditions, decreasing the energy required for direct reactions at high temperatures and pressures (Mehta *et al.,* 2017) Lipases are generally stable in organic solvents, with just a few exceptions for activation or inhibition (Patil *et al.,* 2011). Bacillus sp. lipases have been shown to be very stable in hydrophobic organic solvents and to be somewhat more efficient when short chain alkanes, benzene, and toluene are present at a rate of 10–50 percent (v/v) (Kumar *et al.,* 2016).

2.4.2 Sources of Lipase

Plants, mammals, and a variety of microbes such as bacteria, actinomycetes, fungus, and yeast all generate lipases (Raveendran *et al.,* 2018).

2.4.2.1 Plant Lipase

Lipases derived from seed plants have lately attracted a lot of interest as biocatalysts (Barros *et al.,* 2010). In some situations, these enzymes offer benefits over animal and microbial lipases due to several intriguing features such as specificity, cheap cost, availability, and simplicity of purification, and therefore constitute a great replacement for future commercial exploitation as industrial enzymes (Polizeli *et al.,* 2008; Barros *et al.,* 2010). Plant lipases, such as those found in oilseeds, are frequently found in energy reserve tissues (Seth *et al.,* 2014). Because of their eukaryotic origin, they operate as biocatalysts with excellent substrate specificity, cheap production costs, and simple pharmacological acceptance (Mazou *et al.,* 2016). Plant lipases consequently have more industrial potential in the organic synthesis, food, detergent, and pharmaceutical sectors (Seth *et al.,* 2014).

2.4.2.2 Animal Lipase

Pig and human pancreatic lipases are better known and researched in animals than any other lipase (Riberio *et al.,* 2011). In these species, they are engaged in various phases of lipid metabolism, including fat digestion, adsorption, reconstitution, and lipoprotein metabolism (Riberio *et al.,* 2011).

2.4.2.3 Microbial Lipase

In biotechnological and biochemical applications, microbial lipases are the most commonly utilized enzyme class (Thakur, 2012). Lipases have been identified from a variety of microbes, including bacteria (both Gram-positive and Gram-negative), fungus, yeast, and actinomycetes (Gunasekaran and Das, 2005). Microbial sources produce more enzymes than plants and animals, which may be owing to the ease with which they can be cultivated and genetically modified (Hasan *et al.,* 2006). Due to their capacity to function at high temperatures, pH and stability in organic solvents, chemo-selectivity, regio-selectivity, and enantio-selectivity, microbial lipases have garnered a lot of industrial interest compared to plant and animal lipases (Chandra *et al.,* 2020). (Sharma and Kanwar, 2014). They've also improved production yields, made genetic modification easier, and accelerated growth on low-cost medium, making manufacturing more appropriate (Thakur, 2012).

Carvalho *et al.* (2008) identified Biopetro-4 as a bacterial strain obtained from petroleum-contaminated soil. After 120 hours of fermentation and testing different inducers on lipase activity, the highest value obtained was 1,675uml-1. Mahanta *et al.* (2008) used the *Pseudomonas aeruginosa* PseA solvent resistant strain to obtain a maximum lipase activity of 1 084 U gds1. After 24 hours of fermentation, Alkan *et al.* (2007) produced extracellular lipase from *Bacillus coagulans* with a maximal lipase activity of 149 U gds1. After 72 hours of fermentation by *B. cepacia*, Fernandes *et al.* (2007) obtained a maximal lipase activity of 108 U gds1.

2.5 Fungal Lipase

Because of their extracellular lipase synthesis, fungi have been regarded the finest lipase sources (Falony *et al.,* 2006; Kumar and Ray, 2014). (Narishman and Bhimba, 2015; Ramos-Sanchez *et al.,* 2015) Fungal lipases have benefits over bacterial lipases since contemporary technology favors batch fermentation and low-cost extraction procedures (Mehta *et al.,* 2017). The strain, the content of the growth medium, culture conditions, pH, temperature, and the

kind of carbon and nitrogen supply all influence the development of fungal lipase (Cihangir and Sarikaya, 2004). The industrial desire for novel lipase sources with varied catalytic characteristics drives the isolation and selection of new strains. Lipase-producing microbes have been found in a variety of environments, including industrial waste, vegetable oil processing plants, agricultural plants, and oil-contaminated soil and oil seeds, among others (Thakur, 2012).

Adinarayana *et al.* (2003) investigated Aspergillus species lipase synthesis and found a maximum enzyme output of 1934U/g. Costa *et al.* (2017) isolated a strain of *Aspergillus niger* from agro-industrial wastes and used solid state fermentation to get an expressive activity of 19.844U/g. Vishnupriya *et al.* (2010) investigated Streptomyces grisesus lipase production and found a maximal enzyme activity of 51.9U/ml. Colen *et al.* (2006) used enrichment culture methods to identify 59 lipase-producing fungus strains from Brazilian savanna soil. An agar plate medium containing bile salts and olive oil emulsion was utilized to isolate and cultivate fungus in primary screening tests. The ratio of the lipolytic halo radius to the colony radius was used to select twenty-one strains. The most prolific strain was Colletotrichum gloesporioides, which was chosen from a total of eleven strains (Colen *et al.,* 2006).

Amin and Bhatti (2014) used canola seed oil cake as a substrate to investigate the effect of physicochemical factors on Penicillium fellutanum lipase production. After 48 hours of reaction time in a medium comprising 10 g canola seed oil cake as a substrate, 2 percent olive oil as an inducer, and 50% moisture content at an initial pH of 4.0, employing a 2 mL inoculum at 30°C, maximum lipase activity of 521 units/gram dry substrate (U/gds) was reached.

2.6 Lipase Production

Lipases are the most adaptable commercial enzymes, causing a wide range of bioconversion processes (Gunasekeran and Das, 2005). Since then, the market for enzyme manufacturing has grown substantially (Prakasan *et al.,* 2016). Because of increased understanding of production biochemistry, fermentation procedures, and recovery technologies, a greater variety of enzymes may be manufactured at a lower cost. Techniques for using enzymes have also improved substantially (Mehta *et al.,* 2017). Furthermore, because enzymes may catalyze so many diverse transformations, the number of enzymes utilized in commerce continues to grow (Thakur *et al.,* 2014). The kind and concentration of carbon and nitrogen

sources, culture pH, growth temperature, and dissolved oxygen content all affect lipase production (Elibol and Ozer, 2001). Both solid state and submerged cultures have generated microbial lipases (Colla *et al.,* 2015).

2.6.1 Solid State Fermentation

Solid state fermentation occurs in the absence of water in a solid media (Singhania *et al.,* 2010), however the substrate requires moisture to enable microbial growth and metabolic activity (Thomas *et al.,* 2013). Solid state fermentation is the most effective method for cultivating microorganisms (Bhargav *et al.,* 2008). Solid state fermentation is being employed as a cost-effective alternative to submerged fermentation in a number of applications, including the synthesis of antibiotics and enzymes (Thomas *et al.,* 2013), since raw materials and processing are less expensive, and there is less processing risk (Guerra *et al.,* 2003). Because it has the ability to generate desired microbial products more effectively, this approach involves the use of agricultural substrates with diverse nitrogen sources, carbohydrates, and nutrients such as starch, cellulose, pectin, and fibers (Kumar and Ray, 2014). Agricultural waste (Table 2.7) such as rice husk, wheat bran, beans, sugar cane, and lemon peel are among the substrates typically fermented by solid state fermentation (Kumar and Ray, 2014). In this method, microorganisms are inoculated and grown on a solid substrate with a low moisture content (Manan and Web, 2017)

Table 2.7: Comparison of lipase production in various fungal microorganisms (Kumar and Ray, 2014)

Microbes	**Solid Substrate**	**Inducers**	**Fermentation conditions**			**Lipase Activity (U/mL)**	**References**
			Temp. (°C)	**pH**	**Time (hr)**		
Fusarium oxysporum	Wheat bran	Cetyl trimethylammonium bromide	40	8.5	96	111.48	Angelo *et al.,* 2014
Aspergillus flavus	Wheat bran and castor oil cake	n.r	30	7.0	96	121.35*	Tosacano *et al.,* 2013
Aspergillus niger	Rice bran	n.r	30.3	6.87		121.53*	Hosseinpour *et al.,* 2012

Candida rugosa	Coconut oil cake	Urea, Peptone and Maltose	n.r	n.r	96	87.76*	Benjamin and Pandey, 2004
Aspergillus niger	Shea butter cake	Tween 20	30	7.0	168	3.35*	Salihu *et al.,* 2013
Penicillium restrictum	Babassu oil cake and Olive oil	n.r	37	7.0	24	5.8	Castilho *et al.,* 2000
Aspergillus terreus	Mustard oil cake	n.r	30	6.0	96	1566.6	Sethi *et al.,* 2013

Code: * U/g; n.r. – not reported

To improve microbial growth and enzyme production, selecting an appropriate solid substrate for the fermentation process is a crucial element that necessitates the screening of diverse agro-industrial materials (Krishna, 2008). The type of solid substrate used in this process is mostly determined by the cost and availability of other solid substrates (Kumar and Kanwar, 2012). However, there are a few key variables that influence the synthesis of fungal lipase in solid state fermentation. Substrate size, inoculum size, moisture content, temperature, inducers, pH, nitrogen supply, surfactants, and spore production are all aspects to consider (Kumar and Kanwar 2012).

2.6.2 Submerged Fermentation

Molasses and broths are examples of free-flowing liquid substrates used by SmF. In the fermentation broth, beneficial substances are secreted. Because the substrates are quickly depleted, they must be renewed or supplied with nutrients on a regular basis. This fermentation method is appropriate for microorganisms that demand a lot of moisture, such as bacteria. Another advantage of this method is that product purification is simplified. SmF is generally used to extract secondary metabolites that must be utilised in a liquid state (Subramaniyam and Vimala, 2012).

2.7 Factors Affecting Lipase Production

Microbial lipases are mostly extracellular, and physicochemical variables such as temperature, pH, and dissolved oxygen have a significant impact on their synthesis (Salihu and Alam, 2012). In the presence of lipid substrates such as oils or other inducers, these enzymes are generally generated as triacylglycerols, fatty acids, hydrolysable esters, tweens, bile salts, and glycerol (Gupta *et al.,* 2004).

2.7.1 Effect of Nutritional Factors

2.7.1.1 Carbon sources

Carbon sources are key substrates for energy production in microorganisms (Salihu and Alam, 2012). Carbon sources are crucial in lipase induction in all types of microbial sources. With mustard seed oil as a carbon source, *Aspergillus terreus* produced a high output of lipase (Sethi *et al.,* 2012). A combination of olive oil cake and sugar cane bagasse is used as a carbon source to increase lipase synthesis in fungal strains (Fatima *et al.,* 2020). In compared to alternative carbon sources, olive oil cakes have shown an increase in lipase synthesis (Zarevucka, 2012). Tween 80 has been shown to aid in the recovery of Acinetobacter sp (Fatima *et al.,* 2020). In addition to lipid carbon sources, non-lipidic carbon sources such as glucose, fructose, sucrose, and others have been shown to stimulate lipase synthesis. Bindiya and Ramana investigated the effect of various non-lipid carbon sources (galactose, glucose, xylose, fructose, lactose, maltose, sucrose, and mannitol) and lipid carbon sources (coconut oil, palm oil, cucumber oil, olive oil, mustard oil, sunflower oil, and neem oil) on the production of lipase by *Aspergillus sydowii* (2012).

2.7.1.2 Nitrogen Sources

In the production of lipase, nitrogen plays a crucial function. Diverse organic and inorganic nitrogen sources have had an essential impact in boosting lipase synthesis in various microbial species (Fatima *et al.,* 2020). Rhizopus sp. was cultivated by adding urea to the lipase culture medium, which increases lipolytic activity (Rodriguez *et al.,* 2006). In a combination of peptone and other nitrogen extracts, Aspergillus sp. was also utilized to produce lipase (Colonia *et al.,* 2019). Lipase activity was also influenced by a mixture of organic nitrogen sources, as described by Ulker et al. (2011), who found that Trichoderma reesei had the highest lipase activity in a medium containing glucose and peptone as carbon and nitrogen sources, respectively, and the lowest activity in a medium containing glucose and yeast extract. However, Bindiya and Ramana (2012) investigated the effect of different nitrogen sources (1 percent w/v) on Aspergillus sydowii lipase production. NaNO3, KNO3, NH4NO3, NH4Cl, (NH4)2SO4, urea, beef extract, malt extract, yeast extract, tryptone, and peptone were employed as nitrogen sources in the study. NH4Cl produced the highest activity (49 U/mL).

2.7.2 Effect of Physical Factors

2.7.2.1 Temperature

The physical characteristics of the cell membrane are altered by temperature, which impacts the release of extracellular enzymes (Valeria *et al.,* 2003). By incubating Aspergillus terreus cultures at various temperatures, including 10 ºC, 20 ºC, 30 ºC, and 45 ºC, Mahmoud *et al.* (2015) examined the effect of temperature on lipase synthesis. At 45 ºC (15 U/mL), lipase activity was highest, followed by 30 ºC (12 U/mL), 20 ºC (9.5 U/mL), and 10 ºC (3.0 U/mL). Mukhtar *et al.* (2015) investigated the effect of various incubation temperatures ranging from 25 to 55 ºC on Aspergillus niger lipase production. The highest production was at 30 degrees Celsius, followed by 35 degrees Celsius, 40 degrees Celsius, 45 degrees Celsius, 25 degrees Celsius, 50 degrees Celsius, and 55 degrees Celsius. Sumathy *et al.* (2012) cultured Aspergillus niger cultures at 25 ºC, 30 ºC, and 37 ºC, then measured lipase activity after 48 hours, 72 hours, 96 hours, and 120 hours. After 96 hours of incubation, the culture incubated at 30 ºC had the highest lipase activity. Aspergillus sydowii lipase production was studied at various incubation temperatures by Bindiya and Ramana (2012). At 32 °C, the highest activity (64 U/mL) was achieved, followed by 30 °C, 28 °C, 26 °C, 24 °C, 22 °C, 20 °C, and 34 °C, in that order.

2.7.2.2 pH

Within the pH range of 6.0 to 8.0, powerful lipolytic fungi such as *Aspergillus sp., Geotrichum sp., Rhizopus sp., Rhizomucor sp., Mucor sp.,* and *Penicillium sp.* may thrive and secrete extracellular lipases (Sharma *et al.,* 2016a). *Aspergillus sydowii* lipase activity was studied by Bindiya and Ramana (2012) at different pHs of fermentation broth. Lipase production was highest at pH 8.0, followed by pH 7.5, 8.5, 7.0, 9.0, 6.5, and 6.0. Similarly, Mahmoud *et al.* (2015) examined the effect of different pH on lipase synthesis by cultivating *Aspergillus terreus* at different pH levels ranging from 2.0 to 12.0. The greatest lipase activity (15 U/mL) was reported at a pH of 8.0. The lipase activity of *Aspergillus terreus* was reported to be 12 U ml-1 at pH 12.0, but not at pH 2.0, indicating that alkaline environments enhance fungal lipase development and synthesis.

2.8 Application of Fungal Lipase

Lipase has seen a spike in popularity in industrial applications due to its inherent flexibility (Geoffry and Rajeshwara, 2018). Due to the diversity of their characteristics and simplicity of mass manufacturing, fungal lipases constitute a large category of biotechnologically important enzymes (Singh and Mukhopadhyay, 2012). Fungal lipases offer a wide range of enzymatic and substrate specificity characteristics, which makes them ideal for commercial use (Ray, 2015). Many researchers have looked at how fungal lipases may be used in industry (Treichel *et al.,* 2010).

2.8.1 Lipase in Pharmaceutical Industry

In the pharmaceutical business, enzymes offer several benefits over chemical synthesis, which is why lipase is in such high demand (Singh and Mukhopadhyay, 2012). Mild conditions that prevent isomerization, epimerization, racemization, and rearrangement processes, as well as enantio and regio selectivity and immobilized lipase reuse, are among the advantages. The pharmaceutical industry is now utilizing lipases' ability to overcome racemic mixtures via production of a single enantiomer for medication development. Pharmaceutical goods are made with medicinal mushrooms (Mohamed *et al.,* 2011). *Agaricus brasiliensis, Ganoderma lucidum, Lentinula edodes, Coriolus versicolor, Pleurotus ostreatus, Grifola frondosa, Termitomyces,* and others are a few important mushrooms having pharmacological characteristics (Ganeshpurkar *et al.,* 2010). Fungi include immunosuppressive, antibacterial, antiviral, nematic, and hypocholesterolemic compounds, among other physiologically active chemicals (Hyde *et al.,* 2019).

2.8.2 Lipase in Food Processing Industries

Lipase is an enzyme that is utilized in food industries to modify and break down biomaterials (Singh and Mukhopadhyay, 2012). Fats and oils are vital food ingredients, and their modification is one of the main areas in the food processing sector where innovative economic and green technologies are needed (Gupta, 2003). A huge number of fat-clearing lipases are produced on an industrial basis. The majority of commercial lipases manufactured are used to increase flavor in milk products and other meals such as meat, vegetables, fruit, smoked carp, milk products, baked goods, and beer products (Mehta *et al.,* 2017). In bakery goods, lipases from *Aspergillus niger, Rhizopus oryzae,* and *Candida cylindracea* have been utilized (Singh and Mukhopadhyay, 2012).

2.8.3 Lipase in Textile Industry

In the textile sector, the usage of fungal lipase is becoming increasingly essential (Mehta *et al.,* 2017). Lipases are used in the textile industry to assist eliminate size lubricants from fabrics in order to increase absorbency and dyeing speed (Gupta *et al.,* 2017). Softness, high strength, washability, stain, stretch, machine abrasion, and wrinkle resistance are all advantages of polyester in the textile business. Synthetic fibers have been enzymatically changed for use in the manufacture of yarns, garments, carpets, and textiles. It entails modifying the characteristics of polyester fibers in such a way that they are resistant to post-modification treatment (Singh and Mukhopadhyay, 2012).

2.8.4 Lipase in Waste Treatment

Lipases are employed in activated sludge and other aerobic waste processes, where thin layers of fat are continually removed from the surfaces of aerated tanks to allow oxygen to pass through. Lipase, such as that from *C. rugosa*, is used to digest this skimmed fat-rich liquid (Mehta *et al.,* 2017). Lipases also help anaerobic digesters operate as they should (Su *et al.,* 2016). Successful solids breakdown and the removal and avoidance of fat blockage or films in waste systems are essential in many industrial processes (Islam and Datta, 2015). Through batch and continuous-flow tests, Jeganathan *et al.* (2009) assessed the hydrolysis of wastewater with high oil and grease (O&G) content from the pet food industry using immobilized *C. rugosa* lipase (CRL) as a pretreatment step for anaerobic treatment.

2.8.5 Lipase in Detergent Industry

Fungal lipases are frequently utilized as additives in commercial laundry detergents and household detergents, and this reduces the environmental impact of detergent products by allowing for lower washing temperatures (Saisubramanian *et al.,* 2006). An estimated 1,000 tons of lipase is added to the nearly 13 billion tons of detergents generated each year. *Humicola lanuginosa* lipase is a suitable detergent additive because of its thermostability, high activity at alkaline pH, and stability against anionic surfactants. *Candida*-derived lipases are also utilized as detergents. Dishwashing, bleaching composition, breakdown of lipid contaminants in solvents for dry cleaning, liquid leather cleaner, cleaning of contact lenses, and washing, degreasing, and reconditioning of water utilizing lipases and oxido-reductases are some of the other uses for detergents (Mehta *et al.,* 2017).

2.8.6 Lipase in Paper Industry

Pitch, a wood lipid component that interferes with the manufacture of paper pulp, is extracted using lipolytic enzymes. Through paper recycling, they also assist in the removal of lipid

stains and the prevention of the growth of sticky materials (Dube *et al.,* 2008; Hasan *et al.,* 2006). Nippon Paper Industries in Japan developed a pitch control method that hydrolyzed up to 90% of the triglycerides using *Candida rugosa* fungus lipase (Sharma *et al.,* 2001). Hata and colleagues at Jujo Paper Company stated in 1990 that lipases might reduce pitch problems by reducing the triglyceride level of pulverized wood pulp. When a lipase derived from *Candida cylindrica* was introduced to the ground wood stock chest, it significantly decreased pitch issues and talc use. In the paper industry, *Candida antartica* lipase A (CALA) was employed to regulate pitch (Maria *et al.,* 2005).

2.8.7 Lipase in Biodiesel Production

Biodiesel is a category of esters produced via a transesterification process between fatty acids and alcohol in the presence of catalysts (Mehta *et al.,* 2017). The manufacture of biodiesel from waste and non-edible vegetable oil reduces the cost of biodiesel production significantly, eliminating conflicts between food security and energy security. It is also considered as an important step in lowering emissions and reusing waste oil (Gashaw *et al.,* 2015; Narwal and Gupta, 2013).

Biodiesel output has increased dramatically in the previous decade, from around 950 liters in 2000 to almost 17,000 million liters in 2010, with the European Union accounting for 53% of worldwide biodiesel production (Eryilmaz *et al.,* 2016). Biodiesel accounted for about 5% of global biofuel output in 2000, and around 20% of total biofuel production in 2011. (Eryilmaz *et al.,* 2016). This trend looks to be continuing, with biodiesel output likely to surpass 41,000 million liters in 2022, according to UN estimates. Lipase's higher thermostability and ability to tolerate short-chain alcohols make it ideal for biodiesel synthesis (Bacovsky *et al.,* 2007; Kato *et al.,* 2007). *A. niger, C. antarctica, C. rugosa, R. miehei, R. oryzae,* and *Thermomyces lanuginose* generate the majority of yeast and fungal lipases used in biodiesel synthesis (Fan *et al.,* 2012).

The use of immobilized *Candida Antarctica* lipase-catalyzed methanolysis of soybean oil to produce biodiesel has been described (Ho *et al.,* 2007). Immobilized lipase from *Candida rugosa* on Sepabeads EC-OD was the most promising biocatalyst for enzyme-catalyzed biodiesel production (Winayanuwattikun *et al.,* 2011). *Candida rugosa* lipase (CRL) in ionic liquid accelerated biodiesel synthesis from Chinese tallow kernel oil in a recent study (Su *et al.,* 2016).

CHAPTER THREE
METHODOLOGY

3.1 Materials and Equipment

The equipment and materials used for the experiment are as follows: glass slides, Petri dishes, cover slips, inoculating needle, conical flask, aluminum foil, dropper, cotton wool, inoculating loop, test tubes. burette, McCartney bottle, pipette, spatula, beaker, syringe, Erlenmeyer flask, Bunsen burner, water bath, incubator and autoclave.

3.2 Culture Media and Reagents

The media used during the experiment were: Potato Dextrose Agar (PDA), Tributyrin Agar. The reagents used during the experiment include: Lactophenol blue, rhodamine blue indicator, ethanol, olive oil, Saline solution (2 g/L KH_2PO_4, 1 g/L $MgSO_4$, $FeSO_4 \cdot 7H_2O$ (0.63), $MnSO_4$ (0.01), $ZnSO_4$ (0.62)), Sodium Hydroxide (NaOH), Hydrochloric Acid (HCL).

3.3 Isolation of Fungal Species

3.3.1 Sampling

Wastewater contaminated oil samples were taken from different locations on Campus (CHMS, CBAS, Cafeteria, and Hostel). Using a sterile spatula, soil samples were taken at a depth of 5 – 10 cm and kept in sterile glass vials (Alhamdani *et al.,* 2016). The bottles were correctly labeled and brought to the laboratory for inspection and analysis (Sagar *et al.,* 2013). When not in use, the samples were kept at 27°C in the incubator (Nwuche *et al.,* 2011).

3.3.2 Serial dilution

For the isolation of lipolytic fungi, serial dilution was first prepared (Rajeshkumar *et al.,* 2013). One gram of each soil sample was weighed and mixed with 9 mL of distilled water in a sterile test tube. Aseptically produced five-fold serial dilutions. The sterile test tubes were divided into 10^{-1} to 10^{-5} dilutions and labeled. 1g of the corresponding soil sample was weighed and added to a 9.0 mL distilled water dilution blank. To the second dilution blank, 1.0 mL of the first dilution blank (10^{-1}) was added (10^{-2}). The tubes were shaken, and successive dilutions were carried out until the last tube dilutions were completed (10^{-5}). In a disposable petri-dish, 0.1 mL of the diluents were innoculated with Potato Dextrose Agar (PDA) using the pour plate technique.

3.3.3 Potato Dextrose Agar

For fungus culture, Potato Dextrose Agar (PDA) was employed. PDA is a general-purpose medium for yeast and mold cultivation that may be treated with antibiotics to prevent bacteria from growing (Sadar, 2019). In a conical flask, 39 g of commercially prepared Potato Dextrose Agar was measured and dissolved in 1 liter of sterile distilled water while maintaining aseptic techniques and circumstances. To inhibit bacterial development, a chloramphenicol antibiotic was added. The mixture was heated to thoroughly dissolve the culture medium. After that, the Agar was autoclaved at 121°C for 15 minutes to sanitize it. Before pouring onto the sterile petri dishes, the medium was allowed to cool to 45°C.

3.3.4 Pour plate method

On PDA (Potato Dextrose Agar), the fungal colonies were cultured using the pour plate technique. 0.1 mL of the serially diluted samples (10^{-1}, 10^{-3}, and 10^{-5}) was placed into sterile petri plates, which were shaken to ensure that the sample was distributed evenly. The molten Potato Dextrose Agar were carefully poured into the appropriate petri dishes after a few minutes. The plates were incubated at room temperature for 3-5 days after solidification. The sterility of the medium was verified by incubating a control agar plate that had not been infected (Wadia *et al.,* 2017). To get a pure culture, distinct fungal colonies were chosen from the heterotrophic colony and transplanted into a fresh culture medium using a sterilized inoculating loop. To avoid bacterial contamination, chloramphenicol antibiotics were added to the culture medium (PDA). After that, the plates were incubated and kept at 4°C in a plastic bag.

3.4 Screening of lipolytic fungal species

Tributyrin agar (HiMedia) of pH7.5, 10.0 mL and 990 mL distilled water was utilized. All of the isolated fungi cultures were inoculated into TBA plates and cultured for 7 days at 27 degrees Celsius. Lipase synthesis by the organisms was indicated by the creation of an opaque zone around colonies (Wadia *et al.,* 2017). Colony diameters (d) and total clear halos diameters (d) were also measured. The strains with the most halos (D-d) were chosen as prospective lipase producing fungus (Griebeler *et al.,* 2009). The lipase producing fungal strain was transferred to agar slants for storage at 4ºC.

3.5 Identification of the screened fungi

The strong fungal cultures were inoculated on Potato dextrose Agar and cultured at room temperature for identification and further research after being screened.

3.5.1 Morphological identification

For growth rate, growth pattern, colony texture, and pigmentation, the morphological characteristics of the chosen isolate cultured on PDA were investigated (Promputtha *et al.,* 2005).

3.5.2 Biochemical identification

The borer and an inoculating needle were used to extract a piece of the colony's growth, which was then placed on the slide with a drop of lactophenol blue and covered with a cover slip. For microscopic photography of the fungus, the preparation was viewed under a light microscope with an attached camera (Motic Mc Camera 2.0-megapixel digital colored camera) linked to a computer. The goal was to study the exact arrangement of the conidiophores as well as the spore production process. These fungi's identities were established by cultural, morphological, and textual comparisons with known representatives of various species (Ellis *et al.,* 2007; Alexopoulos *et al.,* 1996).

3.6 Lipase production

Lipase production in submerged fermentation was also quantified using spectrophotometric assay method. The production medium consists of 2 (g/L): Peptone, 20.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; K_2HPO_4, 2.0; Olive oil, 10; Glucose, 15.0, pH 7.5 The Fermentation was carried out using a shaker incubator of 100 rpm at 25°C for 3 - 7 days (Adham and Ahmed, 2009).

3.7 Lipase assay

The culture filtrate after 3 - 7 days of growth was collected using Whatman No. 1 filter paper. The supernatant (crude enzyme) was tested for enzyme activity. Lipase activity was determined spectrophotometrically using the method of *Saeed et al.* (2005) where two solutions were prepared for the assay as follows. The Solution 1 contained 90 mg of p-Nitophenyl Palmitate (pNPP), dissolved in 30 mL propan-2-ol and acetonitrile (1:1), while Solution 2 contained 2.0 g Triton X-100 and 0.5 g gum Arabic dissolved in 450 mL of 50 mM Tris-HCl buffer (pH 8.0). The assay solution was prepared by adding 1.0 mL of Solution 1 to 9.0 mL of Solution 2 drop-wise to get an emulsion. Finally, the assay mixture contained 900 µL of the emulsion and 100 µL of the appropriately diluted enzyme solution. The lipase activity was determined by measuring the amount of liberated p-nitrophenol at 410 nm using spectrophotometer. One unit (U) of lipase activity was defined as the amount of enzyme that releases one micromole (1 µmol) of p-nitrophenol from the substrate.

$$\text{Lipase activity (U/mL)} = \frac{(\Delta ABS) * (V_R)}{\varepsilon * V_E * t} \text{ X } 103$$

Where 'ΔABS' is the change in Absorbance @410nm, 'V_R' is the volume of the assay, 'ε' is the molar extinction coefficient for p-NPP ($M^{-1}cm^{-1}$), 'V_E' is the volume of the enzyme used and 't' is the reaction time.

3.8 Effect of physico-chemical parameters on lipase production

The effects physico-chemical parameters such as carbon sources, nitrogen sources, temperature and pH on the lipase produced by the isolate were investigated.

3.8.1 Effect of carbon sources on lipase production

Under submerged fermentation, the influence of carbon sources such as fructose, galactose, glucose, sucrose, mannitol and starch as carbon sources was examined on lipase synthesis was studied. The production medium had 10 g/L carbon source, KH_2PO_4 (0.2 percent w/v), $MgSO_4$ (0.1 percent w/v), and 2 percent yeast extract as a nitrogen source inside 300 mL Erlenmeyer's flasks holding 50 g of the medium. As a lipase inducer, 2% olive oil was added (Colla *et al.,* 2014). After that, the medium was autoclaved for 20 minutes at 103 kPa. After inoculation, the cultures were cultured at 30°C for 4 days with 120 rpm agitation.

3.8.2 Effect of nitrogen source on lipase production

The effect of yeast extract, peptone, beef extract, ammonium sulphate, sodium nitrate, potassium nitrate and urea as nitrogen source was investigated on lipase production under the solid-state fermentation. The production medium was similar to the one described above containing 10g/L carbon source, KH2PO4 (0.2% w/v), $MgSO_4$ (0.1% w/v) and 2% of nitrogen source inside 300mL Erlenmeyer's flasks containing 50g of the medium. A 2% aliquot of olive oil was added as lipase inducer (Colla *et al.,* 2014). Afterwards, the medium was autoclaved at 103 kPa for 20 mins. After inoculation, the cultures were incubated for 4 days at 30℃ with agitation of 120 rpm.

3.8.3 Effect of temperature on lipase production

The effect of incubation temperatures on lipase production were investigated by varying the temperature of incubation in 5 ^oC interval in the range of 25 – 50 ^oC. The pH of the medium was adjusted to 7.0 followed with sterilization at 121^oC, 15 psi for 15mins. The broths were inoculated and incubated at 25 ^oC under constant shaking at 100 rpm for 5 days. Lipase assay were determined at the end of incubation period as earlier described (Saeed *et al.,* 2005).

3.8.4 Effect of pH on lipase production

The effect of pH on lipase production were determined by varying the pH of the production medium over a range of 3 – 10. The pH of the basal media was adjusted using buffer

appropriately. Where necessary, the pH was further adjusted using 1 N NaOH or 1N HCl as appropriate followed by sterilization, inoculation and incubation of the broth at 25 ºC for 5 days. Lipase assay were determined at the end of incubation period as earlier described (Saeed *et al.,* 2005).

3.9 Statistical Analysis

Each parameter's values were computed and displayed as means of duplicates. For significance, the data was analyzed using Analysis of Variance (ANOVA) with Duncan Multiple Range Test at $P \leq 0.05$. There was no indication of the standard deviation. Tables were also used to show the data.

CHAPTER FOUR

RESULT

4.1 Colony Count

Twelve (12) morphologically different fungi were isolated. Table 4.1 showed the colony count of 10^{-1}, 10^{-3} and 10^{-5} serial dilutions for the soil samples from different locations in MTU dump sites. Samples from CHMS, CBAS, Cafeteria and Hostel dump sites ranged from 8 – 58, 8 – 48, 9 – 51, and 10 – 39 cfu/mL respectively.

4.2 Morphological characterization of the isolates

Table 4.2 shows the morphological identities of the isolates, including; color, shape, edge and elevation. The observed colours included: white, cream, black, pink, light green. The shapes were circular, rhizoid. The edges were filamentous, circular and irregular, while the elevations were raised and convex. The surfaces of the colonies were observed and the colonies appeared wooly and dull.

Table 4.1: The colony count of the sampling plates after serial dilution

Soil Sampling Locations	10^{-1} (cfu/mL)	10^{-3}(cfu/mL)	10^{-5}(cfu/mL)
CHMS dump site	58	30	8
CBAS dump site	48	31	8
CAFETERIA dump site	51	44	9
HOSTEL dump site	39	25	10

Table 4.2: Morphological Characterization of the Isolates

Isolates	Color	Form	Margin	Elevation	Surface
APD1	White	Filamentous	Entire	Raised	Wooly
APD2	Light green	Circular	Entire	Raised	Dull
APD3	White	Circular	Entire	Convex	Dull
APD4	Pink	Circular	Entire	Convex	Dull
APS1	Black	Circular	Entire	Raised	Dull
APS2	White	Irregular	Entire	Raised	Dull
APS4	White	Circular	Entire	Raised	Dull
APS5	Cream	Irregular	Entire	Raised	Dull
APS6	Black	Circular	Entire	Convex	Dull
APS7	Cream	Filamentous	Entire	Raised	Dull
APS8	Cream	Circular	Entire	Raised	Wooly
APS9	White	Circular	Entire	Convex	Wooly

4.3 Screening of fungal isolates for lipase activity

Table 4.3 shows the result of the screening of the isolates for lipase production on solid agar. At the end of incubation periods, the diameter of growth of the isolates ranged from 23.0^{k} – 53.0^{a} mm. Isolate APD1 had the highest diameter of growth while isolate APS4 had the lowest. Moreover, there was a significant difference ($p \leq 0.05$) in lipase activity of the isolates. Lipase production measured based on the zone of clearance of the isolates ranged from 2.0^{g} – 10.5^{a} mm. Isolate APD1 had the highest lipase activity while isolate APS6 had the lowest activity. Four (4) isolates including APD3, APS1, APS2 and APS9 did not show any lipase activity throughout the incubation period. Isolate APD1 exhibited excellent lipase activity and was selected for further study.

4.4 Identification of isolate APD1

Figure 4.1 shows the macroscopic identification of the selected isolate (APD1). The isolate is a rapidly growing mould which matures within 3 to 5 days. Growth begins as fluffy white tufts which then compact and appear woollier.

Figure 4.2 illustrates the microscopic image of the selected isolate, following staining with lactophenol blue dye. The microscopic appearance showed septate and hyaline hyphae. Conidiophores appeared rather short, branching at wide angles while Phialides are flask or ampule shaped (inflated at the base), which again extend from the conidiophore at wide angles. Conidia are round to ellipsoidal and can be smooth or rough walled depending on the species. Based on the results of morphological and biochemical characterizations, the probable identity of the isolate was concluded to be *Trichoderma reesei*.

Table 4.3: Screening of fungal isolates for lipase activity

Isolates	Diameter of growth (mm)	Lipase activity (mm)
APD1	53.0[a]	10.5[a]
APD2	30.0[j]	9.0[b]
APD3	50.0[c]	0.0
APD4	31.0[i]	4.5[f]
APS1	32.0[h]	0.0
APS2	51.0[b]	0.0
APS4	23.0[k]	5.0[e]
APS5	41.0[g]	8.0[c]
APS6	41.0[g]	2.0[g]
APS7	48.0[d]	10.5[a]
APS8	46.0[e]	7.0[d]
APS9	43.0[f]	0.0

Mean followed by different superscript within a column are significantly different (P≤0.05)

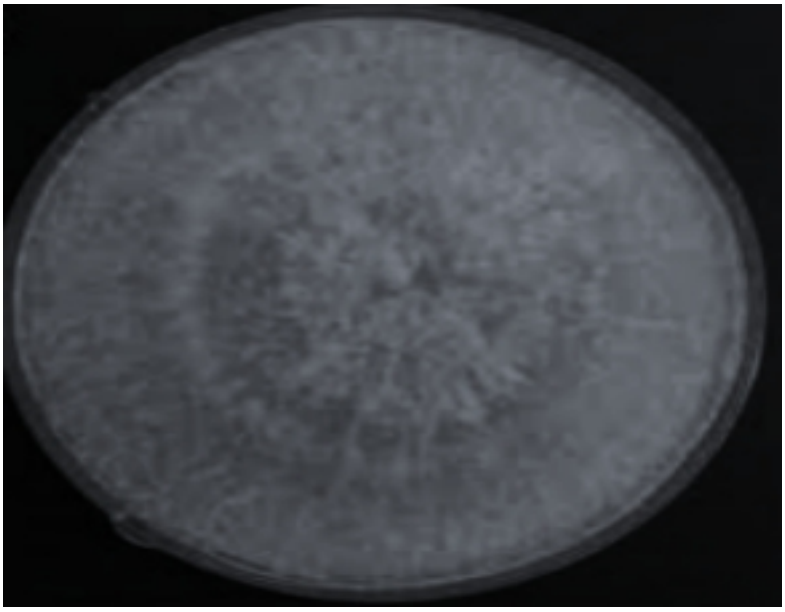

Figure 4.1: Macroscopic appearance of isolate APD1

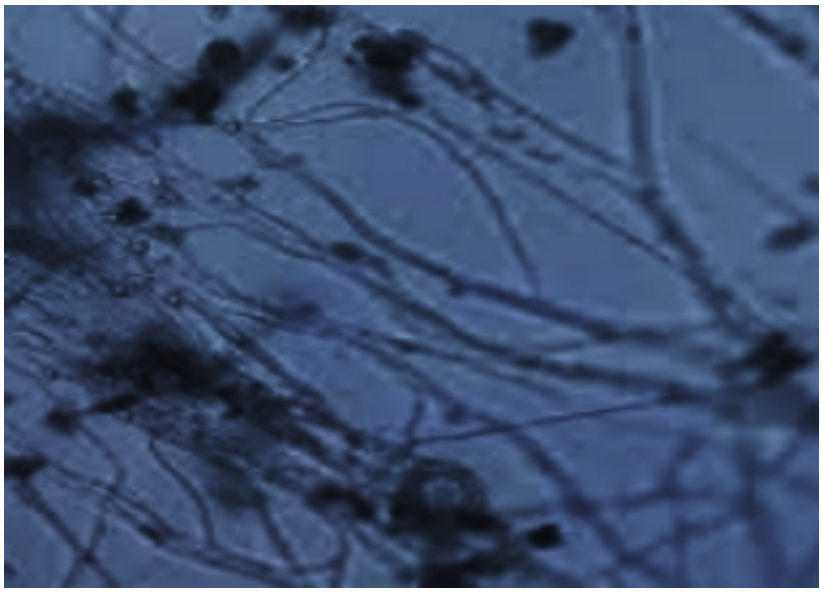

Figure 4.2: Microscopic appearance of isolate APD1.

4.5 Effects of physico-chemical parameters on isolate APD1

The effects physico-chemical parameters such as carbon sources, nitrogen sources, temperature and pH on the lipase produced by the isolate are shown below.

4.5.1 Effect of carbon sources on lipase production

From Figure 4.3, there was a significant difference ($p<0.05$) in lipase production resulting from different carbon sources. Glucose (75.95^{a} U/mL) supported the highest lipase production follow in order by fructose (70.77^{b} U/mL), galactose (59.95^{c} U/mL), mannitol (51.76^{d} U/mL), and sucrose (54.27^{c} U/mL), while Starch (37.07^{e} U/mL) showed the least supports for lipase production in APD1 isolate.

4.5.2 Effect of nitrogen sources on lipase production

From Figure 4.4, there was a significant difference ($p<0.05$) in lipase production resulting from different nitrogen sources. Peptone (76.16^{a} U/mL) supported the highest lipase production follow in order by yeast extract (68.99^{b} U/mL), ammonium sulphate (64.51^{c} U/mL), sodium nitrate (55.66^{d} U/mL), and potassium nitrate (52.24^{e} U/mL), while urea (44.48^{f} U/mL) showed the least supports for lipase production in APD1 isolate.

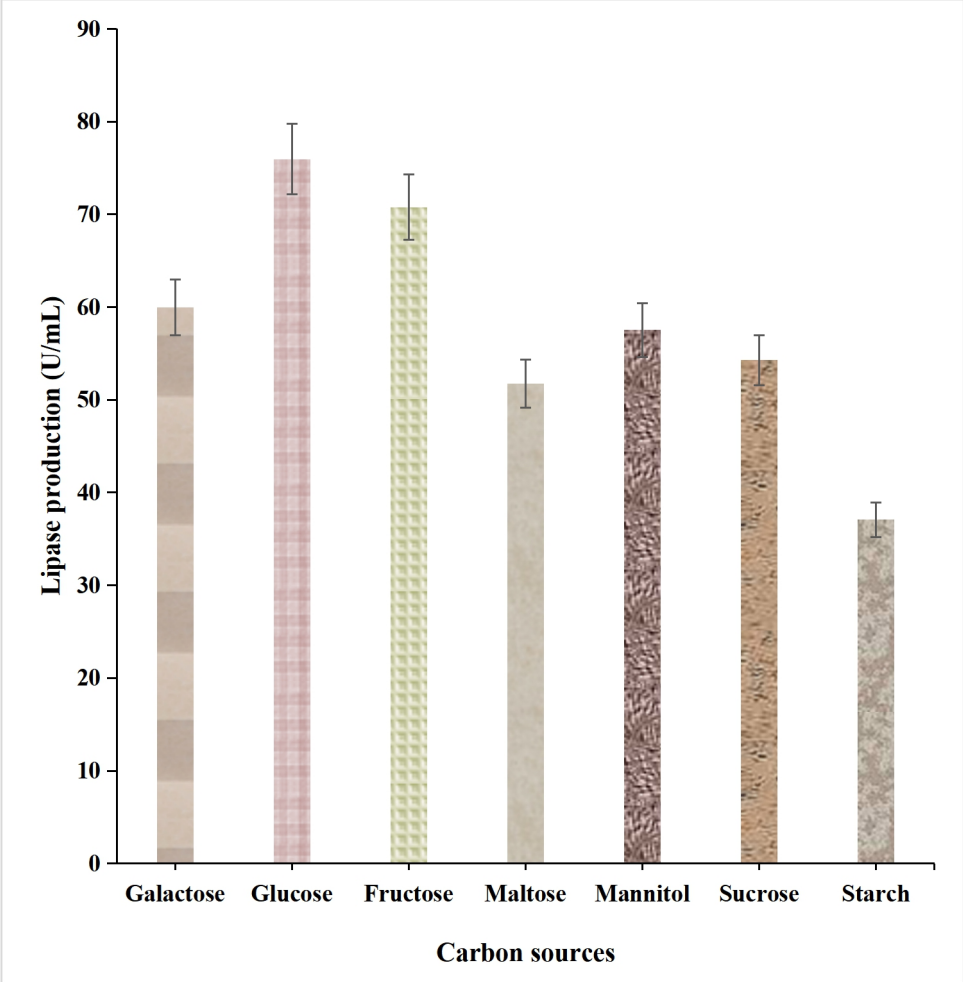

Figure 4.3: Effect of carbon source on lipase production by *T. reesei* APDI

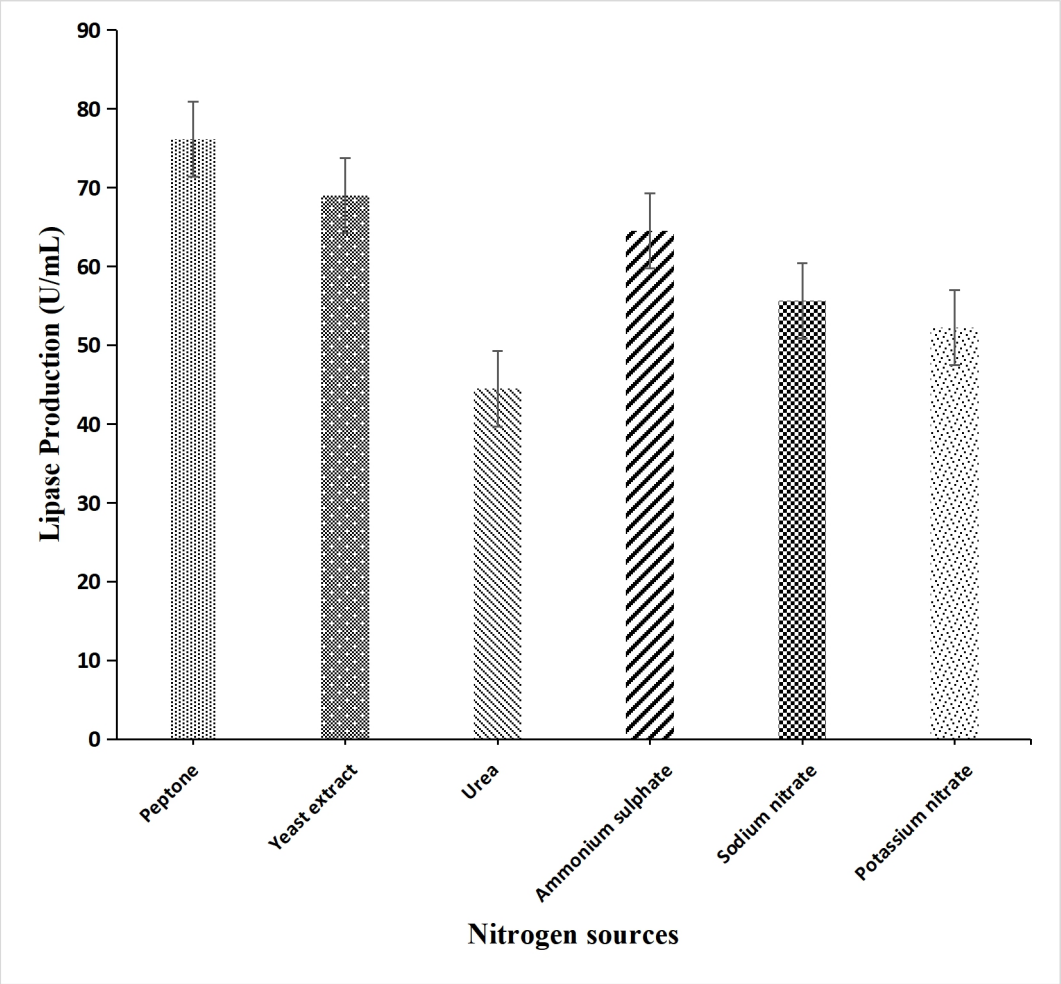

Figure 4.4: Effect of nitrogen source on lipase production by *T. reesei* APDI

4.5.3 Effect of incubation temperature on lipase production

From Figure 4.5, there was a significant difference ($p<0.05$) in lipase production resulting from different temperatures. 30^{o}C (59.41^{a} U/mL) supported the highest lipase production follow in order by 35℃ (54.14^{b} U/mL), 25℃, (50.48^{c} U/mL), 40℃ (47.07^{d} U/mL), and 45℃, (37.04^{e} U/mL), while 50℃, (34.19^{f} U/mL) showed the least supports for lipase production in APD1 isolate.

4.5.4 Effect of initial pH on lipase production

From Figure 4.6, there was a significant difference ($p<0.05$) in lipase production resulting from different pH values. pH 6 (70.64^{a} U/mL) supported the highest lipase production follow in order by pH 5 (61.60^{b} U/mL), pH 7, (60.61^{b} U/mL), pH 8 (51.10^{c} U/mL), and pH 9, (45.17^{d} U/mL), while pH 3, (31.02^{e} U/mL) showed the least supports for lipase production in APD1 isolate.

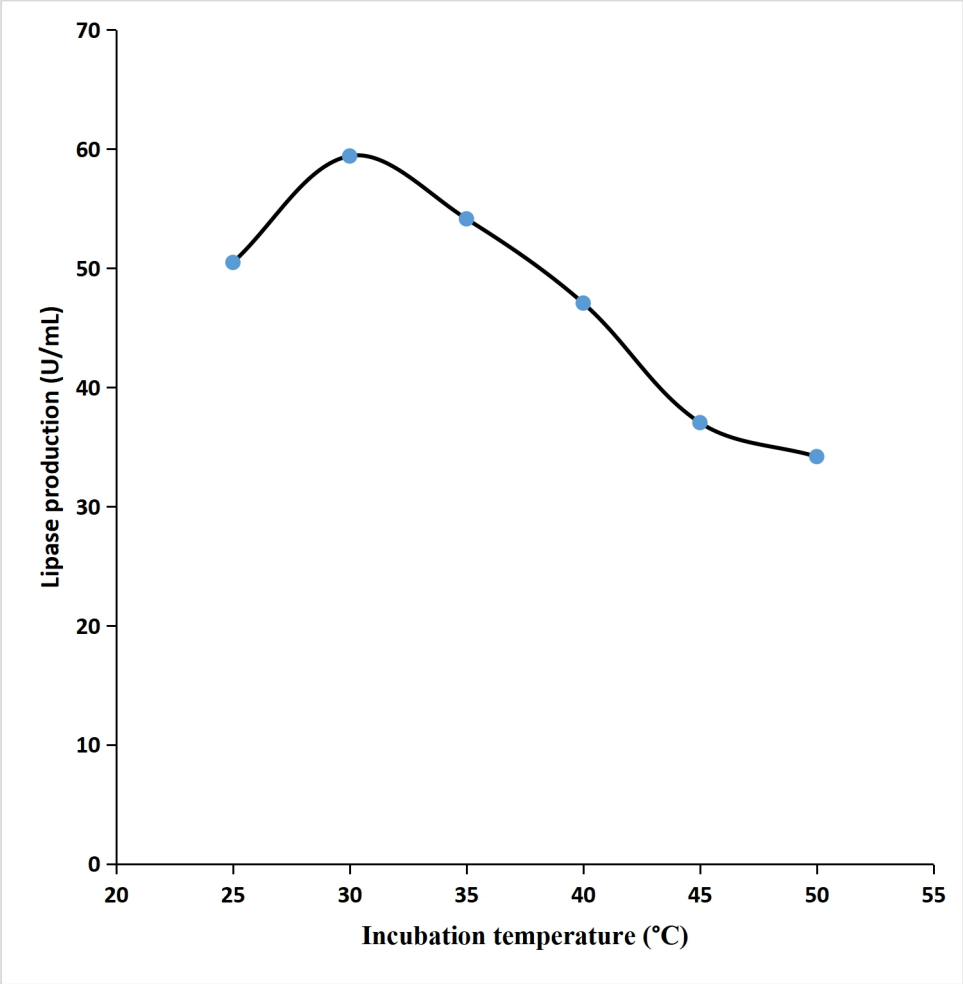

Figure 4.5: Effect of incubation temperature on lipase production by *T. reesei* APDI

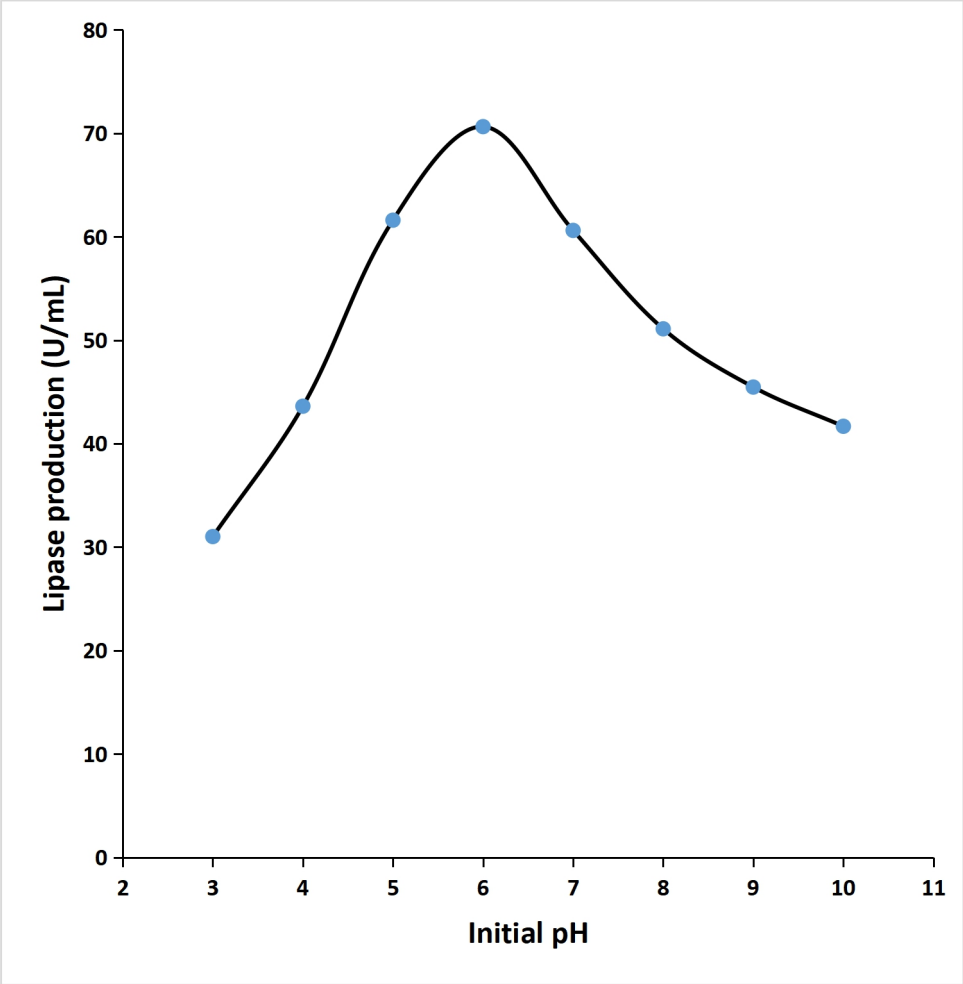

Figure 4.6: Effect of initial pH on lipase production by *T. reesei* APDI

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Lipase-producing fungi have already been found in environmental samples (Ramos-Sánchez *et al.,* 2015; Rihani and Soumati, 2019). Similarly, Akyil and Cihangir (2018) isolated lipolytic fungus in environmental samples. Musa and Adebayo-Tayo (2012) also reported 17 different fungal strains isolated from soil with the ability to produce lipases including *Trichoderma* sp. similar to this finding. Likewise, Yalcin *et al.* (2014) found similar results when they screened 120 filamentous fungi for lipase production from active samples of petroleum sludge, soil, and trash obtained from petroleum refineries and soils contaminated by petroleum. A good supply of lipase was found in twelve (12) of this fungus.

Extracellular lipase is abundant in fungi. The morphological and biochemical characterisation of the chosen isolate (APD1) in this investigation was comparable to Nwuche and Ogbonna's (2011) findings on *Trichoderma sp. Trichoderma* has previously been found to be an excellent source of lipase (Akyil and Cihangir, 2018; Wang *et al.,* 2018, Rantasalo *et al.,* 2019). Rihani and Soumati (2019) identified *T. reesei* isolated from oil-contaminated soil as a suitable source of lipase for industrial production, which is similar to our work. Ulker *et al.* (2011) isolated *T. reesei* from soil samples taken from several areas in Turkey, which exhibited good lipolytic activity.

Lipase expression is influenced by a number of variables. Media components such as carbohydrates and nitrogen, oils, fatty acids, and sugar esters are important contributors to lipase synthesis (Salihu *et al.,* 2011). Carbon or nitrogen sources, as well as their engrossments, have long piqued the interest of manufacturers and scientists seeking low-cost media composition (Singh *et al.,* 2016).

The choice of carbon source by fungi strains differs for optimum lipase production. The highest lipase activity was found in the presence of glucose as a carbon source in this research. Rehman *et al.* (2019) discovered comparable results for lipase production by *Pleurotus ostreatus* utilizing different carbon sources. Lipase activity peaked (2654 U/gds) when glucose was introduced as a carbon source. According to Rihani and Soumati (2019), *T. reesei* generated 1.58 U/mL of lipase when the medium was supplied with olive oil in the absence of glucose and Tween 80. Sugar substrates stimulate the development of

microorganisms but not the synthesis of lipase, according to Falony *et al.* (2006) and Ramos-Sánchez *et al.* (2015), whereas oleic acid and olive oil enhance the synthesis of lipase.

The highest lipase activity (128.01 U/mL) was reported with peptone as the nitrogen source. Ulker *et al.* (2011) found a comparable increase in lipase activity (1.25 g/L) when glucose and peptone were employed as carbon and nitrogen sources, respectively. Akeed and Al-halaby (2018) observed in a comparable study that peptone enhanced lipase production by 94.8 percent when compared to cultivation without any nitrogen supply. Kebabci and Cihangir (2012), on the other hand, observed that adding ammonium compounds boosted lipase production the greatest. Similarly, when ammonium sulphate was added to the fermentation medium, Lopes et al. (2016) discovered the greatest lipase activity (486 U/g).

At pH 6.0, the highest lipase production was reported (Figure 4.6). Brooks and Asamudo (2011) found similar results in their study of the effects of pH on lipase activity, finding that one of the isolates was most active at pH 6.5. Most enzymes lose their activity completely when their pH is too high or too low. The pH of enzymes is also a determinant in their stability. Each enzyme has a pH optimal stability area, similar to its activity. In line with this study, Jaiswal *et al*. (2017) reported the maximum lipase enzyme activity was produced at pH 6. The maximum lipase enzyme activity obtained was 8.5U/mL on 4th day at pH 6 media by *P. mirabilis.* When organic nitrogen sources are employed, such as peptone and yeast extract, which have been used for lipase production by several fungi, microorganisms produce significant yields of lipase. Kasra-Kermanshahi *et al.,* (2011) reported that among the various nitrogen sources used peptone (2 g/l) was found to be the most suitable source for maximum lipase activity.

At a temperature of 30°C, the highest level of lipase production was found. Similarly, Kulkarni and Gadre (2002) found that the highest lipase production (108.0 U/mL) occurred when the incubation duration was 30°C. At 35°C, the lipase enzyme was shown to have high activity, according to Gutarra *et al.,* (2009). Low temperatures also inhibit microorganism development, resulting in lower enzyme output (Kumar *et al.,* 2011; Oliveira *et al.,* 2016). As a result, understanding how temperature affects fungal lipase synthesis is critical. Numerous studies have indicated that unique optimal temperatures exist for specific fungal organisms, and that even a small change in the optimal temperature has a significant detrimental impact on yield. Temperature is a vital parameter, and it affects extracellular enzyme secretion by altering the physical characteristics of the cell membrane. According to Jaiswal *et al.* (2017), the maximum lipase enzyme activity obtained in all fermentation broth cultures at 37°C was 6.5-8.5U/mL.

5.2 CONCLUSION

In conclusion, samples of wastewater polluted soils were used for this experiment, cultured and screened for lipolytic fungi. Out of 12 isolates screened, APD1 isolate showed the highest growth, thus lipase activities on solid agar. The probable identity of the isolates as *Trichoderma reesei* was established based on its morphological and biochemical characteristics. Both glucose and yeast extract supported the highest lipase productions among the carbon and nitrogen sources investigated. Relatively, incubation temperature of 30℃ and pH 6.0 significantly increased lipase production. Isolate APD1 is a good source of lipase production and can be harness for further study with the aim for industrial production.

5.3 RECOMMENDATIONS

Based on the research carried out, the following recommendations were made:

- That *Trichoderma reesei* APD1 isolated from contaminated soil can produce lipase which can be harnessed for industrial purposes.
- That further strain development can be carried out on the isolate to increase its optimum production and further reduction in production cost for commercial purpose.
- Cheaper sources of carbon and nitrogen can be employed for further reduction in production cost.

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APPENDIX: STATISTICAL ANALYSIS

Table 1: Effect of carbon sources on lipase production by *Trichoderma reesei*

Carbon sources	Lipase production (U/mL)
Galactose	59.95[c]
Glucose	75.95[a]
Fructose	70.77[b]
Maltose	51.76[d]
Mannitol	57.52[c]
Sucrose	54.27[c]
Starch	37.07[e]

Table 2: Effect of nitrogen sources on lipase production by *T. reesei*

Nitrogen Sources	Lipase production (U/mL)
Peptone	76.16[a]
Yeast extract	68.99[b]
Urea	44.48[f]
Ammonium sulphate	64.51[c]
Sodium nitrate	55.66[d]
Potassium nitrate	52.24[e]

Table 3: Effect of incubation temperature on lipase production by *T. reesei*

Incubation temperature (°C)	Lipase production (U/mL)
25	50.48[c]
30	59.41[a]
35	54.14[b]
40	47.07[d]
45	37.04[e]
50	34.19[f]

Table 4: Effect of initial pH on lipase production by *T. reesei*

pH	Lipase production (U/mL)
3.0	31.02[e]
4.0	43.62[d]
5.0	61.60[b]
6.0	70.64[a]
7.0	60.61[b]
8.0	51.10[c]
9.0	45.47[d]
10.0	41.68[d]