SCREENING, PRODUCTION AND CHARACTERIZATION OF LIPASE FROM *Saccharomyces cerevisiae*

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES,

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DECLARATION

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

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AKINDELE, BLESSING OLUWADAMILOLA

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Date

CERTIFICATION

This is to certify that this research project titled "**SCREENING, PRODUCTION AND CHARACTERIZATION OF LIPASE FROM *Saccharomyces cerevisiae***" was carried out by Akindele, Blessing Oluwadamilola with matriculation number 17010101009. This project report meets the requirements governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology, Department of Biological Sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation

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Dr (Mrs) O.T. Kayode (Head of Department)

DEDICATION

I dedicate this research to Almighty God for the strength, wisdom and divine direction given unto me to carry out this project successfully.

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ABSTRACT

Lipases (E.C. 3.1.1.3) are hydrolytic enzymes with biotechnological potentials. They play important roles in the hydrolysis of triglycerides to free fatty acids and glycerol as commercial biocatalysts. In this study, lipase producing yeast isolated from palm wine and fermented fruits were screened by assessing various biochemical and physico-chemical parameters. The best isolate was identified, and it's morphological and biochemical properties were evaluated. The effect of different carbon sources, nitrogen sources, temperature and pH were investigated on the lipase production by the isolate. A total of 11 yeast species were isolated and screened for lipase production. Isolate BPWP2 produced the highest lipase activity and was identified as *Saccharomyces cerevisiae*. Carbon sources, nitrogen sources, incubation temperature and initial pH had significant effects ($p<0.05$) on lipase production. Glucose (75.55^a U/mL), yeast extract (88.05^a U/mL), 30℃ (79.50^a U/mL) and pH 6.0 (85.63^a U/mL) supported the optimum lipase production by the selected isolate.

In conclusion, the results of this study suggest conditions to ensure a high yield in the production of lipase enzyme using *Saccharomyces cerevisiae* isolated from the fermented fruits and palm wine.

Key words: Lipase production, carbon source, nitrogen source, temperature, *Saccharomyces cerevisiae*

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Lipases (E.C. 3.1.1.3) are water-soluble hydrolases that catalyse the hydrolysis of insoluble triacylglycerols to produce free fatty acids, diacylglycerols, monoacylglycerols, and glycerol. They catalyse a wide range of processes, including hydrolysis, transesterification, and interesterification of other esters, as well as ester synthesis, and have a variety of regio-, enantio-, and stereo-selective transformation characteristics (Gupta *et al*., 2007; Sharma *et al*., 2011; Svendsen, 2000). Lipase has emerged as a fantastic enzyme to catalyse dynamic reactions, with applications in organic chemical processing, detergent formulations, biosurfactant synthesis, oleochemical industry, dairy industry, agrochemical industry, paper manufacturing, nutrition, cosmetics, and pharmaceuticals (Martinelle *et al*., 1995; Liese *et al*., 2006). Lipases are found in plants, animals, and microbes and have a wide range of functions. Because of their functional ability at severe temperatures, pH, and stability in organic solvents, as well as chemo-selectivity, regio-selectivity, and enantio-selectivity, microbial lipases have gotten a lot more attention in the industrial world than those originating from plants and animals. Furthermore, they have higher production yields, are easier to genetically manipulate, and grow quickly on low-cost media, allowing for more fitting manufacturing (Thakur, 2012). Filamentous fungi are good extracellular lipase producers among microbial sources, and the extraction and purification process is quite simple. Because of their excellent stability and broad substrate specificity, they are widely used in industrial applications (Treichel *et al*., 2010; Gupta *et al*., 2003).

Enzyme-catalyzed reactions are typically carried out at mild circumstances, making them ideal substitutes for a variety of classical chemical reactions. Free enzymes, on the other hand, are typically unstable to pH, heat, and other conditions, making them difficult to recover and reuse (Kim *et al*., 2006). As a result, methods to improve enzyme stability and reusability are in high demand. Immobilization procedures have been extensively used to prepare a variety of high-performance and cost-effective biocatalysts with improved stability and resuability. Immobilized enzymes can work in a much wider pH and temperature range and have higher thermal stability than native enzymes due to multipoint, multi-subunit immobilisation or the creation of favorable

environments, which could be attributed to multipoint, multi-subunit immobilisation or the creation of favorable environments.

Saccharomyces cerevisiae known as brewer's yeast has been in existence since ancient times. The biotechnological importance of this organism in winemaking, baking, and brewing industries are indispensable. It is thought to have been isolated from grape skins. *S. cerevisiae* is a model organism that can be used in a variety of basic research projects. Several clinical and experimental research have indicated that *Saccharomyces cerevisiae var. boulardii* is effective in the prevention and treatment of a variety of gastrointestinal disorders, to varying degrees (Kelesidis *et al*., 2011). The most frequent yeast species in bread and sourdoughs is *S. cerevisiae*, usually known as baker's yeast or simply "the yeast" (Joseph and Bachhawat, 2014). Apart from the industrial use of *S. cerevisiae* in brewing and baking industries, its production of important metabolites such as enzymes has also been reported (Schousboe, 1976). As such, *S. cerevisiae* isolated in this study was used for the production of lipase. The effect of physico-chemical parameters on the isolate was also investigated.

1.1 Statement of Problem

Despite recent progress, the need to identify and isolate novel fungus strains that produce lipases with specific catalytic properties suitable for commercial processes remains a pressing concern. The cost of enzyme production has been the major challenges of industrialist, as such, calls for progressive study. Furthermore, understanding medium composition, which has a significant impact on microorganism growth and metabolite production, as well as the high cost of lipase production, has remained a difficulty in large-scale commercial production. In order to upscale the necessary lipase productivity, there is a need for a thorough examination of the existing fungus strain such as *Saccharomyces cerevisiae* as a lipase producer.

1.2 Justification

Continuous isolation and selection of novel strains has been energized by the cost-effective pursuit of new sources of lipases with diverse catalytic properties. Yeast has been identified as extraordinary microorganisms in our environment due to their release of beneficial products such as the lipase enzyme and their involvement in biodegradation and other bioactivities. They can

be found in a variety of places in the environment, and isolating and screening yeast with effective hydrolytic activity is a pressing need.

1.3 Aim and Objectives

The aim of this study is to produce lipase using *Saccharomyces cerevisiae*.

The objectives of the research include:

i. Isolation of yeast from fermented fruits and palm wine samples using pure culture techniques
ii. Screening of the isolates for lipase production and selection of the best lipase producer.
iii. Identification of the selected isolate using morphological and biochemical characterizations
iv. Physiochemical characterization of the selected isolate

CHAPTER TWO

LITERATURE REVIEW

2.1 LIPASES

Lipases are glycerol ester hydrolases (EC 3.1.1.3) that catalyse the hydrolysis of monoacylglycerols, diacylglycerols, and triacylglycerols to liberate free fatty acids and glycerol (Sheldon, 1993). They belong to the hydrolase fold superfamily and can function at both the aqueous and non-aqueous interface, which distinguishes them from esterases (Verger, 1997; Schmid and Verger, 1998). Lipases have recently received a lot of attention due to their ability to participate in a wide range of reactions such as hydrolysis, inter-esterification, alcholysis, acidolysis, esterification aminolysis, and so on, and these multifaceted enzymes are also playing a role in a variety of industrial applications such as dairy, detergents, medical and pharmaceutical, fat and oleo-chemical, food, cosmetic and perfume industry, and so on (Bjorkling *et al*., 1991; Ghosh *et al*., 1996).

Any enzyme that catalyses the hydrolysis of lipids (fats) is referred to as a lipase. Lipases are a subclass of the esterases. In most, if not all, living creatures, lipases play an important role in the digestion, transport, and processing of dietary lipids (e.g. triglycerides, fats, and oils). Lipase-encoding genes can even be found in viruses (Afonso *et al*., 1999). Most lipases (A1, A2, or A3) act at a specific point on the glycerol backbone of a lipid substrate (A1, A2, or A3) (small intestine). Human pancreatic lipase (HPL) (Winkler *et al*., 1990), for example, breaks down dietary fats in the human digestive system by converting triglyceride substrates found in ingested oils to monoglycerides and two fatty acids. Phospholipases (Diaz and Arm, 2003) and sphingomyelinases (Goni and Alonso, 2002) are two further forms of lipase activities seen in nature, however they are normally addressed independently from "traditional" lipases. During an infection, pathogenic microbes express and produce certain lipases. *Candida albicans*, forinstance, exhibits a variety of lipases, presumably showing broad-lipolytic activity, which may contribute to the virulence and persistence of *C. albicans* in human tissue (Hube *et al*., 2000).

Lipases are found in almost all animals and plants, and the majority of them flourish in microbes (Gupta *et al*. 2004, 2015; Nagarajan 2012). Temperature, pH, and substrate specificity all

influence microbial lipase activity, resulting in quick reaction times, which are crucial in commercial applications such as food processing, fine chemical synthesis, and biodiesel production. Natural bacterial and fungal strains, as well as their recombinant strains, provide the majority of enzymes utilized in biotechnological applications and organic chemistry(Gupta *et al*. 2004; Hasan *et al*. 2006; Nagarajan 2012; Thakur 2012). Extracellular bacterial lipases are the most commonly used commercial lipases because they are easier to produce in large quantities. *Rhizopus oryzae, Candida antarctica, Mucor miehei,* and *Pseudomonas cepacia*, among others, are important extracellular lipase producers (Gupta *et al*. 2015; Gog *et al*. 2012; Hasan *et al*. 2006). Only a few bacteria, such as Bacillus, Achromobacter, Arthrobacter, Alcaligenes, Chromobacterium, Burkholderia, and Pseudomonas, have been confirmed as commercial sources (Kapoor and Gupta 2012). Lipases from Pseudomonas bacteria are the most useful for a variety of biotechnological applications (Kapoor and Gupta 2012). Lipases from different origins have variable pH stability, organic solvent tolerance, and cold activity, and hence play a key role in converting various organic materials and biomass into usable products under conditions that other biomolecules would not tolerate (Maiangwa *et al*., 2015). Lipases are therefore frequently utilised in a variety of industries, including organic synthetic chemicals, detergents, food, feeds, paper, biodiesel production, perfumes, cosmetics, leathers, enantiopure medicines, and medical diagnostics (Aguieiras *et al*., 2015).

Many bacteria and higher eukaryotes manufacture lipases. Microbial lipases account for the majority of economically relevant lipases. Lipase-producing microbes have been discovered in a variety of environments, including industrial waste, vegetable oil processing plants, dairies, oil-contaminated soil, oilseeds, and decaying food, compost heaps, coal ash, and hot springs (Sztajer*et al*., 1988). Bacteria, fungi, yeasts, and actinomyces are all lipase-producing microbes. Lipase-producing microbes have been discovered in a variety of environments, including industrial waste, vegetable oil processing plants, dairies, oil-contaminated soil, oilseeds, and decaying food, compost heaps, coal ash, and hot springs (Wang *et al*., 1995).Lipases' interfacial activation normally takes place at the lipid-water contact, which is one of lipases' unique structural properties. The lipases' active site is protected by a helical oligopeptide unit. When this unit lid comes into contact with a hydrophobic interface, it goes through a series of modifications and exposes the active site, allowing unfettered access to the substrate (Villeneuve *et al*., 2000). The lipase active site is primarily made up of acyl enzyme complexes containing histidine,

serine, and aspartate. Lipases have received a lot of interest as biocatalysts in many commercial applications, notwithstanding their biological usage in plants, microorganisms, and higher animals. Lipases are commonly used in organic chemistry to catalyse the regio, chemo, and stereoselective hydrolysis of carboxylic acid esters. The current study focuses primarily on the practical applications of lipases in the revolution of organic processes, particularly in terms of overall methodological developments.

Filamentous fungi are known to produce high-quality lipases with excellent catalytic characteristics, which are critical in a wide range of industrial applications (Aravindan *et al*., 2007). They are known to produce lipase enzyme with more intensity and diversity in terms of enzyme characteristics and substrate specificity, which is a critical trait required to make the industrial process easier (Villeneuve *et al*., 2000). Lipases are able to hydrolyze ester bonds, transesterify triglycerides, resolve racemic mixtures, and synthesise the ester link in non-aqueous organic solvents orat the substrate-water interface, among other catalytic properties (Illanes *et al*., 2008). Though chemical procedures for accomplishing such catalysis are time-efficient, some are difficult to implement due to low yield and recovery, but enzyme-catalyzed alternatives, notably those involving fungal lipase, have proven to be feasible. Chemical, physical, and biological variables (temperature, pH, substrate, type of organic solvent) have been found to have a significant impact on lipase catalysed reactions (Antczak *et al*., 2009).

2.2 SOURCES OF LIPASES

Lipases are mostly obtained from the forestomach tissue of calves or lambs, as well as the pancreatic tissues of hogs and pigs. The presence of trypsin in pig pancreatic lipases, which results in bitter tasting amino acids, the existence of leftover animal hormones or viruses, and their unwanted effects in the processing of vegetarian or kosher meals are just a few of the drawbacks of utilising animal lipases (Vakhlu and Kour, 2006). Plant lipases are also accessible, however due to the yield and processes involved, they are not commercially exploited. As a result of their technological and economic advantages, microbial lipases are currently attracting increased attention, where the microorganisms are cultivated in medium with adequate nutrient content under regulated conditions (Srivastava, 2008). Lipase synthesis by microorganisms also varies depending on strains, growth medium composition, cultivation circumstances, pH, temperature, and carbon sources and nitrogen sources (Treichel *et al*., 2010; Souissi *et al*., 2009).

Although *Pseudomonas, Candida, Rhizopus, Mucor,* and *Geotrichum sp*. stand out as the principal commercially viable strains, yeast, bacteria, fungus, and actinomycetes are acknowledged as ideal sources of extracellular lipases, allowing enzyme recovery from the culture broth (Ertugrul *et al*., 2007).

2.3 Screening of Lipase Production

2.3.1 Direct screening (Qualitative)

Direct method is one of the best method for analysing fungal growth as well as the ability to produce extracellular enzymes has shown to be a solid agar plate. Furthermore, it is a handy fast way of evaluating individual fungus of genetic variations for the absence or presence of an enzyme like lipase (Hankins and Anagnostakis, 1975). Various recommended agar plate assays that are simple to execute have been created based on the substrates used (Griebeler *et al*., 2011). Furthermore, several researchers choose lipase-inducing substrates such as tributyrin, olive oil, Tween 20 and Tween 80. Tributyrin (C4:0), a triglyceride, is an excellent substrate because it disperses well in water without the use of emulsifiers when shaken or stirred. Tributyrin agar plate has been extensively utilized for detection as a strong surface-active material (Nwaguma *et al*., 2016). The hydrolysis of lipase may be identified by the creation of a clear zone surrounding the colonies on a Tributyrin agar plate, and this has allowed for the selection of powerful fungus (Gopinath *et al*., 2013). Kotogán *et al*. (2014) performed an extracellular lipolytic activity against Tributyrin screening of fungal strains, including some cold resistant isolates, and found that nineteen strains produced exceptional lipase. In addition to employing an agar plate directly for screening, the cup-plate method has been shown to be effective for screening putative fungal lipase producers. The lipase-containing production broth is cup-plated, and the results are examined according to the substrates used.

2.3.2 Indirect screening (Quantitative)

Fungus was cultivated on liquid medium supplemented with inducer substrate to investigate potential lipase producers. This process was used to find low producers of lipase that could not be located using the solid agar plate method. Furthermore, lipase activity varies in the solid agar plate method, with some strains showing fast growth and mycelium covering the halos generated by substrate lipolysis (Kotogán *et al*., 2014). It is required to grow the strains in liquid medium and compare the lipase activity of the filtrates, as the strains must be grown in liquid medium and the lipase activity of the filtrates must be compared. Sethi *et al*., (2013) discovered this by screening and selecting the best lipase-producing *Aspergillus species* using a solid agar media mixed with olive oil and combined with a dye. The media, on the other hand, were not suitable for the synthesis of real enzymes and could not clearly distinguish between cell-bound and extracellular activity. According to Rodrigues *et al*., (2016), who assessed extracellular lipase activity, quantitative screening has proven to be effective in identifying fungi that consistently produce lipase.

2.4 Methods of Lipase Production

2.4.1 Fungal lipases production in solid state fermentation

Solid state fermentation (SSF) is a microbial organism culture procedure that takes place in solid insoluble substrates with little or no water (Idrees and Rajoka, 2002). Due to recent breakthroughs in the field of biotechnology, it has sparked scholars' interest and attention. This has shown commendable ability and is recommended for fungal culture due to optimum production circumstances that are nearly identical to natural conditions (Nigam *et al*., 1994). Many researchers have used SSF for lipase synthesis because fungi are an appropriate source of extracellular lipase for most industrial uses. The ease of use, higher product recovery, low degree of catabolite repression and end-product inhibition, low waste water out, and good quality product are all factors that contribute to the popularity of this technology (He *et al*., 2004). Furthermore, lipases purified from the SSF method had much better thermal stability than those generated by the SMF technique (Sethi *et al*., 2016). Filamentous fungi have been found to be more responsive to SSF techniques, especially when it comes to lipase synthesis. This is owing to their hyphal way of fungal development, which makes them suited for bioconversion of solid

substrates due to their low moisture tolerance and high osmotic pressure condition (Colla *et al*., 2016). Given that culture medium accounts for 35-50 percent of overall output, numerous studies have indicated that using 11 agro-industrial leftovers as alternative substrates is adaptable to SSF and reduces the cost of lipase production while also addressing the problem of environmental contamination (Contesini *et al.*, 2010).

2.4.2 Fungal lipase production in submerged fermentation

The submerged fermentation process is a method of cultivating microbial organisms in a media that is generally liquid. It has shown certain benefits, including increased homogeneity of the culture media and the ability to manage variable parameters like temperature and pH (Pandey *et al*., 2000). Furthermore, extracellular enzyme recovery and biomass determination can be accomplished with simple filtration and centrifugation. Furthermore, using oil as a carbon source affects submerged fermentation, and olive oil is an expensive component of any lipase production medium. Because agro industrial waste is not frequently employed as a substitute carbon source in SMF, the basal medium was modified with extra natural oils. (Sethi *et al*., 2016). As a result, different natural oils have been used to create lipase by *A. lerreus*, with the results revealing that palm oil, sunflower oil, and almond oil provide high lipolytic activity.

2.5 TYPES OF LIPASES

2.5.1 Gastric lipase

Gastric lipase is an acidic lipase produced by gastric chief cells in the stomach's fundic mucosa. It thrives at a pH range of 3–6. Gastric lipase is one of two acidic lipases, along with lingual lipase. Unlike alkaline lipases (such as pancreatic lipase), these lipases do not require colipase or bile acid to function properly. Acidic lipases breakdown 30% of lipids in adult humans during digestion, with gastric lipase contributing the most of the two acidic lipases. In neonates, acidic lipases play a much larger role, accounting for up to 50% of total lipolytic activity.

Gastric lipase hydrolyzes the ester bonds of triglycerides in the stomach. Fatty acids and diacylglycerols are produced during this process. Gastric lipase may be hampered from hydrolyzing additional triglycerides if long-chain free fatty acids are present. In this case, gastric acid will be responsible for less than 30% of lipid hydrolysis (Pafumi *et al*., 2002) These

enzymes are found in the cytoplasm and cell membranes of stomach cells. The bulk of triglyceride hydrolysis does not require the use of gastric lipase.. It is essential for the hydrolysis of the membranes of milk fat globules (Bourlieu *et al*., 2020). In neonates with an undeveloped pancreas, LIPF plays a higher role in lipid digestion than in adults with a fully functional pancreas. LIPF production often increases when the pancreas is unable to operate at full capacity (Aloulou and Carrièrem, 2008). Low levels of LIPF are frequent in the tumors of gastric cancer patients (Kong *et al*., 2016).

2.5.2 Lingual lipase

The digestive enzyme lingual lipase belongs to the triacylglycerol lipases (EC 3.1.1.3) family, which uses the catalytic triad of histidine, aspartate, and serine to hydrolyze long-chain triglycerides andmedium into partial free fatty acids andglycerides. The enzyme catalyzes the first reaction in the digestion of dietary lipids, with diglycerides being the most common reaction result, and is then released into the mouth with saliva (Hamosh and Scow. 1973). The lipolytic activity of lingual lipase, however, continues through to the stomach due to its unique properties, which include a pH optimum of 4.5–5.4 and the capacity to catalyse processes without bile salts (Cleghorn and Shepherd, 1989). The autonomic nervous system tells the serous glands beneath the circumvallate and foliate lingual papillae on the tongue's surface to produce lingual lipase into the grooves of the circumvallate and foliate papillae, which are co-localized with fat taste receptors, to release lingual lipase (Chandrashekar *et al*., 2006). Because long-chain triacylglycerides cannot be absorbed, fat absorption by the small intestine requires hydrolysis of dietary fats. Within 1 to 20 minutes of intake, lingual lipase alone can hydrolyze up to 30% of fat. Lingual lipase is one of two acidic lipases, along with gastric lipase.

2.5.3 Pancreatic lipase

Pancreatic lipase is a pancreatic enzyme that is also known as pancreatic triacylglycerol lipase or steapsin. It is one of the most essential digestive enzymes in the human digestive system, hydrolyzing (breaking down) dietary fat molecules. It breaks down triglyceride substrates found in ingested oils into monoglycerides and free fatty acids (Peter *et al*., 1990). Bile salts, which are generated by the liver and stored in the gallbladder, are released into the duodenum, where they coat and emulsify big fat droplets, reducing their overall surface area and allowing lipase to break them up more effectively. The resultant monomers (two free fatty acids and one 2-

monoacylglycerol) are subsequently transported along the small intestine by peristalsis before being absorbed into the lymphatic system by a lacteal channel. Unlike some pancreatic enzymes that are activated by proteolytic cleavage, pancreatic lipase is released in its final state (e.g., trypsinogen). However, it is only effective if colipase is present in the duodenum. In humans, the PNLIP gene codes for pancreatic lipase (Davis *et al*., 1991). Pancreatic lipase is secreted into the duodenum via the pancreatic duct system. Its serum concentration is generally quite low. When pancreatic function is severely disrupted, such as by pancreatitis or pancreatic cancer, the pancreas may begin to autolyze and release pancreatic enzymes into the bloodstream, including pancreatic lipase. Acute pancreatitis can thus be identified by measuring pancreatic lipase levels in the blood (Koop, 1984).

2.5.4 Lipoprotein lipase

The lipase gene family includes pancreatic lipase, hepatic lipase, and endothelial lipase. The lipase gene family includes lipoprotein lipase (LPL) (EC 3.1.1.34). It's a water-soluble enzyme that breaks down lipoprotein triglycerides into two free fatty acids and one monoacylglycerol molecule from lipoprotein triglycerides. Chylomicrons and very low-density lipoproteins contain it (VLDL). It also helps cells absorb chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids (Mead *et al*., 2002). LPL requires ApoC-II as a cofactor (Kim *et al*., 2006).

LPL is attached to the luminal membrane of endothelial cells in capillaries by the proteins glycosylphosphatidylinositol HDL-binding protein 1 (GPIHBP1) and heparan sulfated peptidoglycans. The largest quantities are seen in adipose, heart, and skeletal muscle tissue, as well as nursing mammary glands (Wong and Schotz, 2002). The LPL gene codes for lipoprotein lipase, which is found in the heart, muscle, and adipose tissue. LPL is a homodimer that functions as a ligand/bridge factor for receptor-mediated lipoprotein uptake as well as a triglyceride hydrolase. Through catalysis, VLDL is converted to IDL and then to LDL. Severe LPL deficiency mutations induce Type I hyperlipoproteinemia, but less severe LPL mutations are linked to a variety of lipoprotein metabolism issues. Lipoprotein lipase deficiency causes hypertriglyceridemia (elevated levels of triglycerides in the bloodstream). LPL overexpression has been shown to cause insulin resistance and obesity in mice (Ferreira *et al*., 2001).

2.6 FACTORS AFFECTING MICROBIAL LIPASE PRODUCTION

2.6.1 Carbon substrate source

Given that the composition of the culture medium can influence lipase synthesis, several studies have found that oil as a carbon source is the most important influencing element in fungal lipase production (Bindiya and Ramana, 2014). Because lipases are abundantly synthesised upon induction, oil carbon sources have always been the most important determinant in lipase activity expression (Tweens, Olive oil, corn oil). Other inducers' roles in lipase synthesis are unknown, as is the overall mechanism of stimulation (Gupta *et al*., 2004; Large *et al*., 1999).

When glucose was used as the only carbon source, no enzyme activity was identified, indicating that the presence of an inducer is required for *Penicillium aurantiogriseum* to create lipases (Lima *et al*., 2003). The microorganism's need for sugar as a carbon source, in addition to lipids, is different. However, Ghosh *et al*. found that medium enriched with glucose and fats increase lipase synthesis in *Rhizopus nigricans* (1996). At 0.1 and 0.7 percent (v/v), respectively, olive oil and Tween-80 increased the synthesis of extracellular lipase in Penicillium citrinum (Maliszewska and Mastalerz, 1992). Tween-80 promotes both lipase biosynthesis and secretion in *C. elegans*, according to Dalmau *et al*. (2000). The yeast *Pseudozymahubeiensis* HB85A lipase was greatly active by 150.8 percent in the presence of Tween-80 as compared to non-Tween-80 supplemented media (Bussamara *et al*., 2010).

2.6.2 Nitrogen source

Lipase synthesis has traditionally relied on both organic and inorganic nitrogen sources. By adding 20 g/L peptone to the production medium of *Aspergillus wentii*, *Mucor racemosus*, and *Ralstonia nigricans*, the yield of lipase was enhanced (Ghosh *et al*., 1996). Inorganic nitrogen supplies like ammonium phosphate, on the other hand, appear to encourage *Rhodotorula glutinis* to produce lipase (Papaparaskevas *et al*., 1992). Salleh *et al*. (1993) discovered that thermophilic fungi, *R. oryzae*, produced the most extracellular lipase when the medium was supplemented with peptone as the nitrogen source. According to Rajendran and Thangavelu, lipase production in *R. arrhizus* requires both peptone and yeast extract (2009). However, for *C. cylindracea* NRRL Y-17506 (Brozzoli *et al*., 2009) and *P. citrinum*, an inorganic nitrogen supply in the form of NH4Cl was shown to be the best (Miranda *et al*., 1999). Corn steep liquor and soybean meal

increased lipase production in *P. citrinum* to a smaller amount than peptone, however urea and ammonium sulphate hindered lipase synthesis (Sztajer and Maliszewska, 1989).

2.6.3 Inorganic minerals

Different microbes require different inorganic elements for their development and lipase synthesis. Inorganic salts in form of MgSO4, (NH4)2SO4, NaCl, K2HPO4, BaCl2 are necessary for maximal lipase synthesis by *Hendersonula toruloidea* (Odibo *et al*., 1995). The optimum culture medium for lipase production in *Candida sp*. 99-125 was found to contain (w/v) soybean oil 4.187 percent, soybean powder 5.840 percent, K2HPO4 0.284 percent, KH2PO4 0.1 percent, (NH4)2SO4 0.1 percent, MgSO4 0.05 percent, and Span 60 0.1 percent, and the absence of any of these components affected the organism's growth and lipase activity (He and Tan, 2006). Magnesium salt is required by most bacteria because it has the ability to play regulatory roles in adenosine triphosphate metabolism and nucleic acid production (Bankar *et al*., 2009). Potassium, which has been proven to be required for osmoregulation in yeast strains, is another requirement for microbial development. Calcium was revealed to be crucial for appropriate lipase stabilization and activity in *Acinetobacter sp*. (Snellman and Colwell, 2004). Irons are mostly used for heme and cytochrome synthesis (Venkateshwar *et al*., 2010).

2.6.4 Temperature and pH

The majority of lipase-producing organisms are mesophilic (growing at a temperature between 25 and 40°C). On the other hand, psychrophilic and thermophilic species have been documented in the literature. After 72 hours of incubation in the presence of abundant residue from the babassu oil business, a wild-type Brazilian strain of *P. simplicissimum* generated lipase with an activity of 90 U/g. The enzyme was discovered to have high activity at temperatures ranging from 35 to 60℃ (Gutarra *et al*., 2009). *Acinetobacter sp., Photobacterium lipolyticum, Achromobacter lipolyticum, Aeromonas hydrophila, Bacillus sphaericus, Morexella sp., Pseudomonas fragi, Pseudomonas fluorescens, Serratia marcescens, Psychrobacter okhotskensis,* and *Staphylococcus epidermidis* have all been reported (Joseph *et al*., 2007). In general, the temperature necessary for lipase synthesis is similar to the temperature required for most microbes to flourish.

The pH of a medium, which is a measure of its acidity or basicity, is crucial in defining the kind of organisms that can colonise it. The majority of lipases active at extremely acidic pH (pH 1.5 to 2.0) are from mammals, such as gastric lipase.

2.7 APPLICATION OF FUNGAL LIPASE

Lipase has seen a spike in popularity in industrial applications due to their inherent adaptability (Geoffry and Rajeshwara, 2018). Due to the flexibility of their features and simplicity of mass manufacturing, fungal lipase is a prominent group of biotechnologically important enzymes (Singh and Mukhopadhyay, 2012). Many researchers have looked at the use of fungal lipases in industry (Treichel *et al*., 2010).

2.7.1 Lipase in pharmaceutical industry

In the pharmaceutical sector, enzymes offer numerous advantages over chemical synthesis, which is why lipase is in such high demand (Singh and Mukhopadhyay, 2012). Mild conditions that prevent isomerization, epimerization, recemisation, and rearrangement processes, as well as enantio and region selectivity and immobilised lipase reuse, are among the advantages. The pharmaceutical industry is actively utilising lipases' ability to overcome racemic mixtures via production of a single enantiomer for medication development.

Pharmaceutical items are made from medicinal mushrooms (Mohammed *et al*., 2011). *Agaricusbrasiliensis, Ganoderma lucidum, Lentinula edodes, Coriolus versicolor, Pleurotusostreatus, Grifolafrondosa, Termitomyces*, and other vital mushrooms having pharmacological qualities include *Agaricusbrasiliensis, Ganoderma lucidum, Lentinula edodes, Coriolus versicolor, Pleurotusostre* (Ganeshpurkar *et al*., 2019). Fungi contain immunosuppressive, antibacterial, antiviral, nematic, and hypocholesterolemic compounds, among other physiologically active chemicals (Hyde *et al*., 2019).

2.7.2 Lipase in detergency

New detergents with various enzyme formulations have been continuously appearing for various applications, particularly in the United States, Europe, and Japan, utilising various enzyme systems such as lipase, protease, amylase, and cellulase. The enzyme must be stable at higher pH and temperatures if it is to be employed in detergent composition. As a result, the formulation includes alkaline lipases and proteases (Khoo and Ibrahim, 2003). A lipase from the Humicola

strain was discovered to be capable of dissolving fatty stains, according to Novo Nordisk. Molecular approaches enabled the gene coding for this lipase to be cloned and introduced into *A. oryzae*, which produced the enzyme in commercially relevant yields and aided in superior washing performances with energy savings (Hasan *et al*., 2006). Alkaline lipases are produced by Bacillus sp. B207 and *Pseudomonas paucimobilis*, which can be employed as additions in detergent formulations (Khoo and Ibrahim, 2003). In the pH and temperature ranges of 7.0 to 9.0 and 30 to 50°C, respectively, both lipases demonstrated outstanding pH and temperature stability. About 1000 tonnes of lipases are added to the approximately 13 billion tonnes of detergents generated each year, according to Jaeger and Reetz (1998).

2.7.3 Lipase in food processing industries

Lipase is a protein that is utilised in the food processing industry to modify and break down biomaterials (Singh and Mukhopadhyay, 2012). Fats and oils are crucial food elements, and their modification is one of the key areas in the food processing sector where innovative economic and green technologies are needed (Gupta, 2003). A vast number of fat-clearing lipases are produced on an industrial basis. Most commercial lipases are used to increase the flavour of milk products and other foods such as meat, vegetables, fruit, smoked carp, milk products, baked goods, and beer products (Mehta *et al*., 2017). In bakery products, lipases from *Aspergillus niger*, *Rhizopus oryzae*, and *Candida cylindracea* have been employed (Singh and Mukhopadhyay, 2012).

2.7.4 Lipase in textile industry

In the textile industry, the use of fungus 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). High strength, softness, stretch, washability, machine abrasion, stain, and wrinkle resistance are all advantages of polyester in the textile business. Synthetic fibres have been enzymatically changed for use in the development of yarns, garments, rugs, and textiles. It entails modifying the characteristics of polyester fibres in such a way that they are resistant to post-modification treatment (Singh and Mukhopadhyay, 2012).

2.7.5 Lipase in waste treatment

Lipases are used in activated sludge and other aerobic waste processes to remove thin layers of fat from aerated tank surfaces to allow oxygen to flow. This skimmed fat-rich liquid is digested by lipase, such as that from *C. rugosa* (Mehta *et al*., 2017). Lipases also help anaerobic digesters work as they should (Su *et al*., 2016). Successful solids breakdown and the removal and avoidance of fat clogging or films in waste systems are crucial in many industrial activities (Islam and Datta, 2015). Through batch and continuous-flow studies, Jeganathan *et al*. (2009) evaluated the hydrolysis of wastewater with high oil and grease (O&G) concentration from the pet food industry using immobilised *C. rugosa* lipase (CRL) as a pretreatment step for anaerobic treatment.

2.8 CHALLENGES OF LIPASE PRODUCTION

Researchers from all over the world have teamed up to increase fungal lipase production by looking at the impact of the production medium and other operational variables, with the goal of improving extracellular lipase yield (Treichel *et al*., 2010). Despite recent developments, the cost of lipase production remains high, with only minimal efficiency in the industrial sector (Yong *et al*., 2016). As a result, it's vital to enumerate and investigate the impact of physico-chemical factors on fungal lipase synthesis in order to broaden the scope and, in turn, improve the condition of production and workability of fugal lipase.

2.9 YEAST

Yeasts are single-celled eukaryotic microorganisms that belong to the fungus kingdom. The first yeast appeared hundreds of millions of years ago, and there are now over 1,500 varieties of yeast (Kurtzman and Fell, 2006). They are thought to account for 1% of all known fungal species (Kurtzman and Pikur, 2006). Yeasts are unicellular creatures that developed from multicellular predecessors (Yong, 2012), with certain species able to generate multicellular traits by generating pseudohyphae or fake hyphae, which are strings of connected budding cells (Kurtzman and Fell, 2005). Yeast diameters vary significantly depending on species and environment, although most yeasts are 3–4 m in diameter, with some reaching 40 m. (Walker *et al*., 2002). Most yeasts reproduce asexually by mitosis, and many of them use the asymmetric division mechanism known as budding to do it. Molds produce hyphae, whilst yeasts have single-celled development habits. Fungal species that may take both forms are known as

dimorphic fungi (depending on temperature or other factors).*Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols during the fermentation process. *S. cerevisiae* is a well-studied eukaryotic microbe that serves as a model organism in current cell biology research. Researchers have cultivated it to learn more about the biology of eukaryotic cells and, ultimately, human biology (Ostergaard *et al*., 2000). Other yeast species, like *Candida albicans*, are opportunistic or harmful pathogens that can infect people. Yeasts have lately been employed to create ethanol for the biofuel business and to generate power in microbial fuel cells. Yeasts are not classified into a single taxonomic or evolutionary category. Although the term "yeast" is frequently used interchangeably with *Saccharomyces cerevisiae* (Kurtzman, 1994), yeasts are classified into two distinct phyla: the Ascomycota and the Basidiomycota. The budding yeasts, often known as "true yeasts," are classified in the order Saccharomycetales under the phylum Ascomycota. Yeasts are chemoorganotrophs, which means they derive their energy from organic compounds instead of sunlight. Carbon is largely derived from hexose sugars like glucose and fructose, as well as disaccharides like sucrose and maltose. Pentose sugars, such as ribose (Barnett, 1975), alcohols, and organic acids are metabolized by some species. Yeast species are either obligatory aerobes (those that require oxygen for aerobic cellular respiration) or anaerobic (those that use oxygen for energy synthesis) (facultative anaerobes). Unlike bacteria, no known yeast species can solely thrive in anaerobic conditions (obligate anaerobes). Most yeasts thrive in a pH range of neutral to slightly acidic. Yeasts have several temperature ranges in which they thrive. *Leucosporidium frigidum*, for example, thrives at 2℃ to 20℃ (28 to 68 degrees Fahrenheit), *Saccharomyces telluris* at 5℃ to 35℃ (41 to 95 degrees Fahrenheit), and *Candida slooffi* at 28℃ to 45℃ (82 to 113 degrees Fahrenheit) (Arthur and Watson, 1976). Under some conditions, the cells can survive freezing, although their viability decreases over time.

Yeasts are often cultured in the lab on solid growth media or in liquid broths. Yeast culture media include potato dextrose agar or broth, Wallerstein Laboratories nutritional agar, yeast peptone dextrose agar, and yeast mould agar or broth..Dried malt extract and agar are commonly used as a solid growth medium by home brewers who produce yeast. Exposure to air is the most common cause. Although it is innocuous, it can impart a disagreeable flavor to pickled vegetables and must be removed on a frequent basis during fermentation (Kaufmann and Schoneck, 2002).

2.9.1 Ecology

Yeasts can be found in abundance in nature and are usually isolated from sweet substances. Plant exudates and naturally occurring yeasts on the skins of fruits and berries (such as grapes, apples, and peaches) are examples (such as plant saps or cacti). Some yeasts can be found on insects or in the soil (Suh *et al*., 2005). Yeasts' ecological function and biodiversity are relatively unknown when compared to other microbes (Herrera and Pozo, 2010). *Rhodotorula rubra, Torulopsis, Candida albicans,* and *Trichosporon cutaneum* have all been found in the skin flora between people's toes (Oyeka and Ugwu, 2002). Yeasts can also be found in the intestinal flora of humans and insects, and even deep-sea settings (Kutty and Philip, 2008). Yeast killer toxins are proteins produced by some strains of yeasts that allow them to destroy competing strains. This might cause problems in the winemaking process, but it could also be leveraged to the winemaker's benefit by using killer toxin-producing strains. Yeast killer toxins may have medical benefits in the treatment of yeast infections (Magliani *et al*., 2006).

Marine yeasts, which are yeasts isolated from seawater rather than freshwater, can develop faster on a seawater-based medium than on a freshwater-based medium (Zaky *et al*., 2014). In 1894, Bernhard Fischer discovered the first marine yeasts in the Atlantic Ocean, which he named *Torula sp*. and *Mycoderma sp.* (Kutty *et al*., 2008). Following this discovery, marine yeasts have been isolated from a number of sources around the world, including seawater, seaweeds, marine fish, and marine mammals (Zaky *et al*., 2016). Some yeast isolates from terrestrial settings were introduced to and survived in marine conditions (grouped as facultative marine yeast). The remaining marine yeasts were classified as obligatory or indigenous marine yeasts since they only live in marine environments *(Kutty et al., 2008)* Marine yeast has been successfully employed to manufacture bioethanol on seawater-based media, potentially lowering the bioethanol's water impact (Zaky *et al*., 2018).

2.9.2 Reproduction

Some yeast isolates from terrestrial settings were introduced to and survived in marine conditions (grouped as facultative marine yeast). The remaining marine yeasts were classified as obligatory or indigenous marine yeasts since they only live in marine environments.

Marine yeast has been successfully employed to manufacture bioethanol on seawater-based media, potentially lowering the bioethanol's water impact (Zaky *et al*., 2018). Some yeasts, such as *Schizosaccharomyces pombe*, reproduce via fission rather than budding, resulting in two similarly sized daughter cells (Balasubramanian *et al*., 2004). In general, haploid cells perish in high-stress settings like starvation; however, diploid cells can go through sporulation, which

involves sexual reproduction (meiosis) and the formation of a variety of haploid spores that can eventually mate (conjugate), reuniting the diploid cell (Neiman, 2005). *Schizosaccharomyces pombe*, a haploid fission yeast, is a facultative sexual microbe that can mate when nutrients are scarce (Davey, 1998). When *S. pombe* is exposed to hydrogen peroxide, a chemical that produces oxidative stress and oxidative DNA damage, mating and the generation of meiotic spores are strongly induced (Bernstein and Johns, 1989).

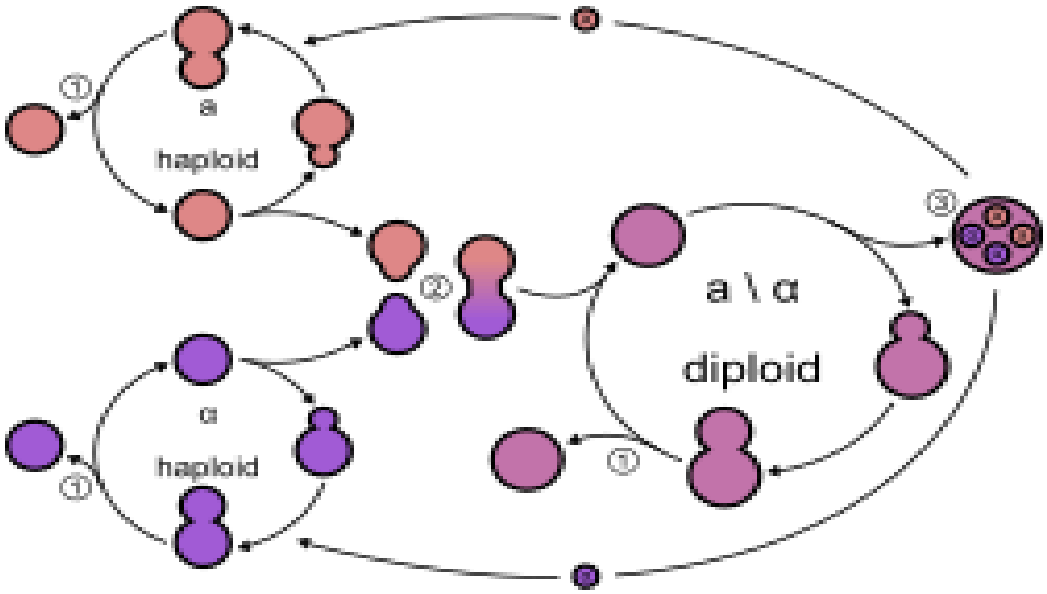

Figure 2.1: Diagram of yeast cell's life cycle (Source: Wikipedia)

Budding, Conjugation, and Spore are all part of the yeast cell's life cycle. When nutrients are abundant, the budding yeast *S. cerevisiae* reproduces as diploid cells by mitosis, but when nutrients are short, the yeast goes through meiosis to produce haploid spores through meiosis (Herskowitz, 1988). Mitosis allows haploid cells to proliferate asexually. In wild *S. cerevisiae* populations, Katz Ezov (Katz *et al*., 2010) provided evidence that clonal reproduction and selfing

(in the form of intratetrad mating) predominate. Out-crossing is uncommon in nature since haploid cells are normally married to make diploid cells by members of the same clonal population (Ruderfer *et al*., 2006). Out-crossing happens only once every 50,000 cell divisions, according to an analysis of the lineage of spontaneous *S. cerevisiae* strains (Ruderfer *et al*., 2006).

Some yeasts have the potential to be useful in the field of bioremediation. *Yarrowia lipolytica*, for example, has been found to breakdown palm oil mill effluent, TNT (an explosive), and other hydrocarbons such alkanes, fatty acids, lipids, and oils (Zinjarde *et al*., 2014). It can also withstand high salt and heavy metal concentrations (Bankar *et al*., 2009), and its use as a heavy metal biosorbent is being researched (Bankar *et al*., 2009). *Saccharomyces cerevisiae* has the capacity to bioremediate hazardous contaminants from industrial effluents such as arsenic (Soares and Soares, 2012). Certain types of yeast have been found to destroy bronze statues (Cappitelli and Sorlini, 2008). Different yeasts from Brazilian gold mines bioaccumulate free and complexed silver ions (Singh, 2006). Nutritional supplements, particularly those sold to vegans, include yeast. When offered as a dietary supplement, it is commonly referred to as "nutritional yeast." Nutritional yeast is usually *S. cerevisiae* that has been deactivated. It's naturally low in fat and sodium, as well as a source of protein and vitamins, including most B-complex vitamins (though without fortification, it doesn't contain much vitamin B12), as well as other minerals and cofactors needed for growth (Thaler and Safferstein, 2014). Some, but not all, kinds of nutritional yeast are supplemented with vitamin B12, which is made separately by bacteria (Duyff, 2012).

Some yeast species are opportunistic pathogens, meaning they can infect people with compromised immune systems. In immunocompromised people, *Cryptococcus neoformans* and *Cryptococcus gattii* are important pathogens. They are the primary cause of cryptococcosis, a fungus that affects around one million HIV/AIDS patients and kills over 600,000 people each year (Cogliati, 2013). These yeast cells are encased in a hard polysaccharide capsule that helps them avoid being recognized and ingested by white blood cells in the human body (O'Meara and Alspaugh, 2012). Human candidiasis is caused by yeasts of the genus Candida, which are opportunistic pathogens that cause oral and vaginal infections. Candida is a commensal yeast found in humans and other warm-blooded animals' mucous membranes. These same strains,

however, can occasionally become pathogenic. A hyphal extension from the yeast cells penetrates the mucosal membrane, causing pain and tissue loss (Deacon, 2008). *C. tropicalis, C. stellatoidea, C. krusei, C. glabrata, C. parapsilosis, C. albicans, C. guilliermondii, C. lusitaniae, C. viswanathii,* and *Rhodotorula mucilaginosa* were listed in declining order of virulence for humans in a book from the 1980s. *Candida glabrata*, after *Candida albicans*, is the second most prevalent Candida pathogen, producing infections of the urogenital tract and bloodstream (candidemia) (Brunke and Hube, 2013). *C. auris*was only recently discovered. Yeasts can develop in meals with a low pH (5.0 or less) and easily digestible carbon sources such as sugars, organic acids, and other carbohydrates (Kurtzman, 2006). As part of their development, yeasts digest some dietary components and make metabolic end products. As a result, the physical, chemical, and sensory characteristics of food are altered, and the food is spoiled. Yeast growth on food surfaces, such as cheeses or meats, and sugar fermentation in beverages and semiliquid products, such as syrups and jams, are both frequent. The genus Zygosaccharomyces has a long history as rotting yeasts in the food business. This is because these species can thrive in high quantities of sucrose, ethanol, acetic acid, sorbic acid, benzoic acid, and sulfur dioxide, all of which are common food preservation methods. The presence of living yeast cells is detected using methylene blue (Downes and Ito, 2001). *Brettanomyces bruxellensis* is the most common spoiling yeast in oenology.

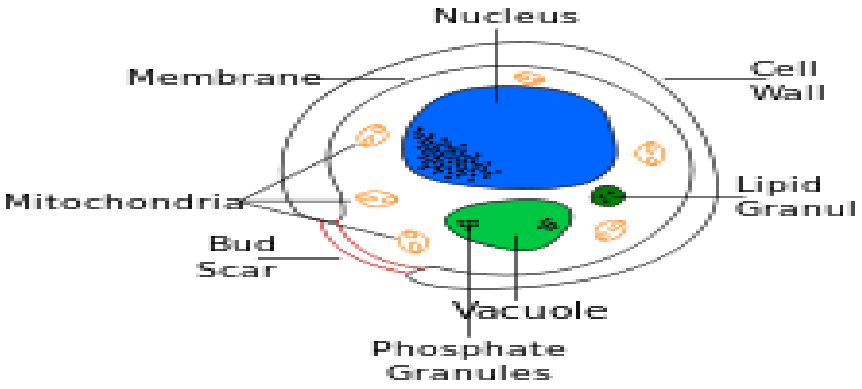

Figure 2.2: Diagram of a Yeast Cell (Source: Wikipedia)

2.10*Saccharomyces cerevisiae*

The yeast *Saccharomyces cerevisiae* is a type of yeast (single-celled fungus microorganisms). Since ancient times, the species has been used in winemaking, baking, and brewing. It is thought to have been isolated from grape skins. It, like the model bacteria *Escherichia coli*, is one of the most studied eukaryotic model organisms in molecular and cell biology. The most prevalent type of fermentation is caused by thisbacterium. "Saccharomyces" is a Latinized Greek word that means "sugar-mold" or "sugar-fungus," with saccharon (v) meaning "sugar" and myces () meaning "fungus." cerevisiae is a Latin word that means "of beer." The cells of *S. cerevisiae* range in size from spherical to ovoid, with a diameter of 5–10 m. The ideal temperature for *S. cerevisiae* growth is 30–35°C (86–95 °F) (Stefanini*et al*., 2012). It multiplies by budding (Feldmann and Horst 2010). The first step in finding cell cycle proteins, signaling proteins, and protein-processing enzymes was to look for their yeast homologs. Berkeley bodies, which are engaged in specific secretory pathways, are only found in *Saccharomyces cerevisiae*, the only yeast cell known to have them. Antibodies against *S. cerevisiae* can be identified in 60–70% of Crohn's disease patients and 10–15% of ulcerative colitis patients (and 8 percent of healthy controls) (Walker *et al*., 2004). The proline and ornithine present in yeast are predecessors of the 2-Acetyl-1-pyrroline, a roast-smelling odorant, in the bread crust, and *S. cerevisiae* has been discovered to contribute to the fragrance of bread (Struyf and Nore, 2017). *Saccharomyces cerevisiae* (*S. cerevisiae*) is a unicellular fungus with 12068 kilobases (kb) of nuclear genomic DNA arranged into 16 chromosomes sequenced the entire genome and discovered that it contains roughly 6000 genes, of which 5570 are projected to be protein-encoding genes (Wood *et al*., 2001). Bioinformatic investigations have indicated that a number of protein-encoding genes are of foreign origin, as defined by lateral gene transfer by Doolittle, 1999 (Doolittle, 1999). These genes are either prokaryotic or eukaryotic in origin and entered *S. cerevisiae's* genome horizontally (Hall *et al*., 2005). Because of its osmotrophic feeding style and the presence of powerful cell walls, cell- and intracellular membranes, this came as a surprise at first. *S. cerevisiae* is a model organism that can be used in a variety of basic research projects. In contrast to other model organisms like *E. coli* or *Caenorhabditis elegans*. *S. cerevisiae* is also a very important species for a number of industrial uses. One aspect of its life style, dubbed "make-accumulate-consume," is one of the key reasons for this attribute (Thomson *et al*., 2005). This trait is based on the Crabtree effect, which asserts that even under aerobic conditions, *S.*

cerevisiae does not use the respiratory machinery to metabolize saccharides and promote biomass development, but instead uses pyruvate to produce ethanol and other two-carbon molecules (Pronk *et al*., 1996). *S. cerevisiae* is a kind of yeast. Yeast is a single-celled fungus that divides asexually through budding or fission and has a big diameter of 5–10m and a small diameter of 1–7m. *S. cerevisiae* cells are pigmented, and in surface-grown colonies, a cream tint can be seen (Walker and White, 2011). In terms of structure and function, yeast cells are completely different from bacterial cells.

2.10.1 Uses of *S.cerevisiae* in medicine

In both humans and animals, *Saccharomyces cerevisiae* is employed as a probiotic. A strain of *Saccharomyces cerevisiae var. boulardii*, in particular, is industrially produced and clinically used as a medicine. Several clinical and experimental research have indicated that *Saccharomyces cerevisiae var. boulardii* is effective in the prevention and treatment of a variety of gastrointestinal disorders, to varying degrees (Kelesidis *et al*., 2011). *Saccharomyces cerevisiae var. boulardii* has been found to lower the risk of antibiotic-associated diarrhea in adults and children (Szajewska and Kolodziej 2015), as well as the risk of *Helicobacter pylori* eradication therapy side effects (Szajewska *et al*., 2015). Limited data also supports *Saccharomyces cerevisiae var. boulardii's* efficacy in the prevention (but not treatment) of traveler's diarrhea and, at least as an additional drug, (McFarland and Lynne 2010) in the treatment of acute diarrhea in adults and children, as well as persistent diarrhea in children. It may also help with allergic rhinitis symptoms.

2.10.2 Saccharomyces species

Previously isolated Saccharomyces species of industrial potentials included: *S. bayanus, S. bonlardii, S. bulderi, S. cariocanus, S. cariocus, S. cerevisiae, S. chevalieri, S. dairenensis, S. ellipsoideus, S. eubayanus, S. exiguous, S. florentinus, S. kiuyveri, S. monacensis, S. norbensis, S. paradoxus, S. pastorianus, S. spencerorum, S. martiniae, S. turicensis, S. uvarum, S. unisporus, S. zonatus* (Walker, 2009).

Table 2.1: Taxonomic hierarch of yeast *S. cerevisiae*

Domain	**Eukarya**
Kingdom	Fungi
Division	Ascomycota
Subdivision	Saccharomycotina
Class	Saccharomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Sub family	Saccharomycetoideae
Genus	Saccharomyces
Species	*Cerevisiae*

2.11 Application of *Saccharomyces cerevisiae*

2.11.1 In the wine industry

The discovery of calcium salts of tartaric acid and terebinth resin in a pottery jar, about 5400–5000 BC (McGovern *et al*., 1996), was the first experimental evidence for the presence of wine in Iran. Cavalieri *et al.* (2003) discovered ribosomal DNA from *S. cerevisiae* in an Egyptian wine jar going back to 3150 BC, demonstrating that wine and *S. cerevisiae* have a long history together. This link was initially identified in 1860, when Louis Pasteur first uncovered the "hidden" world of yeast activity during wine fermentation, and then in 1890, when Müller-Thurgau proposed the controlled wine fermentations with starter cultures process. (Marsit and Dequin, 2015). This unique approach, which gained widespread adoption in the 1970s after almost a century, transformed the wine business and improved wine quality by providing better control and, as a result, higher repeatability and reliability of fermentations (Marsit and Dequin, 2015). Non-Saccharomyces species, as well as a variety of *S. species*, were used as starters. Cerevisiae strains greatly increased physicochemical properties, the composition and concentration of the wine's volatile elements, i.e. flavor, aroma of the finished product, glycerol content, and others. Ciani *et al*., 2016 (Ciani *et al*., 2016) examined what had been done up to that point.

2.11.2 In the bread industry

Making bread is one of the world's oldest biochemical processes. Although there is evidence that yeast was used to create bread as early as 10.000 BCE, the first archaeological evidence for leavened breads was discovered in Egypt in the second millennium BC and in North Western China in the first millennium BC. Bread was generally baked at home until the Middle Ages, when population growth prompted the construction of communal mills and ovens, as well as the development of professional bakers (Heitmann *et al*., 2018). The most frequent yeast species in bread and sourdoughs is *S. cerevisiae*, usually known as baker's yeast or simply "the yeast" (Joseph and Bachhawat, 2014) Baker's yeasts were acquired from the residues of the beer manufacturing process and have been utilized as a starting culture since the 19th century (Nielsen, 2019). The first compressed yeasts for baking and brewing were developed in England in 1792, and they were available in northern Europe by 1800. In 1868, a compressed yeast of a better strain was introduced in the United States, enabling for large-scale bread production

(Menezes *et al*., 2015). Water, Flour, and sourdough must be mixed together to make bread. Depending on the culture and geographic area, flours such as wheat, barley, einkorn, emmer, khorasan, spelt, rye, teff, maize, or sorghum were used, whereas sourdough was a mixture of flour and water containing fermenting yeast and Lactic Acid Bacteria (LAB) (Carbonetto *et al*., 2018). *S. cerevisiae* is typically inoculated into bread dough at a concentration of 2% of total components. In a matter of minutes, the yeast cells consume the oxygen contained in the dough during mixing, and under the anaerobic conditions that arise, yeast cell multiplication slows down and the fermentation process happens. Fresh cells need more time to ferment than older cells, hence the best temperature for dough fermentation is around 34–38 °C, pH 4.0–5.2. When fat, salt, or spices are added, yeast may proliferate more slowly (Hidalgo and Brandolini, 2014).

2.11.3 In the chocolate industry

Theobroma cacao beans are the principal raw material used in the production of chocolate (Schwan and Whaels, 2004**)**. Raw cocoa beans, on the other hand, are inedible because they are bitter and astringent, and their aroma and flavors are not those of chocolate; as a result, they are fermented to reduce bitterness and astringency, as well as develop flavors that determine the fine organoleptics of cocoa and chocolate (Aprotosoaie *et al*., 2016).

2.11.4 In the brewing industry

Saccharomyces cerevisiae is a top-fermenting or top-cropping yeast that is used in the brewing of beer. Its hydrophobic surface causes the flocs to stick to CO2 and ascend to the top of the fermentation vessel during the fermentation process. Top-fermenting yeasts ferment at higher temperatures than lager yeast *Saccharomyces pastorianus*, resulting in beers with a distinct flavor profile from those fermented with lager yeast. If the yeast is exposed to temperatures near 21 °C (70 °F) or if the beverage's fermentation temperature varies during the process, "fruity esters" may occur. Lager yeast ferments at a temperature of around 5 °C (41 °F), at which point *Saccharomyces cerevisiae* goes dormant. *Saccharomyces cerevisiae var. diastaticus,* a yeast variation, is a beer spoiler that can produce secondary fermentations in packaged products.

CHAPTER THREE

METHODOLOGY

3.1Materials and Equipment

The following materials and equipment were used for the experiment: petri dishes, cover slips, glass slides, distilled water, test tube, conical flask, inoculating loop, beaker, spirit lamb, aluminum foil, measuring cylinder, McCartney bottle, syringe, water bath, incubator, autoclave.

3.2 Culture Media and Reagent

The media used during the experiment were: Potato Dextrose Agar (PDA), and Tributyrin Agar. The reagents used during the experiment include: diluted methylene blue, Alcohol (70% ethanol), rhodamine blue indicator, Tween solution, Saline solution (2g/L KH_2PO_4, 1g/L $MgSO_4$, $FeSO_4.7H_2O$ (0.63), Sodium Hydroxide (NaOH), Hydrochloric Acid (HCL).

3.3 Isolation of Yeast Species

3.3.1 Sampling

The fruit samples of Orange, Pineapple, and Mango as well as Palm wine were bought from local market and kept for two days before culturing. The samples were taken to the laboratory for examination and subsequent analysis.

3.3.2 Serial dilution

Serial dilution was performed for the samples (Rajeshkumar *et al*., 2013). 1 mL of the samples were placed in a sterile test tube with 9mL of distilled water. Five-fold serial dilutions were prepared aseptically. The sterile test tubes were taken and labeled per dilutions ranging from $10^{-1}$to 10^{-5}. 1.0 mL of the first dilution blank (10^{-1}) was added to the second dilution blank (10^{-2}). The tubes were shaken and serial dilution done till the last tube dilution (10^{-5}). 0.1 mL of the diluents was inoculated using the pour plate method in a disposable petri dish with Potato Dextrose Agar (PDA).

3.3.3 Preparation of potato dextrose agar

Potato Dextrose Agar (PDA) was utilized for fungal cultivation. 39g of Potato dextrose agar (according to the manufacturer's instructions) was dissolved in 1 liter of distilled water. Cotton wool wrapped with aluminum foil was used to plug the mouth of conical flask before sterilization. The mixture was heated for a short time to completely dissolve the powder before autoclaving at 121°C, 15 lb for 15 minutes. The medium was then cooled to 45– 50°C before pouring into sterile petri dishes and allowed to solidify. Chloramphenicol antibiotics were added to the medium to prevent bacterial growth.

3.3.4 Culturing

Pour plate method was used to culture the fungal colonies on PDA. 0.1 mL from the serially diluted samples (10^{-1}, 10^{-3} and 10^{-5}) was poured into the sterile petri plates. The petri plates were gently shake for uniform distribution of the sample. After few minutes, the molten Potato Dextrose Agar was poured gently into the respective petri plates. After solidification, the plates were incubated at room temperature for 3-5 days. The sterility of the media was confirmed by incubating an uninoculated agar plate labeled as the control (Wadia *et al*., 2017). Distinct yeast colonies were selected from the heterotrophic colony and were transferred using a sterilized inoculating loop into a new culture medium to get a pure culture. The plates were then incubated and stored in a plastic bag at 4^{o}C.

3.4 Screening of lipolytic yeast species

Tributyrin agar (HiMedia) of pH7.5, 10.0 mL and 990 mL distilled water was utilized. All of the isolated yeast cultures were inoculated into TBA plates and cultured for 7 days at 27 degrees Celsius. Lipase synthesis by the organisms is 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 4^{o}C.

3.5 Identification of the screened isolate

After screening, the potent fungal cultures were inoculated on Potato Dextrose Agar and incubated at room temperature for identification and further studies.

3.5.1 Morphological identification

The morphological characteristic of the selected isolate grown on PDA was studied for growth rate, growth pattern, colony texture and pigmentation (Promputtha *et al*., 2005).

3.5.2 Biochemical identification

A single colony of yeast was placed on a glass slide with a drop of sterile distilled water and spread until the smear dried off. After that, the smear was stained with diluted methylene blue dye, air dried, and examined under a light microscope at a magnification of 100 times.

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 7.5The Fermentation was carried out using a shaker incubator of 100 rpm (Remi, India).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.

Lipase activity (U/mL) = $\frac{(\Delta ABS) * (V_R)}{\varepsilon * V_E * t} \text{X } 10^3$

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 of carbon sources, nitrogen sources, temperature and pH on the lipase produced by *S. cerevisiae* were determined.

3.8.1 Effect of carbon sources on lipase production

Under submerged fermentation, the influence of, glucose, galactose, fructose, maltose, sucrose, mannitol, and starch as carbon sources was examined on lipase synthesis. The production medium had 10 g/L carbon source, KH2PO4 (0.2 percent w/v), MgSO4 (0.1 percent w/v), and 2 percent yeast extract as nitrogen source inside 300 mL Erlenmeyer's flasks containing 50g 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. The fermented brans were stored at -20°C until they were needed.

3.8.2 Effect of nitrogen source on lipase production

The effect of yeast extract, peptone, ammonium sulphate, sodium nitrate, potassium nitrate and urea as nitrogen source was investigated on lipase production under the submerged fermentation. The production medium was similar to the one described above containing 10g/L wheat bran, 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. The fermented brans were kept at -20℃ until use.

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^{o}C 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 effects 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 ^{o}C 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 calculated and displayed as means of duplicates. ANOVA was used to analyze the data, with the Duncan Multiple Range Test for significance set at $P \leq 0.05$. There was no indication of the standard deviation. Tables were also used to convey the data.

CHAPTER FOUR

RESULTS

4.1Colony count

Eleven (11) morphologically different yeast were isolated. Table 4.1 showed the colony count of10^{-1}, 10^{-3} and 10^{-5} serial dilutions for the fermented fruits and palm wine from different locations from different local market. Orange, pineapple, mango and palm wine samples ranged53-142, 41-137, 69-108 and 53-117 respectively.

4.2 Morphological characterization of the isolates

From table 4.2, the isolates were identified based on their color, shape and edge. The isolates were white and cream in color, circular and rhizoid in shape, and had entire edges and the elevations were raised and convex. The surfaces of the colonies were observed and the colonies appeared dull. BPWP2 isolate was identified as a fast-growing yeast, maturing in 3 to 5 days. The isolate had a creamy color and was opaque, smooth, fluffy, and regular colony. Under a light microscope with a magnification of 100 times (lOOx), isolate BPWP2 had an ellipsoid or ovoid shape, which was typical of *Saccharomyces cerevisiae* (Greame, 2005). Fifteen yeast colonies had a similar ability to budding, with 4-5 cells budding out of ten.

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

Samples	**10^{-1}**	**10^{-2}**	**10^{-3}**
Orange	142	87	53
Pineapple	137	78	41
Mango	108	92	69
Palm wine	117	81	53

Table 4.2: Morphological Characterization of the Isolates

Isolates	Color on PDA	Shape	Edge	Elevation	Surface
BOP1	Cream	Circular	Entire	Raised	Dull
BOP2	Cream	Circular	Entire	Raised	Dull
BOP3	White	Circular	Entire	Convex	Dull
BPP1	Cream	Circular	Entire	Convex	Dull
BPP2	Cream	Circular	Entire	Raised	Dull
BPP3	Cream	Circular	Entire	Raised	Dull
BPWP1	White	Circular	Entire	Raised	Dull
BPWP2	Cream	Circular	Entire	Raised	Dull
BPWP3	Cream	Circular	Entire	Convex	Dull
BMP1	Cream	Circular	Entire	Raised	Dull
BMP2	Cream	Circular	Entire	Raised	Dull

4.3 Screening of fungal isolates for lipase activity

Table 4.1 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 BPWP2 had the highest diameter of growth while isolate BOP1 had the lowest. Moreover, there was a significant difference ($p \geq 0.05$) 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 BPWP2 had the highest lipase activity while isolate BMP1 had the lowest activity. Four (4) isolates including BOP3, BPP2, BPWP1 and BMP2 did not show any lipase activity throughout the incubation period. Isolate BPWP2 exhibited excellent lipase activity and was selected for further study.

4.4 Identification of the selected isolate

The surfaces of the colonies were observed and the colonies appeared dull. BPWP2 isolate was identified as a fast-growing yeast, maturing in 3 to 5 days. The isolate had a creamy color and was opaque, smooth, fluffy, and regular colony. Under a light microscope with a magnification of 100 times (lOOx), isolate BPWP2 had an ellipsoid or ovoid shape, which was typical of *Saccharomyces cerevisiae* (Greame, 2005).

Table 4.3: Screening of yeast isolates for lipase production

Isolates	Diameter of growth (mm)	Lipase activity (mm)
BOP1	23.0[k]	5.0[e]
BOP2	30.0[j]	9.0[b]
BOP3	43.0[f]	0.0
BPP1	31.0[i]	4.5[f]
BPP2	41.0[g]	0.0
BPP3	51.0[b]	8.0[c]
BPWP1	41.0[g]	0.0
BPWP2	53.0[a]	10.5[a]
BPWP3	32.0[h]	10.5[a]
BMP1	48.0[d]	2.0[g]
BMP2	50.0[c]	0.0

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

4.5 Effects of physico-chemical parameters on the selected isolate

4.5.1 Effect of carbon sources on lipase production

Figure 4.1 shows the effect of various carbon sources on lipase production by *S. cerevisiae* BPWP2. There was significant difference ($p<0.05$) in lipase production by the isolate. Lipase production ranged from 47.27^{g}– 75.55^{a}U/mL. Glucose (75.55^{a}U/mL) supported the highest production of lipase by *S. cerevisiae* BPWP2 follow in order by galactose (69.15^{b} U/mL), and fructose (63.07^{c} U/mL) while starch (47.27^{g}U/mL) gave the least support.

4.5.2 Effect of nitrogen sources on lipase production

Figure 4.2 shows the effect of the different inorganic and organic nitrogen sources on the activity of extracellular lipaseproduced by*S. cerevisiae* BPWP2. There was significant difference($p<0.05$) in lipase production in the presence of different organic and inorganic nitrogen sources. Lipase production ranged from 54.11^{f}– 88.05^{a} U/mL. Yeast extract (88.05^{a} U/mL) supported the highest production of lipase by *S. cerevisiae* BPWP2 follow in order by peptone (86.66^{b} U/mL), and ammonium sulphate (74.31^{c} U/mL) while urea (54.11^{f} U/mL) gave the least support.

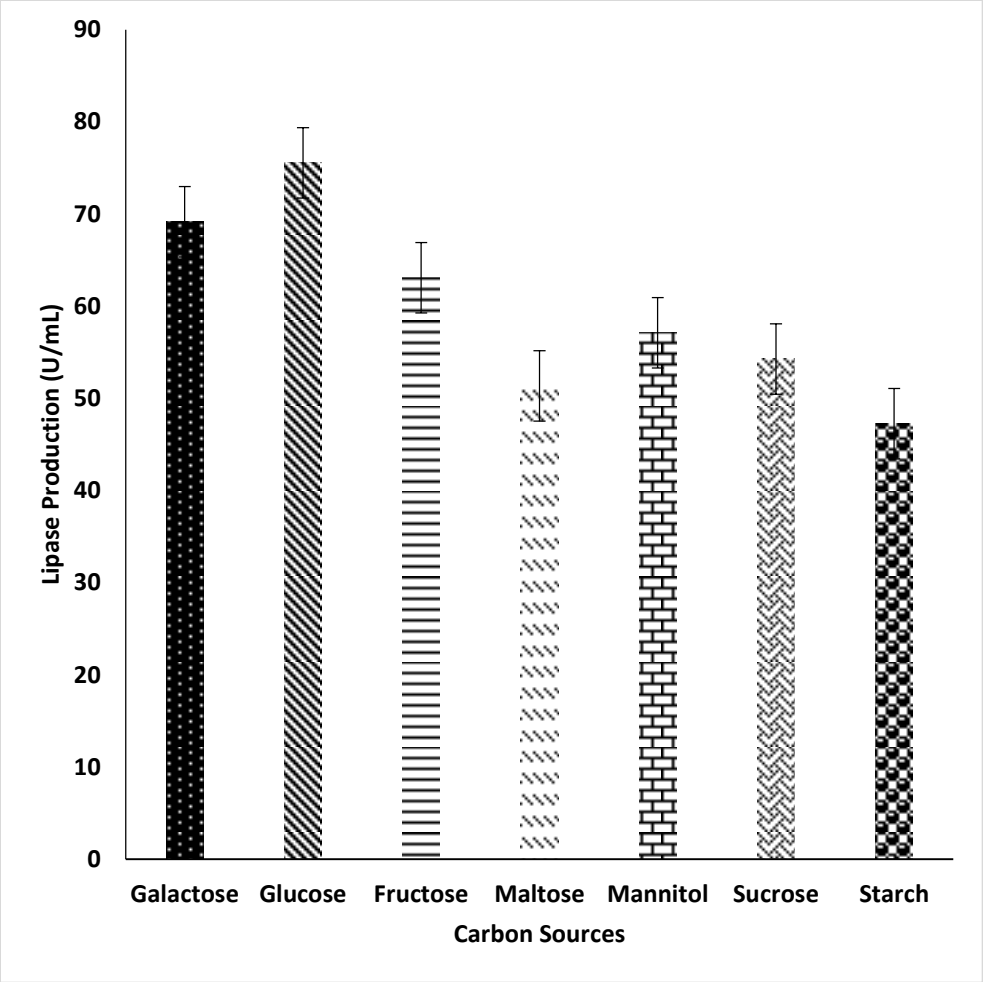

Figure 4.1: Effect of carbon sources on lipase production by BPWP2 isolate, *S. cerevisiae*

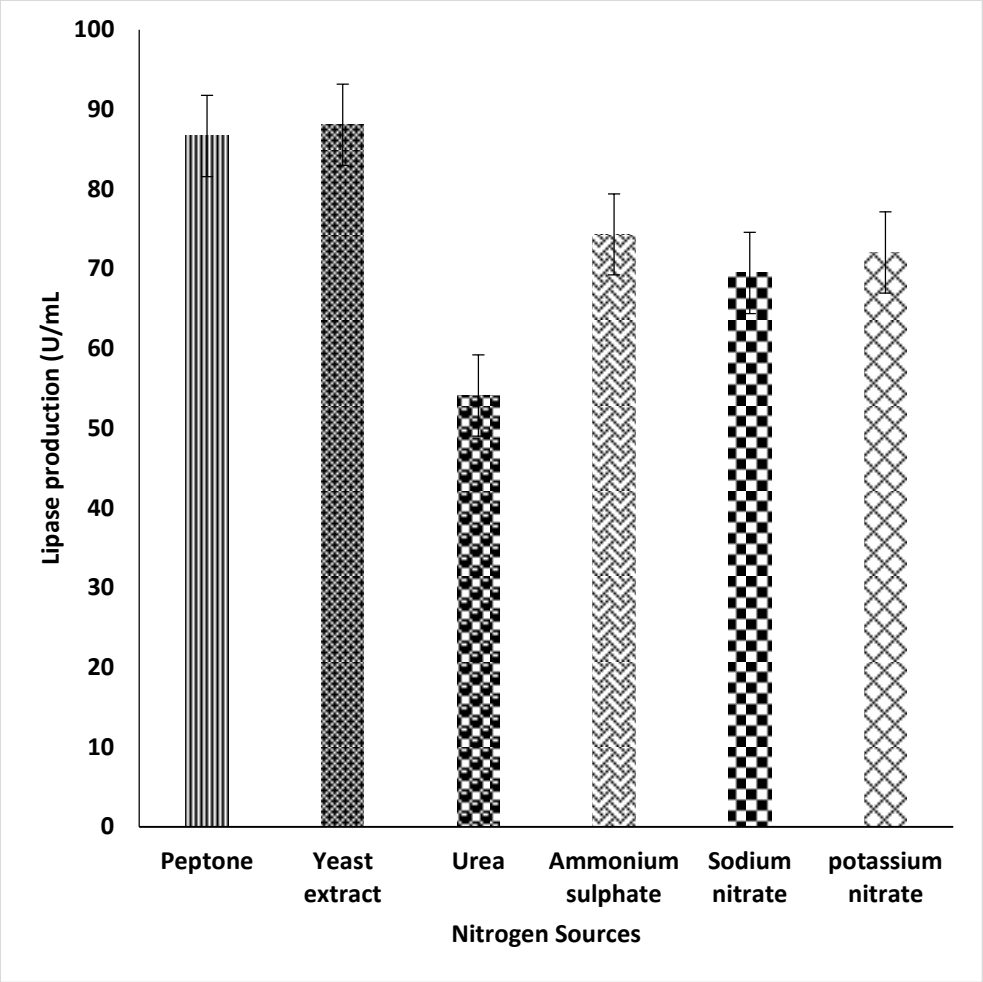

Figure 4.2: Effect of nitrogen sources on lipase production by BPWP2 isolate, *S. cerevisiae*

4.5.3 Effect of temperature on lipase activity

Figure 4.3 shows the effect of the different temperature on the activity of extracellular lipase produced by *S. cerevisiae* BPWP2. There was significant difference ($p<0.05$) in lipase production in the presence of different temperature. Lipase production ranged from 34.28^{f} - 79.50^{a} U/mL. Temperature 30℃ (79.50^{a} U/mL) supported the highest production of lipase by *S. cerevisiae* BPWP2 follow in order by 25℃ (70.57^{b} U/mL), 35℃ (64.23^{c} U/mL) while 50℃ (34.28^{f} U/mL) gave the least support.

4.5.4 Effect of pH on lipase activity

Figure 4.4 shows the effect of the different pH on the activity of extracellular lipase produced by *S. cerevisiae* BPWP2. There was significant difference ($p<0.05$) in lipase production in the presence of different pH. Lipase production ranged from 31.03^{h}-85.63^{a} U/mL. pH 6 (85.63^{a} U/mL) supported the highest production of lipase by *S. cerevisiae* BPWP2 follow in order by pH 7 (80.62^{b} U/mL), pH 8 (64.23^{c} U/mL) while pH 3 (31.03^{h} U/mL) gave the least support.

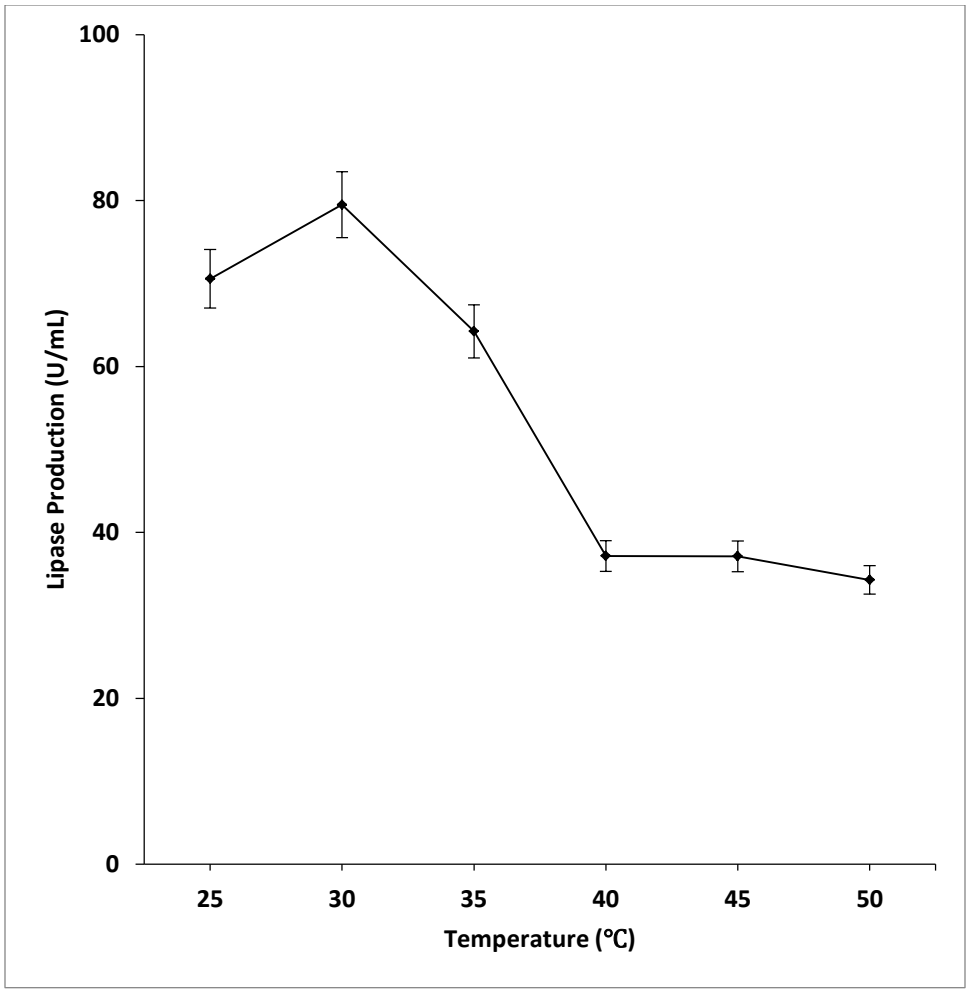

Figure 4.3: Effect of incubation temperature on lipase production by *S. cerevisiae* BPWP2

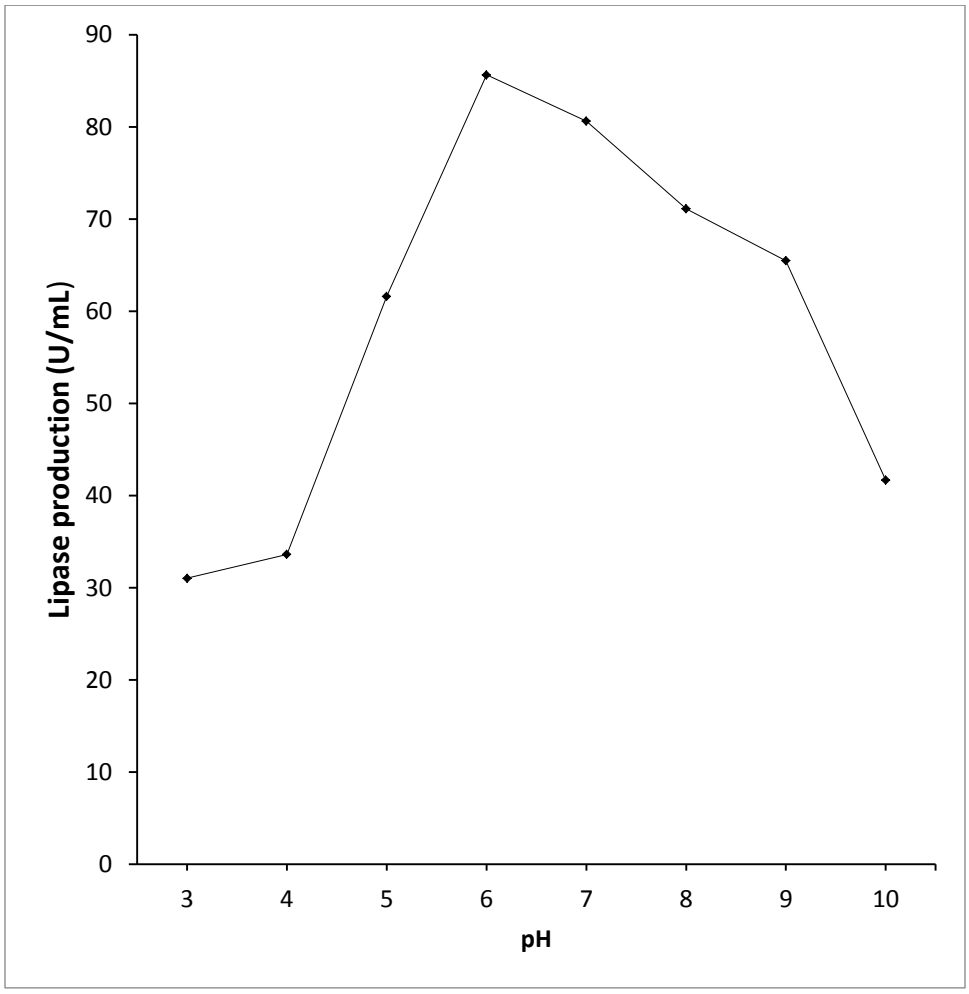

Figure 4.4: Effect of initial pH on lipase production by *S. cerevisiae* BPWP2

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Lipase expression is influenced by a number of factors. 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 always attracted the interest of manufacturers and scientists seeking low-cost media formation (Singh *et al*., 2016). In this study, 11 fungi were isolated from the fermented fruits and palm wine samples with potential for lipase synthesis. The morphological and biochemical characterization of the selected isolate, BPWP2, in this investigation was similar to Kevin's (2005) report, which stated that typical *Saccharomyces cerevisiae* colonies were creamy and had a regular colony shape. According to Kockova (1990), Cavalieri *et al.* (2001), and Kuthan *et al.*, (2003) colonies generated by wild *Saccharomyces cerevisiae* cells have a fluffy appearance (2003). The absence of an extracellular matrix, the compactness of the colony, and the changes in cell shape within the colony all played a role in the morphological differences of yeast colonies (Kuthan *et al*., 2003). Therefore, the isolate, BPWP2, is *Saccharomyces cerevisiae.*

The effect of the various carbon sources on enzyme synthesis varies. The maximum lipase activity (156.42^{a} U/mL) was found in the presence of glucose as a carbon source in this investigation. Rehman *et al*. (2019) found similar results using different carbon sources for lipase synthesis by *Pleurotusostreatus*; when glucose was used as a carbon source, lipase activity reached its peak (2654 U/gds). Also, Rihani and Soumati (2019) observed that *Trichoderma harzianum* produced 1.58 U/mL of lipase when the medium was supplemented with olive oil but without glucose and Tween 80. When compared to the control media, studies on the effect of sugars added as an extra carbon source had no influence on lipase synthesis. The synthesis of lipase was suppressed by galactose, maltose, and sucrose. Other sugars tested reduced lipase enzyme synthesis as compared to the control media. Falony *et al*. (2006) and Ramos-Sánchez *et al*. (2015) found that sugar substrates promote the development of microorganisms but not the synthesis of lipase, whereas oleic acid and olive oil promote the synthesis of lipase.

With yeast extract as the nitrogen source, the highest lipase activity (88.05[a] U/mL) was reported (Figure 4.2). Ulker *et al*. (2011) found that employing peptone as a nitrogen source resulted in the highest lipase activity (1.25 g/L). Lipase synthesis has traditionally relied on both organic and inorganic nitrogen sources. In a similar study, Akeed and Al-halaby (2018) discovered that peptone increased lipase synthesis by 94.8 percent when compared to cultivation without any nitrogen source. However, Kebabci and Cihangir (2012) discovered that adding ammonium compounds increased lipase synthesis the most. Similarly, when the fermentation medium was added with ammonium sulphate, Lopes *et al*. (2016) found the highest lipase activity (486 U/g).

At pH 6.0, the highest lipase production (85.63[a]U/mL) was reported (Figure 4.4). 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 (79.50[a] U/mL) was found (Figure 4.3). Similarly, Kulkarni and Gadre (2002) found that the greatest 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.

5.2 CONCLUSION

In conclusion, samples of fermented fruits (mango, pineapple and orange) and palm wine were used for this experiment, cultured and screened for lipolytic yeast. Out of 11 isolates screened, BPWP2 isolate showed the highest growth and lipase activities on solid agar. The probable identity of the isolates as *Saccharomyces cerevisiae* 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 BPWP2 is a good source of lipase production and can be harness for further study with the aim for industrial production.

5.3 RECOMMENDATIONS

In this study, lipase was produced from *S. cerevisiae* BPWP2. Based on this research, I hereby make the following recommendations:

- *S. cerevisiae* BPWP2 can be harnessed for lipase production for industrial usage
- Lipase production from *S. cerevisiae* BPWP2 can be used for the production of biotechnologically important products such as detergents, soaps and so on.
- Further strain improvement can be carried out on the organism for better production so as to meet the expected commercial purposes.

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APPENDIX

STATISTICAL ANALYSIS OF RESULTS

Table 4.4: Effect of carbon sources on lipase production by *S. cerevisiae* BPWP2

Carbon sources	**Lipase production (U/mL)**
Galactose	69.15[b]
Glucose	75.55[a]
Fructose	63.07[c]
Maltose	51.36[f]

Mannitol	57.12[d]
Sucrose	54.27[e]
Starch	47.27[g]

Table 4.5: Effect of nitrogen sources on lipase production by *S. cerevisiae* BPWP2

Nitrogen Sources	Lipase production (U/mL)
Peptone	86.66[b]
Yeast extract	88.05[a]
Urea	54.11[f]
Ammonium sulphate	74.31[c]
Sodium nitrate	69.47[e]
potassium nitrate	72.04[d]

Table 4.6: Effect of incubation temperature on lipase production by *S. cerevisiae* BPWP2

Incubation temperature (^{o}C)	Lipase production (U/mL)
25	70.57[b]
30	79.50[a]
35	64.23[c]
40	37.16[d]

45	37.13[e]
50	34.28[f]

Table 4.7: Effect of initial pH on lipase production by *S. cerevisiae* BPWP2

Ph	**Lipase production (U/mL)**
3.0	31.03[h]
4.0	33.63[g]
5.0	61.61[e]
6.0	85.63[a]
7.0	80.62[b]
8.0	71.11[c]
9.0	65.48[d]
10.0	41.69[f]