SCREENING AND PRODUCTION OF LIPASE FROM *Streptomyces sp.*

BY

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CERTIFICATION

This is to certify that this research project titled **"SCREENING AND PRODUCTION OF LIPASE FROM *Streptomyces* sp"** was carried out by ABBA, Praise Oluwatobiloba, with Matriculation number **17010101012.** This report meets the requirements governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that the project report was written under the supervision of Dr. G. E. Adebami and it is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and the list of references provided. This researched project report has not been presented for the award of any degree.

Abba Praise O. ______________________

Signature and Date

DEDICATION

This project is dedicated to the Almighty God, the giver of life, and to my parents, Mr. and Mrs. Abba.

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I am greatly indebted to God, for his marvelous doings, his faithfulness and his unconditional love towards me and for his provision over my life.

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ABSTRACT

Lipases (EC 3.1.1.3) is a triacylglycerol acylhydrolase that works on carboxylic ester linkages. They belong to the class of serine hydrolases and do not require any cofactor. In this study, bacterial isolates from domestic wastewater contaminated soils 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 15 bacterial species were isolated and screened for lipase production. Isolate PSN07 showed the highest lipase activity and was identified as *Streptomyces* sp. Carbon sources, nitrogen sources, incubation temperature and initial pH had significant effects ($p<0.05$) on lipase production. Glucose (65.95[a] U/mL), yeast extract (88.99[a] U/mL), 30°C (69.41[a] U/mL) and pH 6.0 (80.64[a] U/mL) supported the optimum lipase production by the selected isolate. *Streptomyces* sp. PSN07 isolated from domestic wastewater contaminated soil was a good lipase producer which can be developed for the industrial production of biotechnologically important products such as food, beverages, leather, textiles, detergents, and soaps.

Keywords: Lipase production, carbon source, nitrogen source, temperature, *Streptomyces* sp.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Streptomyces are saprophytic gram-positive bacteria that are a major source of natural compounds with a variety of biological activities, including antibiotics. It also has a big impact on infectious disease control, enzymes, vitamins, anticancer drugs, enzyme inhibitors, and the development of new medicinal molecules (Knetsch et al., 2011).The interest in microbial lipase of *Streptomyces* production has increased in the last decade (Mehta *et al.,* 2017), because of its large potential in manufacturing applications as food additives, fine chemicals, waste water treatment, cosmetics, pharmaceutical, leather and medicine, alongside the quest for sustainable production to meet up with the increasing demand for lipase (Kumar and Ray, 2014).

Any enzyme that catalyses the hydrolysis of fats, also known as lipids, is known as lipase (Svendsen, 2000). Lipases are primarily manufactured by microbes, and bacterial lipases, in particular, play an important role in commercial operations. Lipases are also defined as glycerol ester hydrolases that catalyse the hydrolysis of triglycerides to free fatty acids and glycerol. Lipases catalyse esterification, inter-esterification, acidolysis, alcoholysis and aminoly-sis in addition to the hydrolytic activity on triglycerides (Joseph et al., 2008). Novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and bio-diesel, the production of enantiopure pharmaceuticals, agro-chemicals, and flavour compounds (Jaeger and Eggert, 2002). In nature, they are pervasive and are obtained from several plants, animals and microorganisms (Thakur, 2012). Due to their capacity to stay active under extremes of temperature, pH and organic solvents, and as a result of their chemo-, regional and enantio-selectivity, microbial lipases have attracted special industrial interest (Chandra *et al.,* 2020). Apart from the hydrolysis of triglycerides, lipases can catalyze a variety of chemical reactions which include esterification, trans-esterification, acidolysis and aminolysis (Joseph *et al.,* 2008).

1.2 Statement of problem

The cost of enzyme production has been a major challenge of industrialist, as such, calls for progressive study. More importantly, composition of production medium, also has significant impact on microorganism growth and metabolite production. It also influences the cost of production, which is a major criterion in large-scale commercial production. In order to upscale the necessary lipase productivity, there is a need for a thorough examination of the existing microbial strains used for lipase production.

1.3 Justification

A complete study is being done to create enhanced lipase production solutions due to the rising pace of industrialization, the multifunctional use of enzymes, and the need for long-term enzyme production. Because of their flexibility, lipases are preferred enzymes for usage in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries. Synthetic lipases are costly to make, difficult to get, and poisonous. As a result, fungus and some filamentous bacteria have been observed to generate extracellular lipases. These microorganisms are extracellular in nature, abundant, and easy to find.

1.5 Aim and objectives of the study

The aim of this research is to produce lipase from bacterial species isolated from contaminated soil samples.

i. To isolate bacteria from contaminated soil samples

ii. To screen the isolates for lipase production and select of the best lipase producer

iii. To identify the selected isolates using morphological and biochemical characterizations

iv. To determine the effects of physio–chemical parameters on lipase production by the selected isolate

CHAPTER TWO

LITERATURE REVIEW

2.1 Streptomyces

Streptomyces are a diverse category of soil-dwelling bacteria with filaments that resemble threads (Bhatti *et al.,* 2017). They are abundant in natural habitats and are involved in a variety of biological and metabolic activities, including the production of extracellular enzymes (Ghorbani-Nasrabadi *et al.,* 2013). About 90% of *Streptomyces* genera have been isolated from soil and are safe for use in a variety of disciplines, including the industrial and medicinal industries (Bawazir *et al.,* 2018). Furthermore, *Streptomyces* generate pigments that are red, green, yellow, brown, and black in color on the media (Narain *et al.,* 2014). *Streptomyces* is thought to be the plausible organism that includes antibiotic synthesis, followed by *Nocardia* and *Micromonospora,* which are inferior in nature when compared to *Streptomyces* in terms of antibiotic production (Manandhar *et al.,* 2017).

Streptomyces may be cultivated on standard laboratory bacteriological medium including nutrition agar, trypticase soy agar, and blood agar (Nanjwade *et al.,* 2010). *Streptomyces* are found all over the world and play an important role in the natural cycle. *Streptomyces* growing on agar may be identified by their morphology (the presence or absence of spores on the substrate mycelium, the development of zoospores in specialized spore vesicles or sporangia), the arrangement of their spores, and the characteristics of their colonies (Oskay *et al.,* 2004).

Odeyemi *et al*. (2013) discovered 32 lipolytic bacteria in the genera when researching microorganisms that may use palm oil as a substrate because they have many fungus-like characteristics (such as mycelial growth) and cause the same eye illness as fungi. Some of the *Streptomyces* species include *Streptomyces coelicolor*, *S. scabies*, and *S. meyeri.* In the agroindustry, *Streptomyces* are being studied as a source of physiologically active chemicals, biocontrol agents, and PGPRs. *Streptomyces* is the most common kind of *Streptomyces*. According to Tanaka and Omura (1993), these bacteria are responsible for roughly 60% of novel pesticides and herbicides. This rising interest stems from their low toxicity and environmental

friendliness; they are biodegradable and extremely specific, making them less harmful to nontarget species.

Streptomyces is a genus of bacteria belonging to the Actinobacteria class. They're all Gram-positive bacteria. *Streptomyces* are facultatively anaerobes (excluding *A. meyeri* and *A. israelii*, which are obligate anaerobes), and they thrive in anaerobic environments (Mendler *et al.,* 2019). Individual bacteria of *Streptomyces* species can create endospores, and colonies of *Streptomyces* form fungus-like branching networks of hyphae. The appearance of these colonies led to the mistaken belief that the creature was a fungus, and the name

Streptomyces species may be found in soil as well as in animal microbiota, including the human microbiome. They are well-known for their importance in soil ecology; they generate a variety of enzymes that aid in the decomposition of organic plant material, lignin, and chitin. As a result, their presence is critical in the composting process. Humans and cattle have commensal flora on their skin, mouth flora, gut flora, and vaginal flora. They are also known to cause illnesses in people and cattle, generally by gaining entrance to the inside of the body through wounds. People with immunodeficiency are more susceptible to opportunistic infections, as they are to other opportunistic diseases. They are comparable to *Nocardia* in all of the features listed above, as well as in their branching filament production (Parks *et al.,* 2020).

Streptomyces species, like many other anaerobes, are finicky and difficult to cultivate and isolate. Although clinical laboratories culture and isolate them, a negative result does not rule out infection since unwillingness to grow in vitro might be the cause.

2.1.1 Classification of Streptomyces

Streptomyces are distinguished by the development of usually branching threads or rods, which frequently result in a unicellular mycelium, particularly during the early phases of growth. The hyphae are usually non-septate; however, septa can be seen in certain forms under particular circumstances. Mycelium can be vegetative, growing in the substrate, or aerial, producing a specific mycelium above the vegetative development. Table 2.1 shows other categories for *Streptomyces*:

Table 2.1: Classification of *Streptomyces* (Chandramohan, 1997)

Section	Characteristics
Nocardio Form Actinomycetes	Aerobic, may be acid-alcohol fast; occur as rods, cocci and branched filaments or form substrate and aerial mycelium that fragment; wall chemotype IV; contain mycolic acids.
Actinomycetes with multilocular sporangia	Aerobic to facultatively anaerobic; mycelium divides in all planes, no aerial hyphae, wall chemotype III.
Actinoplanetes	Aerobic spored *Streptomyces* nonmotile, spores may be enclosed within vesicles; no aerial mycelium; wall chemotype II; whole-organism hydrolysates contain arabinose and xylose.
Streptomycetes and related genera	Aerobic spored *Streptomyces*; form an extensively branched substrate and aerial mycelium.
Thermomonospora and related genera	Aerobic spored *Streptomyces*; form an extensively branched substrate and aerial mycelium, both of which may carry single of chains of spores; spores either motile or non-motile; wall chemotype III
ThermoActinomycetes	The stable filaments produce aerial growth. Single spores (endospores) are formed on both aerial and vegetative filaments. All species are thermophilic. The cell wall contains meso-DAP but no characteristic amino acids or sugars.
Other genera	They all produce aerial growth bearing chains of spores

2.2 Application of *Streptomyces* Genera

Lipase-producing bacteria can be used in a variety of industrial and non-industrial processes, including food processing, environmental cleaning, bioremediation, cosmetics and makeup, bioengineering, and many other applications. Among the other uses are:

Several insect-hostile microorganisms, such as fungus, bacteria, nematodes, and viruses, have been discovered as possible biological control agents. *Streptomyces* serve an important role in the biological control of insects, such as the house fly *Musca domestica*, by synthesizing insecticidally active compounds (Hussain *et al.,* 2002). When *Streptomyces* treatments were used, larval and pupal mortality was extremely high, reaching as high as 90% in some cases (Hussain *et al.,* 2002). The use of *Streptomyces* to control *Culex quinquefasciatus* proved successful (Sundarapandian *et al.,* 2002). *Streptomyces* are a crucial bacterial group, not only because they decompose organic materials in the natural environment, but also because they produce antibiotics and other commercially important chemicals (Saugar *et al.,* 2002).

2.2.2 Phytochemicals agro-active properties

Streptomyces are the most prolific source of microorganisms when it comes to all kinds of bioactive metabolites, including those that are agroactive. Over 1000 secondary metabolites originating from *Streptomyces* were discovered between 1988 and 1992. The bulk of these compounds are produced by a number of distinct *Streptomyces* species. *Streptomyces* is the source of around 60% of new insecticides and herbicides published in the previous five years (Tanaka and Omura, 1993). With the exception of one, nearly three-quarters of all *Streptomycetes* species, according to some estimates, are capable of generating antibiotics. (Alexander 1977).

2.2.3 Biosurfactants from *Streptomyces*

Several insect-hostile microorganisms, such as fungus, bacteria, nematodes, and viruses, have been discovered as possible biological control agents. *Streptomyces*serve an important role in the biological control of insects, such as the house fly *Musca domestica*, by synthesizing insecticidally active compounds (Hussain *et al.,* 2002). When *Streptomyces* treatments were used, larval and pupal mortality was extremely high, reaching as high as 90% in some cases (Hussain *et al.,* 2002). The use of *Streptomyces* to control *Culex quinquefasciatus* proved successful (Sundarapandian *et al.,* 2002). The emulsion produced by the cell to aid the absorption of insoluble substrate is made up of all surface-active chemicals. The sole product on the market is Emulsan, a bioemulsifier generated by Acineotobacter species. Bioemulsifiers are produced in large part by *Streptomyces*. Wagner and his colleagues have spent a lot of time studying the *Trehalosedimycolates* generated by *Rhodococcus erythropolis*. Biosurfactants have

a number of benefits over chemically produced surfactants. They have a high degree of specificity, are less hazardous, and are biodegradable. They work in a wide range of temperature, pH, and salinity conditions.

2.3 Lipase

The hydrolases family includes lipases (EC 3.1.1.3), a triacylglycerol acylhydrolase that works on carboxylic ester linkages (Fetzner and Steiner, 2010). They belong to the class of serine hydrolases and do not require any cofactor (Beisson *et al.,* 2000). Natural lipases hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. Esterases, as well as lipases, may hydrolyze carboxylic esters linkages (Pascoal *et al.,* 2018). Lipases catalyze the hydrolysis of ester bonds at the boundary between an insoluble substrate phase and an aqueous phase where the enzymes remain liquefied under normal conditions. However, *Pseudomonas aeruginosa, Candida anatarctica, and Burkholderia glumae* possessed a lid but did not show interfacial activation (Stergiou *et al.,* 2013). Esterification, transesterification, interesterification, acidolysis, alcoholysis, and aminolysis conversion reaction takes place by lipases (Jiao *et al.,* 2018).The presence of a lid and the interfacial activation are not the suitable criteria for to categorize a true lipase, carboxylesterase simply defined that catalyzes the hydrolysis and synthesis of long-chain acylglycerols (Lei *et al.,* 2016).

Plant lipases, animal lipases (Hamosh *et al.,* 1984), and microbial lipases, notably bacterial and fungal lipases, have all been studied extensively (Desnuelle, 1972). Despite the fact that pancreatic lipases have been utilized for a variety of reasons in the past, it is widely known that microbial lipases are favored for commercial applications owing to their multifaceted characteristics, ease of extraction, and possibility for an infinite supply (Macrae and Hammond, 1985). In this paper, we will examine some of the most significant areas of research in bacterial lipases and their characteristics, as well as discuss fungal lipases in greater depth, including their beneficial features, uses, and commercial applications. Lipases are not involved in any anabolic processes in vivo. Since these enzymes act at the oil-water interface, they can be used as catalysts for the preparation of industrially important compounds (Tombs, 1991). As lipases act on ester bonds, they have been used in fat splitting, inter- esterification (transesterification), development of different flavors in cheese, improvement of palatability of beef fat for making

dog food, etc. A current application involves the use of lipases in water deficient organic solvents for synthesizing different value-added esters from organic acids and alcohols. Lipases which are stable and work at alkaline pH, say 8 to 11 , which are usually the suitable wash conditions for enzyme-containing detergent powders and liquids, have also been found, and these hold potential for use in the detergent industry (Macrae and Hammond, 1985).

Lipases belong to the class of serine hydrolases and, therefore, do not require any cofactor. The natural substrates of lipases are triacyl- glycerol, which have very low solubility in water. Under natural conditions, lipases catalyze the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase where the enzyme remains dissolved (Macrae and Hammond, 1985).

2.3.1 Applications of Lipase

Lipases are one of the most versatile enzymes available, and they are widely used in a wide range of applications including food industry, oleochemical industry, pharmaceutical industry, cosmetic industry, region selective vaccinations and so many more. Applications of lipase include:

2.3.1.1 In dairy industry

Lipases are commonly used in the dairy industry for hydrolysis of milk fat, modification of fatty acid chain lengths, and enhancement of cheese flavor (Balcao and Malcata 1998). It is currently used to accelerate cheese ripening and fat, butter, and cream lipolysis. Various products, particularly soft cheeses with specific flavor characteristics generated with free fatty acids, are produced by the action of lipases on milk fat (Hamdy *et al.,* 2017). The engineered industry developed a variety of microbial lipases for making cheese from *M. miehei, A. niger, A. oryzae*, and other bacteria (Li *et al.,* 2012). Individual microbial lipases or their combinations are used in the production of a high-quality range of cheeses. Enzyme Modified Cheese (EMC) is made by incubating cheese at a higher temperature in the presence of enzymes in order to extract a concentrated flavor using lipase catalysis (Law, 1999). EMC has a 10 times greater fat content than regular cheese and is utilized in a variety of goods including dips, sauces, soups, and snacks. (Chandan, 2008)

Flavor components such as acetoacetate, -keto acids, flavor esters, methyl ketones, and lactones are made from free fatty acids by beginning simple chemical processes (Priebe *et al.,* 2011). Lipase facilitated the hydrolysis and alcoholysis of ester linkages in vitamin A and E esters. For the study of immobilized *C. antarctica*, the Supercritical Fluid Extraction (SFE) method is employed to extract the oxidation-prone vitamins A and E. (Turner *et al.,* 2001). The SFE technique should be used to determine vitamins D2/D3, K53, and -carotene in milk powder and baby formulas. Gastric lipases are used to accelerate the ripening and increase the flavor of a variety of cheeses, including cheddar, provolone, and ras cheeses (Aravindan *et al.,* 2007). After the addition of lipase, the rate of fatty acid delivery increases, hastening the formation of flavor. (Omar *et al.,* 2016).

With the addition of calf lipase, fatty acid release rose considerably, as did the ripening temperature (from 7 to 53 °C) (Karami *et al.,* 2004). The improved procedure's released fatty acid profiles were indistinguishable from the control, and the total quantities of short-chain liberated fatty acids (C4 to C6) were important for improving cheddar cheese flavor during maturation, as shown by the observations (Kondyli *et al.,* 2003). The lipase enzyme remains active during maturation, which can result in the development of a pronounced rancid flavor. When a cock-tail of fungal protease and lipase were employed in the cheddar cheese industrialization, the cheese had more soluble proteins and free fatty acids and had a superior flavor within 3 months of ripening (Grummer *et al.,* 2013). A high amount of enzyme during ripening may result in too much enzymatic response communicating an unwanted specific and lowering production (McSweeney and Sousa, 2000).

Liposome technology was used to speed up the ripening of cheese, reducing bitterness and productivity losses (Alkhalaf *et al.,* 1988). Bacterial intracellular enzymes are unconstrained by cell lysis and contribute to flavor via lipolysis and other enzymatic activities (Schilling *et al.,* 2002). Takeout milk coagulation can benefit from the use of cell-free extracts microcapsules in milk fat. When cheese is made with intact capsules, it includes much more enzymatic end products than when enzymes are added directly to the cheese (Fraga *et al.,* 2018). Capsule stability can be improved by encapsulating in a lipid with a high melting percentage. The natural milk lipase found in unpasteurized milk impacts the significant lipolytic activity of cheese (Kailasapathy and Lam, 2005). Lipolytic cheeses, such as Blue-vein and Camembert, generate

lipases utilizing the culture and secondary microflora, such as P. roqueforti and P. camembertii, respectively (Lessard *et al.,*2012).

2.3.1.2 Lipase in fat and oil industry

Oil and fats amendment is one of the primary sectors in food processing manufacturing that requires economically green technology, and it is a highly important element of foods (Titus *et al.,* 2018). Lipases that change the position of fatty acid chains allow us to change the lipid assets in glycerides and replace one or more of them with new ones (Otzen *et al.,* 2011). In this approach, a relatively inexpensive and less suitable lipid can be upgraded to a better value fat. The fat catalyzes the hydrolysis, esterification, and inter esterification of oils and fats (Gunstone *et al.,* 1999). Between the lipolytic transformation of oils and fats like specialty fats and partial glycerides using positional and fatty acid detailed lipases, esterification and inter esterification are used to get value added products like specialty fats and partial glycerides, and have a better industrial potential than fatty acid production in bulk through hydrolysis (Cobb *et al.,* 2013). An immobilized lipase membrane reactor was built for fat and oil hydrolysis, which generated products and required less downstream processing, lowering total processing costs (Giorno *et al.,* 2000). Highly selective microbial phospholipases is a new environmentally friendly technique for removing phospholipids from vegetable oils that has just been industrialized (de-gumming) (Singhania *et al.,* 2015).

To develop a food-grade, cost-effective, immobilized 1, 3-regioselective (lipozyme TL 1 M) lipase for the production of frying fats and the inter-esterification of commodity oil reductions and lard components, using granulation to immobilize lipases (Ibrahim *et al.,* 2005). It cannot be obtained by predictable chemical inter-esterification to produce modified acylglycerols lipases catalyzed inter-esterification of fats and oils to produce modified acylglycerols lipases (Rønne *et al.,* 2005). Immobilized lipases from *C. antarctica* (CAL-B), *C. cylindracea* Ay30, *H. lanuginosa*, *Pseudomonas* sp., and *G. candidum* were used to esterify functionalized phenols and produce lipophilic antioxidants in sunflower oil (Joseph *et al.,* 2007). Many studies have shown the effects of lipases in their pure form, immobilized form, or cell bound form on the breakdown of fats and oils (Murty *et al.,* 2002). The use of triacylglycerol lipase obtained from hereditarily modified A. oryzae as a processing aid in the oils and fats productiveness for oil de-gumming, and in the food industry to progress emulsifying possessions was scientifically accepted by the

Australia New Zealand Food Authority (ANZFA) in 2002. (Rumble *et al.,* 2003). A new procedure for immobilizing lipases based on silica granulation has drastically shortened the development time and reduced the procedure cost. Such innovative approaches are currently widely used in the production of commodity fats and oils that are free of trans-fatty acids (De Clercq *et al.,* 2012). For the interesterification of soybean oil with 22.7 percent oleoyl and 54.3 percent linoleoyl moieties as molar acyl in hexane using an immobilized Sn-1, 3-specific lipase (Lipozyme IM) from *M. miehei* and oleic acid, a continuous packed bed rector was designed and operated (Haraldsson *et al.,* 1989). The loss of Lipozyme IM's catalytic activity in soybean oil slowed the rate of change in oleoyl and linoleoyl moiety arrangements in the oil.

2.3.1.3 In cosmetics and personal care products

By 2024, the worldwide cosmetic market share may reach USD 680 billion, including goods relating to hair care, skin care, scent, personal hygiene, and dental care. The industry's expansion may be fueled by sophisticated cosmetic products. Supply dynamics may be hampered by a lack of regulatory rules, putting downward pressure on the lipase market price trend (opaciuk *et al.,* 2013). Unichem International (Spain) manufactures isopropyl myristate, isopropyl palmitate, and 2- ethylhexylpalmitate for use as a palliative in maintenance products such as cutaneous and sun-tan ointments, bath oils, and so on (Fiume *et al.,* 2013). Rose oxide is a fragrance component that is made from numerous microbiological sources via lipases and the transesterification of 3, 7-dimethyl-4, 7-octadien-1-ol. As a biocatalyst, *Rhizomucor meihei* lipase was immobilized (Knezevic *et al.,* 2003). Instead of using a traditional acid catalyst, the business claims that the employed enzyme offers the necessary lowest downstream refining and abundant enhanced value. Wax esters (esters of fatty acids and fatty alcohols) have similar functions in personal care products and are similarly enzymatically produced (Croda Universal Ltd.), and the firm utilizes *C. cylindracea* lipase in a batch bioreactor (Lehtinen *et al.,* 2018). According to the producer, the production cost is often slightly more than that of traditional processes, and the improved quality of the finished product justifies the expense. Retinoids (Vitamin A and its derivatives) have a lot of economic promise in cosmetics and medicines like skin care products. Water-soluble retinol derivatives were made using an immobilized lipase catalytic process (Zasada *et al.,* 2019). Lipases are also utilized for hair stressing, as a component of topical antiobese emulsions, and as an oral administration. They're also utilized in the cosmetics industry for cleansing, moderating, smelling, and coloring (Safford *et al.,* 2015). Lipases are involved in the synthesis of surfactants

and aromas, and are utilized in cosmetics and perfumery. A patent Nippon Oil and Fats received from for the manufacture of propylene glycerol mono fatty acid ester in the presence of lipase is also used as an emulsifier and a pearling agent in cosmetics and meals (Seino *et al.,* 1984).

2.3.1.3 In bioremediation

Bioremediation is a method used to decontaminate samples from oil spills, oil-wet soils, industrial wastes, and wastewater tainted with lipids (Lailaja *et al.,* 2007). The wastewater that reaches the natural environment without previous treatment may be harmful. Lipase-producing organisms such as *Staphylococcus pasteurii* COM-4A, *Bacillus subtilis* COM-B6, and *Arthrobacter sp.* have been reported to successfully reduce pollutants (Maclean *et al.,* 2019). *Pseudomonas* species, such as *P. aeruginosa*, have shown to be particularly effective as accessible devices for microbial remediation. Statistical techniques have been used to optimize the lipase production and oil hydrolysis processes. *Bacillus spp*. pool obtained from matured petroleum-contaminated soil and *B. stearothermophilus* recovered from slaughterhouse waste are potential bioremediation candidates (Olusesan *et al.,* 2009). Under acidic circumstances, the bacterial genera *Burkholderia sp.* and *Raoultella planticola* have the ability to destroy edible oil (Gao *et al.,* 2019). Previous bacterial consortia have been proposed as possible inoculum for the treatment of high intensity Oil and Gas wastewater, including *P. aeruginosa*, *Bacillus sp*., *Halomonas sp., Citricoccus alkalitolerans,* and *Acinetobacter caloaceticus*, as demonstrated in other research (Chandra *et al.,* 2016). *B. subtilis, B. licheniformis, B. amyloliquifaciens, S. marsescens, P. aeruginosa,* and *S. aureus* made up the other group (Phulpoto *et al.,* 2020).

2.3.1.4 As biosensors in food industry

The physico-chemical transducer is employed as a quantifiable signal, and the second compartment has biological origin for the purpose of giving particular analysis (Xu *et al.,* 2011). The Lipase is one of the biologically derived components. And tributyrin, a frequent substrate for the beginning of different lipases. For the lipases, *Bacillus subtilis* and *Chromobacterium viscosum* are listed, as well as the fungi *Rhizomucor miehei, R. oryzae,* and *Fusarium solani* hydrolases tributyrin to dibutyrin and butyrate (Esteban-Torres *et al.,* 2016). Immobilized lipases are utilized as a biosensor for the quantitative detection of triacylglycerol because of their precision and efficiency. Lipases play an important role in the food sector, particularly in fats

and oils, soft drinks, pharmaceuticals, and beverages, as well as in medical diagnostics (Zehani *et al.,* 2014). The triacylglycerol is broken down into glycerol using lipase enzymes as a biosensor in analytical and quantitative techniques. A surface acoustic wave impedance biosensor was developed for the detection of organo-phosphorous pesticides utilizing lipase hydrolysis (Després *et al.,* 2009). It's also used to look for traces of the pesticide Dichlorvos in vegetables. Lipases may be mounted onto pH/oxygen electrodes by combining with glucose oxidase, and they can be utilized as lipid biosensors to analyze triglycerides and blood cholesterol samples (Bantscheff *et al.,* 2012). Pesticidal residues in water and food grains can now only be quantified and determined using chromatographic and spectroscopic techniques. The estimate and detection of triglycerides is a clinically important measure that is linked to the disorder of heart-related issues (Koesukwiwat *et al.,* 2008). A potentiometric biosensor based on *C. rugosa* lipase was also developed for the measurement of methyl-parathion and tributyrin. *C. rugosa* lipase was purified and cross-linked with glutaraldehyde on a glass electrode (Barrios *et al.,* 2014). 4-nitrophenyl laurate was hydrolyzed to 4-nitrophenol and laurate, 4-nitrophenyl oleate to 4-nitrophenol and oleate, 4-nitrophenol palmitate to 4-nitrophenol and palmitate, 4-nitrophenyl propionate to 4-nitrophenol and propionate, and -naphthyl acetate to -naphthol and acetate, Micrococcus sp. was shown to convert methyl acetate to methanol and acetate, methyl butyrate to methanol and butyrate, methyl laurate to methanol and laurate, methyl palmitate to methanol and palmitate, methyl propionate to methanol and propionate, and methyl stearate to methanol and stearate. (Pohanka *et al.,* 2019).

2.3.1.5 In wine production

Wine's color, flavor, and fragrance all contributed to a complex combination of hundreds of components (Sumby *et al.,* 2010). In recent years, wine scent has gotten a lot of study, and numerous sensory components have been identified (Lambrechts and Pretorius, 2000). The ethyl esters have gotten a lot of attention because of their huge impact on flavor (Petruzzi *et al.,* 2017), and the ethyl acetate ester is the most common molecule found in wines. Other esters include ethyl decanoate, ethyl 2-methyl-propionate, ethyl 3-methyl-propionate, ethyl 3-methylbutanoate, ethyl cinnamate, methyl-butyl acetate, 2-phenyl–ethyl acetate, hexyl acetate, 2-ethyl hidroxpropionato, diethyl butanediol (Caven-Quantrill *et al.,* 2017). Those produced enzymatically between an alcohol and an acid created by chemical esterification during the aging

process of the beverage are divided into two types (Mingorance-Cazorla *et al.,* 2003). Stability in the presence of ethanol, sodium metabisulfate, malic, tartaric, citric, and lactic acid, as well as strong activity at pH 5–7, as well as the particular characteristics of esterases and lipases used to make ethyl acetate, ethyl butanoate, ethyl hexanoate, and ethyl octanotate (Esteban-Torres *et al.,* 2015). The genetic improvement of *Escherichia coli* BL21 was achieved by the insertion of a gene encoding the lipase/esterase enzyme derived from *Lactobacillus plantarum* WCFS, which was chosen for its lipase/esterase producing properties (Barros *et al.,* 2010). The microbes generated enzymes and exhibited excellent activity at low pH and stability in the presence of ethanol, sodium metabisulfite, tartaric, lactic, and citric acids, indicating that they have great potential for use in the winemaking process (Belda *et al.,* 2017). The impact of successive inoculation of yeasts, *Williopsis saturnus* var. mrakii NCYC2251 and *Saccharomyces cerevisiae* var. bayanus R2, on ester generation during the preparation of papaya wine was studied (Jagtap and Bapat, 2017).

2.3.1.6 In dietetics

Because of the risks associated with high fat intake, there is a growing demand for low caloric fats and fat replacers. As a result, consumer awareness has increased in the current scenario (Lindley, 1993). The majority of reduced caloric fats and fat substitutes available today are fatty acids, which are not naturally present in edible oils and fats but match the chemistry and functions of natural fats (Lim *et al.,* 2010). However, the lack of nutritionally important essential fatty acids (EFA) is a drawback of such goods (Sargent *et al.,* 1995). The structured triglycerols constituted a positional analysis, with primary positions proliferating in parallel to secondary positions in favor of lipase activity (Müllner and Daum, 2004; Lee *et al.,* 2005). Targeted structured triglycerols with palmitoyl moieties in the Sn-2 position, as well as medium chain acyl moieties in the Sn-1, 3 positions, should be useful for baby nutrition and clinical food formulation, as well as parental sustenance (Watanabe *et al.,* 2015). The biocatalyst for the acidolysis was commercially immobilized Sn-1, 3-specific lipase, Lipozyme RM IM, which was isolated from *R. miehei*. For both oleic and stearic acids, the amount of incorporation increased with reaction time (Fernandez-Lafuente, 2010; Foresti and Ferreira, 2010). The SLs generated may be used in baby formulas, and the researchers called for further collaboration between industry and academics to improve the commercialization of enzymatic processes (Bristrian,

1997). With the alteration of vegetable oils, infant formula may be made with more absorbable TAGs (Floros *et al.,* 2010). In the same locations and quantities as those present in human milk, polyunsaturated fatty acids (PUFAs) and medium chain fatty acids (MCFA) are detected (Straarup *et al.,* 2006). Enzymatic acidolysis between tripalmitin, hazelnut oil fatty acids, and stearic acid produced structural lipids (SLs) with palmitic, oleic, stearic, and linoleic acids, comparable to human milk fat (HMF) (Teichert and Akoh, 2011). The gram-positive bacteria Staphylococcus epidermidis was rendered inactive by lipase-treated formulations (Koesukwiwat *et al.,* 2008). Current research demonstrates that the lipid fraction of baby formulas is not only a source of nutrients, but also has antiviral and antibacterial activity when incubated with lipases (Isaacs *et al.,* 1992).

2.5 Factors Affecting the Production of Lipases

2.5.1 Factors relating to the environment

Because the presence of a substrate influences the stability of the bacteria, the pH of the culture medium and the pH of the assay medium are both important considerations when screening bacteria for lipase production (Talon *et al.,* 1995). In addition to the others, pH, temperature, inoculum age and size, agitation, and incubation time are also important characteristics to consider. The synthesis of lipase by thermophilic *Bacillus sp*. continued for more than 24 hours during the stationary phase, according to Handelsman and Shoham (1994), and the ideal length of time required for the maximum synthesis of lipase was determined to be 36 hours. Contrary to popular belief, the lipolytic activity of bacteria like *B. cereus* and *B. coagulans* peaks after two days of continuous agitation (El-Shafei and Rezkalla, 1997).

Shake flasks are widely used for the production of extracellular enzymes because they enhance the rate of aeration in aerobic organisms. Lipase production requires a 4:1 air-to-medium ratio, with aeration resulting in the highest activity. Lipolytic activity is seen in *Bacillus sp*. The concentration peaked after two days of incubation with continuous agitation (El-Shafei and Rezkallah, 1997).

2.5.2 Nutritional Factors

2.5.2.1 Nitrogen source

Lipase titers in the production broth are affected by the kind of nitrogen supply in the medium (Ghosh *et al*. 1996). Several *Bacillus spp.* (viz. *Bacillus* strain A30-1, *B. alcalophilus*, *B. licheniformis* strain H1) and *pseudomonads* (viz. *Pseudomonas sp., P. fragi, P. fluorescens* BW 96CC), *Staphylococcus haemolyticus*; Wang L62 *haemolyticus*) have utilized peptone and yeast extract as nitrogen sources for (Oh *et al*. 1999). Inorganic nitrogen sources such as ammonium chloride and diammonium hydrogen phosphate have been found to help some bacteria (Gilbert *et al.,* 1991). 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).

2.5.2.2 Carbon Source

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. Lipases are inducible enzymes (Lotti *et al.,* 1998), therefore they are usually produced in the presence of a lipid source, such as oil, or any other inducer, such as triacylglycerols, fatty acids, hydrolyzable esters, tweens, bile salts, and glycerol (Ghosh *et al.,* 1996). Sugars, sugar alcohol, polysaccharides, whey, casamino acids, and other complex carbon sources have a significant influence on their synthesis (Gilbert *et al.,* 1991). 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,s sucrose, and others have been shown to stimulate lipase synthesis.

2.6 Sources of Lipase

Plants, mammals, and a variety of microbes such as bacteria, *Streptomyces*, fungus, and yeast all generate lipases (Raveendran *et al.,* 2018). Lipase can be obtained from a variety of sources, including:

2.6.1 Microbial lipase

Microbial lipases are ubiquitous in nature and have a higher economic value than animal and plant lipases due to their lower production costs, improved stability, and greater availability

(Borrelli and Trono, 2015). Microbial lipases, either naturally occurring or recombinant, are commonly employed in a variety of bioengineering applications (Mayordomo *et al.,* 2000). Nature provides a diverse range of microbial resources, allowing microorganisms to adapt to harsh environments such as the Dead Sea, Antarctica, alkaline lakes, hot springs, volcanic vents, and polluted soils, resulting in exceptional potential for lipase production with distinct characteristics (Elend *et al.,* 2007). An immense spin-off in terms of enantioselectivity hydrolysis and carboxyl ester formation has resulted in quick availability. The production of enzymes and protein active substances is more capable in marine microfloras. Lipase is a kind of extracellular secretion produced by fungus and bacteria (Boutaiba *et al.,* 2006).

Candida antarctica lipase B (CALB) is the most often utilized enzyme in biocatalytic processes and has the most patents. *Candida rugosa* lipase (CRL) is a scientifically relevant lipase from the yeast that is a combination of various isoforms that is commercially available. This grounding is known as "Generally Recognized As Safe" (GRAS) and is utilized in the food sector (Moura *et al.,* 2015). PLA1s and PLA2s from *Fusarium oxysporum, T. lanuginosus, Aspergillus niger, and Trichoderma reesei* are utilized in the degumming of vegetable oils and have been marketed. PLA1s, PLA2s, and PLBs isolated from *A. oryzae* and *A. niger* are primarily employed in the food sector (Magarvey *et al.,* 2004). PLDs derived from *Actinomycete*s strains are commercially accessible and employed in a variety of industrialized techniques due to their strong transphosphatidylation and hydrolytic activity (Munsch-Alatossava *et al.,* 2018). *Pseudomonas, Bacillus,* and *Streptomyces* have been identified as the most common bacterial genera for producing lipases and phospholipases, followed by *Burkholderia, Chromobacterium, Achromobacter, Alcaligenes,* and *Arthrobacter* (Geoffry and Achur, 2018). 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 *Streptomyces* (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). They've also

improved production yields, made genetic modification easier, and accelerated growth in low-cost media, making production more suitable (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. The solvent resistant strain *Pseudomonas aeruginosa* (PseA) produced the highest lipase activity of 1,084 U gds1 (Mahanta *et al.,* 2008). *Bacillus coagulans* produced extracellular lipase with a maximal lipase activity of 149 U gds1 after 24 hours of fermentation (Alkan *et al.,* 2007). After 72 hours of fermentation, *B. cepacia* had a maximal lipase activity of 108 U gds1 (Fernandes *et al.,* 2007)

2.5.2 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).

2.5.3 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). 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).

2.7 Types of Lipase Enzymes

2.7.1 Gastric lipase

LIPF is another name for this protein. This enzymatic protein is encoded by the LIPF gene in humans (Bodmer, 1987). Gastric lipase is an acidic lipase that is produced by the gastric main cells in the stomach's *Fundic mucosa* and is responsible for fat and protein breakdown. For this chemical, the pH range of 3–6 is ideal. Gastric lipase and lingual lipase make up the two acidic lipases. Unlike alkaline lipases (such as pancreatic lipase), these lipases do not require the presence of bile acid or colipase to operate properly. In the adult human, acidic lipases contribute for 30% of the lipid hydrolysis that happens during digestion, with gastric lipase accounting for the majority of the two acidic lipases present. Acidic lipases are significantly more important in neonatal development, accounting for up to 50% of total lipolytic activity in these young animals. Gastric lipase is a digestive enzyme that breaks down triglyceride ester linkages in the stomach. Because the pancreas of a baby is still growing, LIPF is more crucial in fat digestion than the pancreatic of an adult who is fully functional. When the pancreas is unable to operate at its maximum capacity, Aloulou and Carrière (2008) discovered an increase in LIPF production. LIPF is often detected at low quantities in the tumors of gastric cancer patients.

2.7.2 Lingual lipase

This enzyme belongs to the triacylglycerol lipases (EC 3.1.1.3) family of digestive enzymes that hydrolyze medium and long-chain triglycerides into partial glycerides and free fatty acids using the catalytic triad of aspartate, histidine, and serine. The lipolytic activity of lingual lipase, however, continues through to the stomach due to its unique characteristics, which include a pH optimum of 4.5–5.4 and the ability to catalyze reactions without bile salts (Cleghorn *et al.,* 1989). After ingestion, the autonomic nervous system signals the serous glands under the circumvallate and foliate lingual papillae on the tongue's surface to secrete lingual lipase into the grooves of the circumvallate and foliate papillae, which are co-localized with fat taste receptors (Chandrashekar *et al.,* 2006). Because long-chain triacylglycerides cannot be absorbed by the small intestine, 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.

2.7.3 Pancreatic lipase family

The ester bonds in triglycerides are hydrolyzed by a group of lipolytic enzymes known as triglyceride lipases (EC 3.1.1.3). Lipases may be found in abundance in the animal, plant, and bacterial worlds. The pancreatic, hepatic, and gastric/lingual isozymes are the only tissue-specific isozymes discovered in higher vertebrates. These lipases, as well as lipoprotein lipase (EC 3.1.1.34), which hydrolyzes chylomicron triglycerides and extremely low density lipoproteins, are closely related (VLDL). It has been established (Blow, 1990) that the most conserved area in all of these proteins is focused on a serine residue, which combined with a histidine and an aspartic acid residue is considered to operate in a charge-relay mechanism. Another enzyme with this structure is lecithin-cholesterol acyltransferase (EC 2.3.1.43) (McLean and colleagues, 1986), which catalyses fatty acid transfers between phosphatidylcholine and cholesterol. This area can also be found in prokaryotic lipases.

2.8 Bacterial Lipase

Lipase was first discovered in 1901, with *B. prodigiosus* and *B. fluorescens*, which are now replaced by *Serratia marcescens* and *P. fluorescens* as the top lipase-producing bacteria (Mobarak-Qamsari *et al.,* 2011; Veerapagu *et al.,* 2014). Bacterial lipases produce glycoproteins and lipoproteins. Certain polysaccharides have been shown to impact enzyme synthesis in the majority of bacteria (Muthazhagan and Thangaraj, 2014). Some bacterial lipases are thermostable, while the majority of bacterial lipases are known to be constitutive and nonspecific in substrate specificity (Tembhurkar *et al.,* 2012). The bacteria *Achromobacter sp., Alcaligenes sp., Arthrobacter sp., Pseudomonas sp., Staphylococcus sp.,* and *Chromobacterium sp.* have all been used to make lipases (Sardessai and Bhosle, 2004).

In comparison to plant and fungal lipases, only a few bacterial lipases have been thoroughly researched and evaluated (Sugiura, 1984). Bacterial lipases are mostly glycoproteins; however, some extracellular lipases are lipoproteins. Bacterial extracellular lipase synthesis is frequently influenced by nitrogen and carbon sources, inorganic salts, and the presence of lipids, temperature, and oxygen availability. Enzyme synthesis in most bacteria is influenced by specific polysaccharides, according to a 1979 study (Winkle *et al.,* 1979). The majority of known bacterial lipases are constitutive and may be blocked by serine hydrolase inhibitors. The majority

of bacterial lipases have broad substrate specificity, and just a few are thermostable (Macrae and Hammond, 1985). Different bacteria genera produce lipase, including *Streptomyces* spp., although the genera *Achromobacter spp., Alcaligenes spp., Arthrobacter spp., Pseudomonas spp.,* and *Chromobacterium spp.* have been successfully explored for lipase production (Godtfredson, S.E. 1990). The bacterial genera *Staphylococcus* and *Pseudomonas* have been studied in particular for commercial purposes. *Staphlococcus aureus* and *S. hyicus* are two significant staphylococci for lipase synthesis. On SDS-PAGE, lipases isolated from *S. aureus* and *S. hyicus* had molecular weights ranging from 34 to 46 kDa. Divalent ions, such as Ca2+, generally activate them, whereas the chelator EDTA inhibits them. These enzymes' pH-optima ranges from 7.5 to 9.0. Lipoproteins are found in *Staphylococcus* lipases (Brune, and Götz, 1992).

2.9 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).

The majority of microbial lipases are extracellular, discharged into the culture media via the cell membrane(s). Temperature, pH, nitrogen, carbon and lipid sources, agitation, and dissolved oxygen content are all variables that influence the quantity of lipase generated. For species such as *Pseudomonas fragi, Aspergillus wentii, Mucor hiemalis, Rhizopus nigricans,* and *M. racemosusi*, a variety of media compositions have been developed. Lipids promote lipase synthesis, which is normally coordinated with the availability of triglycerides. Certain inducers also have a significant impact on lipase production stimulation. Triglycerides, free fatty acids, hydrolysable esters, bile salts, and glycerol are among the inducers. *R. oligosporus* grew well and

produced lipase after emulsifying oil-containing culture medium with gum acacia. In immobilized protoplasts, triolein, olive oil, tributyrin, and oleic acid butylester were able to induce lipase, but Tween 80 increased lipase activity. Lipase activity was increased by a variety of fatty acyl esters, including triglycerides, spans, and Tweens, but was suppressed by long-chain fatty acids, such as oleic acid. The kind of nitrogen supply in the medium has been found to influence lipase production levels in general. On the case of *Pseudomonas spp*., high lipase synthesis occurs in a peptone-supplemented medium, albeit the efficacy of various peptones varies. A concentration of peptone between 0.5 and 2 percent, on the other hand, had no effect on Micrococcus sp. growth or lipase synthesis. Lipase production was highest in *Aspergillus wentii, M. racemosus,* and *Rhizopus nigricans* when peptone was at 2%. Lipases are generated at temperatures ranging from 20 to 45 degrees Celsius. The optimal temperature for lipase synthesis and growth in *R. nigricans* was 30°C, but it was approximately 45°C in *Talaromyces emersomi*. Different species produce lipase in different ways as a result of aeration. Bacterial lipase can be obtained from a variety of microbes or sources.

Table 2.10: Sources of bacterial lipase

Bacterium	**References**
Achromobacter sp.	Mitsuda *et al*. 1988
A. lipolyticum	Brune and Gotz 1992; Davranov 1994
Acinetobacter sp.	Wakelin and Forster 1997; Barbaro *et al*. 2001
A. calcoaceticus	Dharmsthiti *et al*. 1998
A. radioresistens	Liu and Tsai 2003
Alcaligenes sp.	Mitsuda *et al*. 1988
A.denitrificans	Odera *et al*. 1986
Arthrobacter sp.	Pandey *et al*. 1999
Archaeglobus fulgidus	Jaeger *et al*. 1999
Bacillus sp.	Nawani and Kaur 2000
B. alcalophilus	Ghanem *et al*. 2000
B. atrophaeus	Bradoo *et al*. 1999
B.megaterium	Hirohara *et al*. 1985
B.laterosporus	Toyo-Jozo 1988

B. pumilus	Jaeger *et al*. 1999
B.sphaericus	Toyo-Jozo 1988
B. stearothermophilus	Bradoo *et al*. 1999; Jaeger *et al*. 1999
B. subtilis	Jaeger *et al*. 1999
B. thaiminolyticus	Toyo-Jozo 1988
B. thermocatenulatus	Jaeger *et al*. 1999; Pandey *et al*. 1999
Brochothrix thermosphacta	Brune and Gotz 1992
Burkholderia glumae	Jaeger and Reetz 1998; Reetz and Jaeger 1998
Chromobacterium violaceum	Koritala *et al*. 1987

CHAPTER THREE

METHODOLOGY

3.1 Materials

The materials used includes; Distilled water, Sterile Petri dishes, Cotton wool, Alcohol (70% ethanol), Inoculating loop, Slides, Test tubes, Measuring cylinder, Conical flask, Durham tubes, Beaker, Aluminum Foil, Dropper, medium bottles, micropipette.

3.2 Culture media

The culture media used includes; Nutrient Broth, Simmon citrate, Sensitivity broth, Methyl red Vogues Proskauer Agar, Nutrient Agar for the isolation of bacteria, Tributyrin Agar. The reagents used during the experiment include: Ethanol, Rhodamine B, physiological saline, Tween solution, 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 Equipment and reagent

Equipment used includes; Oven, Incubator, Autoclave, Weighing Balance, Thermometer, water bath, Colony counter, Spectrophotometer. Reagents used include; Gram iodine, Kovacs Reagent, Crystal violet, Methyl red.

3.4 Collection of soil samples

The experiment was carried out by the collection of soil samples from dump site and from a cooking site.

3.5 Preparation of nutrient agar slant

According to the manufacturer's instruction, Nutrient Agar medium was prepared 28g of nutrient agar was measured on a weighing balance into a sterile conical flask; 1000ml of distilled water was dispensed into the conical flask. 7g of nutrient agar was measured in 250 ml of water. Swirling was done to the solution in the conical flask to dissolve the medium. The solution was then boiled in the water bath to ensure homogenization after mixing has been properly done. After boiling, the medium was autoclaved for 15mins at 121℃. Immediately after autoclaving,

the medium was poured in the plates after serial dilution was done. The medium was allowed to solidify in this position.

3.6 Isolation of microorganisms from soil sample

Initially, one gram of soil sample was taken and serially diluted up to 10^{-5}. From this, an aliquot of 0.1 mL of each diluted sample was taken and distribute the samples spread evenly over the surface of the plates and pouring the nutrient agar on it using the pour plate technique (Athalye *et al.,* 1981). Subsequently, plates were incubated at 37°C for 24hours and were observed for the appearance of colonies. Afterwards, positive colonies were identified and further purified by streak-plate technique and the pure cultures were maintained on Starch Casein Nitrate Agar (SCA) slants at 4°C. Culture of *Streptomyces* spp. previously isolated from soil samples. The isolates was kept on Nutrient agar slants and kept in the refrigerator. Constant sub culturing was carried out to ensure the viability of the isolates.

3.7 Pure culture technique

From the primary plates, different isolates were sub-cultured aseptically by streaking onto the prepared nutrient agar plates. For 24hours the plates were incubated at 37°C. These resulted in pure culture of the isolated organism. Streaking of the pure culture of isolates was done on a prepared sterile set agar slant in MacConkey bottles and kept in the refrigerator for further tests and identification.

3.8 Selection of lipase-producing microorganisms

Lipolytic activity was tested using culture medium proposed by Kouker and Jaeger (1987), with modifications (0.5% peptone, 0.1% yeast extract, 0.4% NaCl, 1.5% agar and 0.1% rhodamine B solution). After sterilization, 2.5% sterile olive oil was added as a carbon source. All isolates were inoculated by the spot method, and plates incubated at 25 °C and 50 °C for up to 72 h. The growth of the isolates was checked daily and the lipid hydrolysis confirmed by the orange fluorescence on the colonies when exposed to UV light at 350 nm. Isolates with faster growth were reinoculated in the culture medium deprived of yeast extract, to induce olive oil uptake and thus to select the best enzyme-producing strains. Incubation took place in the same conditions as described before. The same assay was performed with canola oil and grape seed oil as a carbon source.

3.9 Identification of the selected isolate

The selected isolate was identified using morphological and Biochemical characterizations.

3.9.1 Morphological identification

Morphological characterizations were done using colonial, cellular and pigment appearances on culture plates.

3.9.2 Biochemical identification

Biochemical characterizations were done using Gram Staining, Oxidase test, Catalase test, Methyl red/ Voges Proskauer test, Coagulase test, Starch hydrolysis test, Sugar fermentation Test, Simmon Citrate test and Indole test.

3.9.2.1 Gram staining

The gram stain is fundamental to the phenotypic characterization of bacteria. A smear was made on a glass slide and heat fixed. The crystal violet which is the primary stain was flooded on the fixed culture for 60 seconds; the stain was washed off with water. Iodine solution was added to the smear for 60seconds and was poured off; then was rinsed with water. A few drops of ethyl alcohol (decolourizer) were added and rinsed with water immediately after 5seconds and finally safranin which is the secondary stain was added for 60seconds and washed off, then the smear was left to air dry. After the drying of the slide, it was observed under the microscope. Gram staining was done to find reactions of the bacterial isolates to Gram reagents. Gram stain helps in distinguishing and classifying bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria.

3.9.2.2 Coagulase test

A coagulase test is a biochemical test that is used to differentiate *Staphylococcus aureus* (coagulase negative) from other Staphylococci species (coagulase negative) on the basis of the ability to produce the coagulase enzyme. This enzyme clots the plasma by converting fibrinogen to fibrin. The test was done by placing a drop of plasma on the slide and the isolated organism was added and mixed gently. Within 10 seconds, positivity was detected by the clumping of bacterial cells.

3.9.2.3 Catalase test

Catalase test is used in differentiating Staphylococci (which produces catalase enzyme) from Streptococci (doesn't produce catalase enzyme). 1 mL of Hydrogen peroxide solution is placed in a test tube, and a small amount of bacteria growth was added by wood stick. The formation of air bubbles indicated positive result. (Cheesebrough, 2000).

3.9.2.4 Oxidase test

Oxidase test is carried out with the use of oxidase strip that has been soaked in few drops of oxidase reagent. A sterile wire loop is used to pick up colonies from the 24hours old pure culture and a smear is made each on the oxidase strip. The appearance of purple coloration within 10 seconds indicates a positive result and no color change indicates a negative result. On a strip 2-3 drops of oxidase reagents were placed. A small amount of the organism was taken using a glass spreader and was streaked on the moist surface of the paper. The presence of Pseudomonas was confirmed by the appearance of intense purple coloration (Hossain *et al.,* 2006).

3.9.2.5 Indole test

This is done by using sterile wire loop to inoculate the isolated organism in peptone water. The tubes were incubated overnight at 37°C. A drop of kovacs reagent was added after incubation period (Cheesebrough, 2006).

3.9.2.6 Simmons citrate test

A 2.14gm aliquot of Simmons citrate agar was dissolved in 500ml of distilled water gently homogenized using magnetic stirrer while swirling gently to dissolve the medium completely. Afterwards, the medium was sterilized by autoclaving at 121°C for 15minutes and allowed to cool at 50°C and poured in sterile test tubes. The tubes were then stabbed with a loopful of each isolates into each test tube and then transferred to the incubator and incubated at 37°C for 24hours. After 24hours, the tubes were observed (Olutiola *et al.,* 2000)

3.9.2.7 Methyl Red/Voges Proskauer (MRVP) test

8.5gm of the MRVP broth was dissolved in 500ml of distilled water, gently homogenized to dissolve the medium completely. 10ml of the broth was distributed into each test tube, covered with corks and sterilized for 15minustes by autoclaving and then allowed to cool at room

temperature. Each isolate was inoculated into each test tube while labeling them accordingly. The tubes were incubated at 37°C and observed after 24hours. Afterwards, 5 drops of methyl red solution was added to each solution. The appearance of red colour indicates positive reaction while the appearance of a yellow colour indicates negative reaction. (Olutiola *et al*, 2000).

3.10.2.8 Urease test

The urea broth medium was inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant was streaked with the test organism. The cap was kept on loosely and incubated in the test tube at 35°C in ambient air for 18 to 24 hours; unless specified for longer incubation. If the organism produces urease enzyme, the color of the slant changes from light orange to magenta. If organism do not produce urease the agar slant and butt remain light orange; medium retains the original colour.

3.10.2.9 Sugar fermentation test

A weight of 5 g peptone, 0.5g of NaCl, 5g of the fermentable sugar (Glucose, Sucrose, Maltose, Lactose and Galactose) and a pint of bromocresol purple was measured into a conical flask and the 500ml of distilled water was added, homogenized, dispensed to 19 test tubes. Inverted Durham tubes were placed in each test tube, covered with corks and sterilized for 15 minutes. Afterwards, each isolates were inoculated into each test tube respectively and incubated at 37°C. After 24hours, the results were observed (Olutiola *et al.,* 2000).

3.10.2.10 Starch hydrolysis test

An aliquot of 20ml of molten starch agar was aseptically poured into each sterile petri dishes allowed to set and was inverted in an incubator at 37°C. The organism was streaked across the surface of the plate and incubated at 37°C for 24-48 hours. Afterwards the plates were flooded with some quantity of Gram's Iodine (Olutiola *et al.,* 2000).

3.11 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).

3.12 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.

3.13 Effect of carbon sources on lipase production

Under submerged fermentation, the influence of maltose, fructose, galactose, glucose, 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.

3.14 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 submerged fermentation. The production medium was similar to the one described above containing 10g/L yeast extract, 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.

3.15 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).

3.16 Effect of pH on lipase production

The effect of pH on lipase production was 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 1N 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).

3.17 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

RESULT

4.1 Colony count

Fifteen (15) morphologically different bacteria were isolated from different dump sites located in the University premises. Table 4.1 shows the colony counts in each of the serial dilution plated for 10^{-1}, 10^{-3} and 10^{-5} after at the end of 48 hours incubation period. The colony counts for 10^{-1}, 10^{-3} and 10^{-5} serial dilutions for CHMS, CBAS, Cafeteria and Hostel dump site ranged from 11 – 54, 9 – 44, 10 – 36, and 22 – 52 (cfu/cm^3) respectively.

4.2 Morphological characterization of the isolates

Table 4.2 shows the morphological characteristics of the isolates including their shape, colour, surface, edge, and elevation. The observed color included yellow, cream, green, brown; the shapes included circular, filamentous and rhizoid; the elevation included raised, convex and flat; the surface included dull, smooth and rough.

4.3 Biochemical characterization of the isolates

Table 4.3 shows the biochemical characterizations of the isolates including; grams staining, starch hydrolysis test, catalase tests, MR-VP tests, coagulase test, indole test, citrate utilization test, urease test and sugar fermentation for glucose and galactose. Both positive and negative reactions to the test reagents were observed.

Table 4.1: Colony counts for the soil samples

Soil sampling locations	**10^{-1}** (cfu/cm^3)	**10^{-3}** (cfu/cm^3)	**10^{-5}** (cfu/cm^3)
CHMS dump site	54	32	11
CBAS dump site	44	12	9
Cafeteria dump site	36	19	10
Hostel dump site	52	34	22

Table 4.2: Morphological characteristics of the isolates

Isolates	Colour on Nutrient Agar	Shape	Elevation	Surface
PSN01	Yellow	Circular	Flat	Smooth
PSN02	Cream	Circular	Flat	Smooth
PSN03	Cream	Circular	Raised	Dull
PSN04	Cream	Circular	Flat	Smooth
PSN05	Yellow	Rhizoid	Flat	Rough
PSN06	Green	Circular	Convex	Smooth
PSN07	White	Filamentous	Flat	Rough
PSN08	Brown	Circular	Flat	Smooth
PSN09	Cream	Circular	Raised	Smooth
PSN10	Yellow	Circular	Flat	Dull
PDSN01	Cream	Circular	Flat	Dull
PDSN02	Yellow	Circular	Flat	Smooth
PDSN03	Yellow	Circular	Flat	Smooth

Table 4.3: Biochemical characterization of the isolates

Isolates	Gram staining	Shape	Catalase	Oxidase	Methyl red	VP Test	Urease	Indole	Citrate	Coagulase	Starch hydrolysis	Glucose	Galactose	Bacterial Isolates
PSN01	+	Rod	+	-	-	-	-	-	+	-	+	-	-	*Bacillus*
PSN02	+	Cocci	+	-	-	-	-	-	-	-	+	-	-	*Staphylococcus*
PSN03	+	Cocci	+	-	-	-	+	-	-	-	+	-	-	*Staphylococcus*
PSN04	+	Cocci	-	-	-	+	-	-	-	-	+	-	+	*Staphylococcus*
PSN05	+	Rod	-	-	-	-	-	-	-	-	+	-	+	*Bacillus*
PSN06	+	Cocci	+	+	-	-	-	-	-	+	-	-	+	*Staphylococcus*
PSN07	+	Rod	+	+	-	-	+	-	-	+	+	-	-	*Streptomyces*
PSN08	+	Rod	-	+	-	+	+	-	-	-	-	-	-	*Bacillus*
PSN09	-	Rod	+	-	-	-	+	-	-	-	+	-	-	*Klebsiella*
PSN10	+	Cocci	-	+	-	+	-	-	-	-	+	-	-	*Staphylococcus*
PDSN01	+	Rod	-	-	-	+	-	-	-	-	+	-	-	*Bacillus*
PDSN02	+	Cocci	+	-	+	-	-	-	-	-	-	-	-	*Staphylococcus*
PDSN03	+	Rod	+	-	-	-	+	-	-	+	+	-	-	*Bacillus*
PDSN04	+	Rod	+	+	+	-	-	-	-	-	+	-	+	*Bacillus*
PDSN05	+	Cocci	+	+	-	-	+	-	-	-	-	-	-	*Streptomyces*

4.4 Screening of the isolates

Table 4.3 shows the result of the screening of the isolates for lipase production on solid agar. 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 (3.0^{h} – 11 $.5^{a}$ mm). Isolate PSN07 had the highest lipase activity while isolate PSN09 had the lowest activity. Three (3) isolates including PSN02, PSN03 and PSN05 did not show any lipase activity throughout the incubation period. Isolate PSN07exhibited excellent lipase activity and was selected for further study. Isolate PSN07 was identified using morphological and biochemical characterizations as *Streptomyces* sp.

Table 4.4: Screening of bacterial isolates for lipase production

Isolates	Lipase activity (mm)
PSN01	9.5^{b}
PSN02	0.0
PSN03	0.0
PSN04	5.5^{f}
PSN05	0.0
PSN06	9.0^{c}
PSN07	11.5^{a}
PSN08	7.0^{c}
PSN09	3.0^{h}
PSN10	11.0^{a}
PDSN01	7.0^{d}
PDSN02	4.5^{g}
PDSN03	6.0^{e}

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

Figure 4.3 – 4.6 show the results of the physico-chemical parameters on lipase production by the selected isolate. These included carbon source, nitrogen source, incubation temperature and pH

4.5.1 Effect of carbon source on lipase production

Figure 4.3 shows the effect of various carbon sources on lipase production by isolate PSN07. There was significant difference ($p \leq 0.05$) in lipase production in the presence of different carbon sources. Lipase production ranged from (17.07^{g} – 65.95^{a} U/mL). Glucose (65.95^{a} U/mL) supported the highest lipase production follow in order by fructose (60.77^{b} U/mL), mannitol (47.52^{c} U/mL) and maltose (41.76^{d} U/mL) while the least production was recorded in starch (17.07^{g} U/mL).

4.5.2 Effect of nitrogen source on lipase production

Figure 4.4 illustrates the effect of different inorganic and organic nitrogen sources on the activity of extracellular lipases. There was significant difference ($p \leq 0.05$) in lipase production in the presence of different organic and inorganic nitrogen sources. Lipase production ranged from 34.48^{e} – 88.99^{a} U/mL. The highest level of lipase activity was recorded in the presence of yeast extract (88.99^{a} U/mL) followed in order by sodium nitrate (65.66^{b} U/mL), ammonium sulphate (64.51^{c} U/mL), and potassium nitrate (62.24^{e} U/mL), while Urea provided the least support (34.48^{e} U/mL).

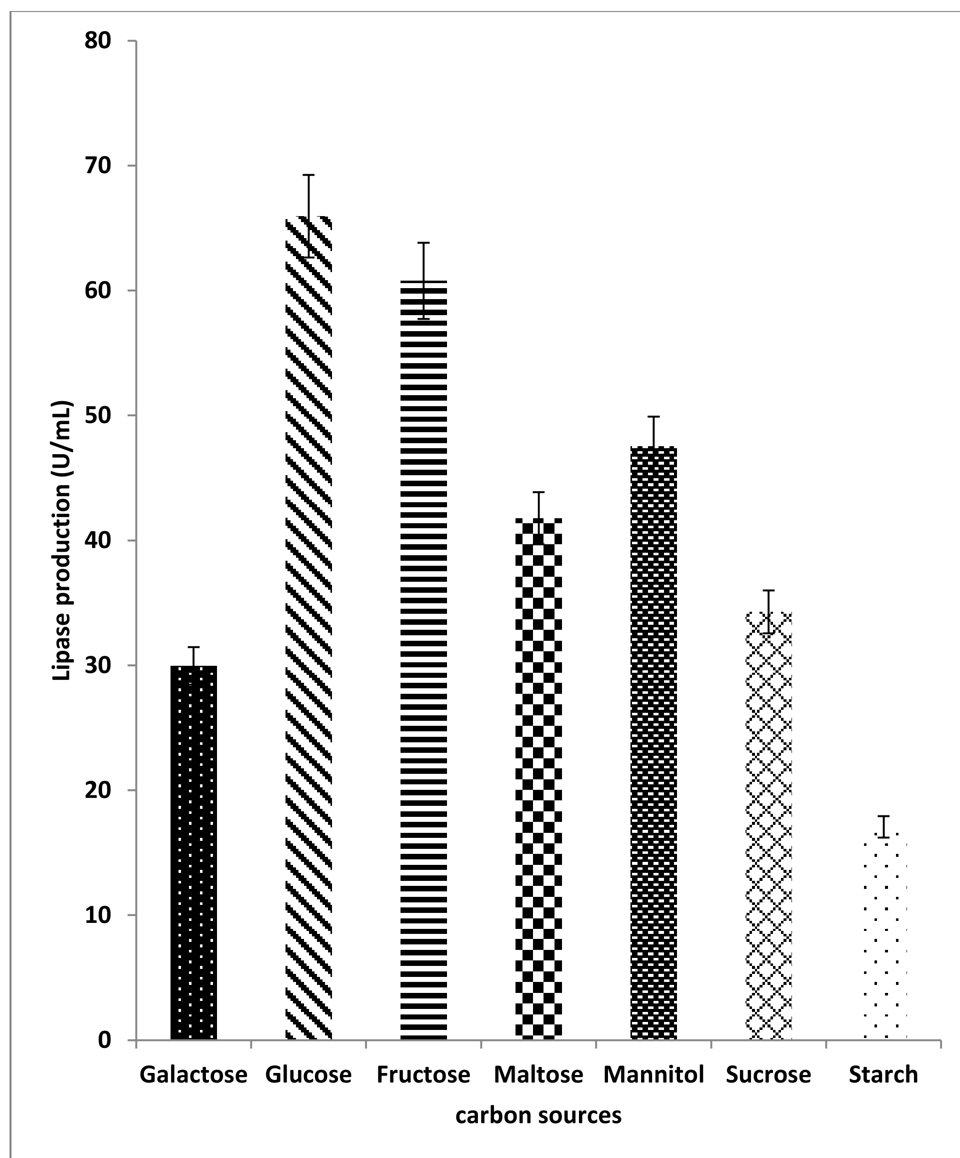

Figure 4.1: Effect of carbon sources on lipase production by *Streptomyces sp.* PSN07

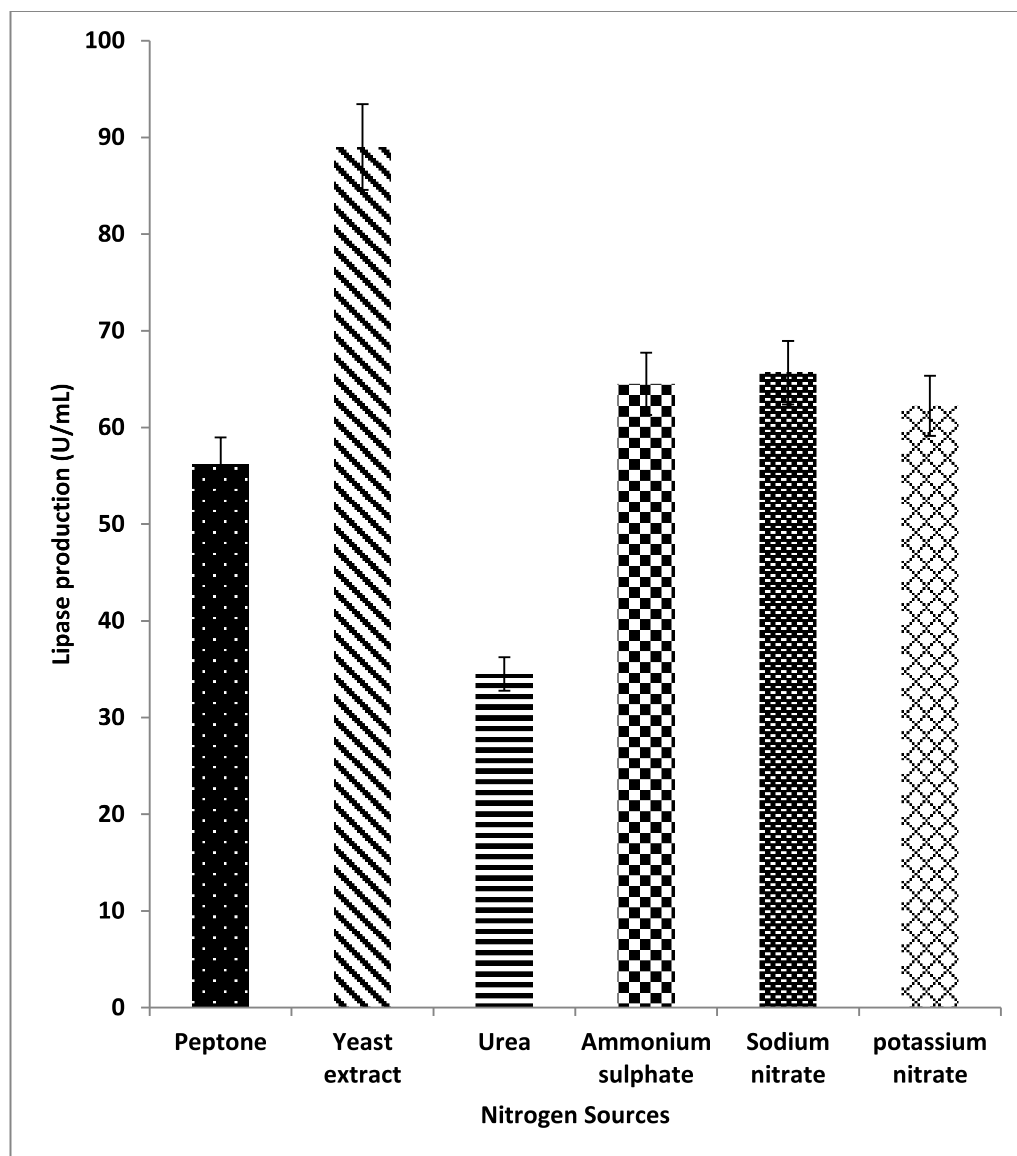

Figure 4.2: Effect of nitrogen sources on lipase production by *Streptomyces sp.* PSN07

4.5.3 Effect of temperature on lipase production

Figure 4.5 illustrates the effect of different incubation temperature on the activity of extracellular lipases. There was significant difference ($p \leq 0.05$) in lipase production in the presence of different incubation temperatures. Lipase production ranged from 24.19^{f} – 69.41^{a} U/mL. The highest level of lipase activity (69.41^{a} U/mL) was recorded at 30°C followed in order by 25°C (60.48^{b} U/mL), 35°C (54.14^{c} U/mL) and 40°C (27.07^{d} U/mL) while 50°C provided the least support (24.19^{f} U/mL).

4.5.4 Effect of pH on lipase production

Figure 4.6 illustrates the effect of different pH values on the activity of extracellular lipases. There was significant difference ($p \leq 0.05$) in lipase production in the presence of different pH values. Lipase production ranged from 21.02^{h} – 80.64^{a} U/mL. The highest level of lipase activity (80.64^{a}U/mL) was recorded at pH 6.0, followed in order by pH 7.0 (70.61^{b} U/mL), pH 8.0 (61.10^{c} U/mL) and pH 9.0 (55.47^{d}U/mL) while pH 3.0 (21.02^{h} U/mL) provided the least support.

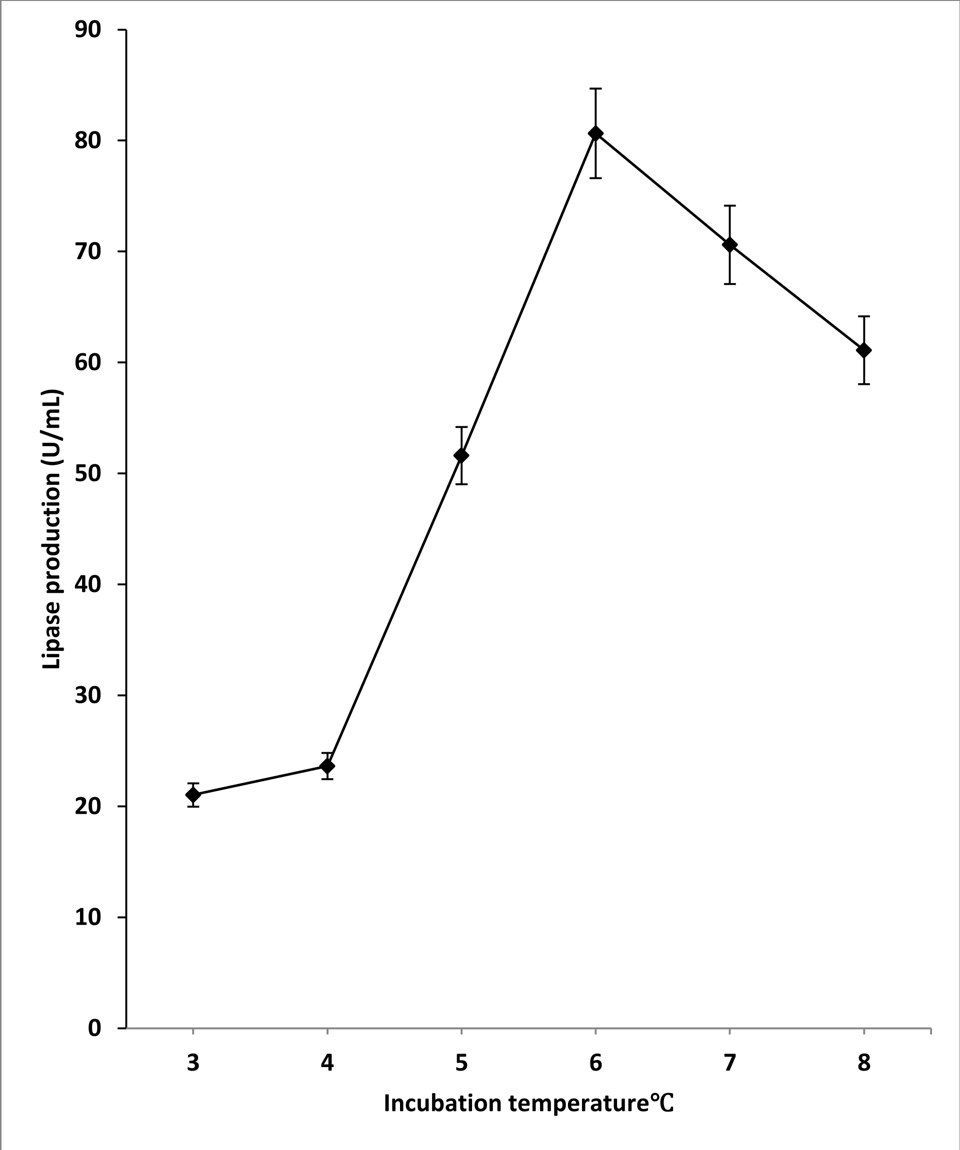

Figure 4.3: Effect of incubation temperature on lipase production by *Streptomyces sp.* PSN07

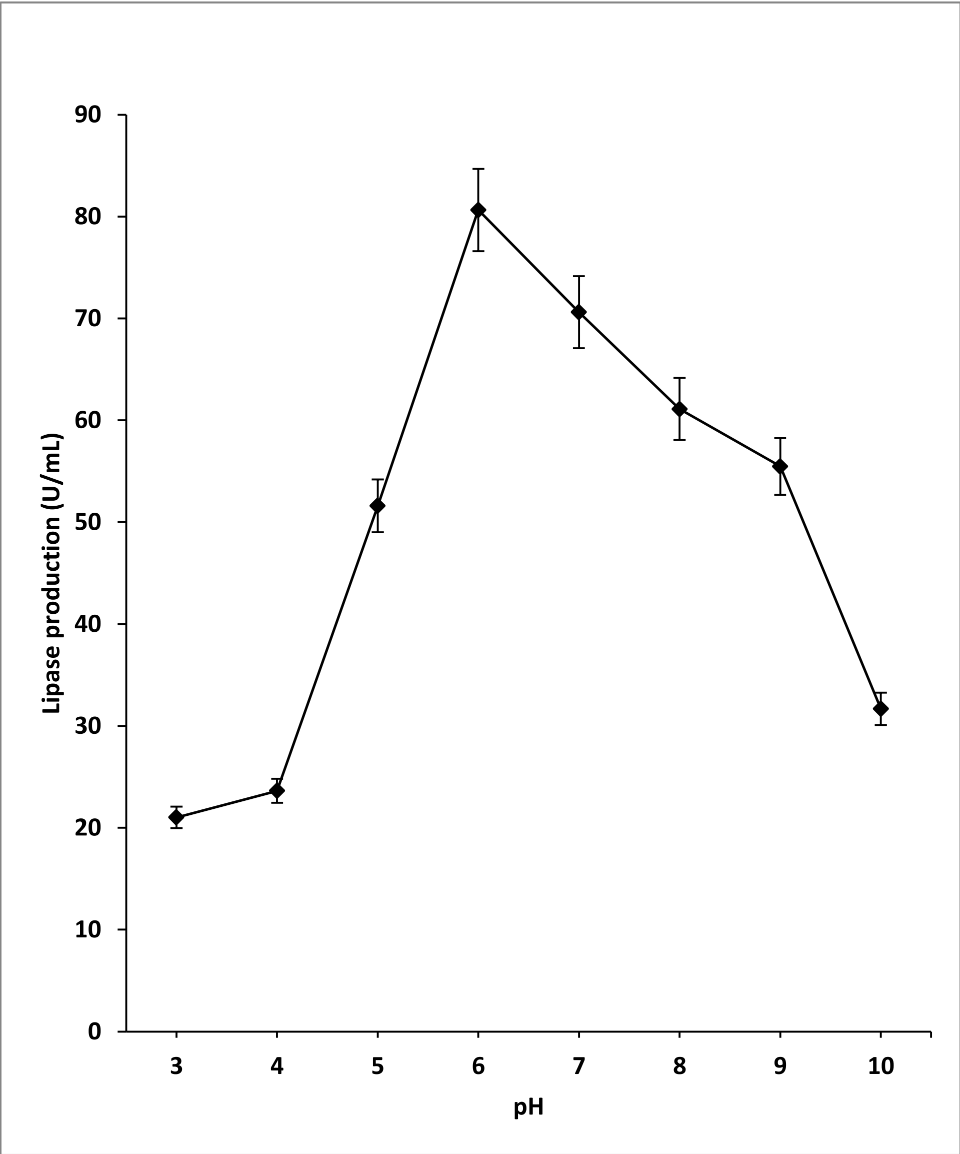

Figure 4.4: Effect of initial pH on lipase production by *Streptomyces* sp. PSN07

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

It has previously been reported that lipase-producing bacteria can be found in environmental samples (Dharmsthiti and Kuhasuntissak, 1998). Musa and Tayo (2012) discovered and described 13 taxa with lipolytic activity using soil samples polluted with different oils, expired food, and other wastes. Ankit *et al*. (2011) identified species of lipolytic bacteria from the genera *Pseudomonas* sp. and *Bacillus* sp. from severely polluted water samples from numerous rivers in the Bhopal region of India, as part of their research into lipolytic microorganisms for industrial application. When investigating microorganisms capable of utilizing palm oil as a substrate, Odeyemi *et al*. (2013) identified 32 lipolytic bacteria in the genera.

In this present study, *Streptomyces* was obtained from a soil samples obtained from a dump site and cooking area at the college of Humanities and Management sciences, Mountain Top University, Ogun state, Nigeria. Lipase is abundant in bacteria (PSN07 isolate). The morphological and biochemical characterization of the chosen isolate, PSN07, was observed and identified to be *Streptomyces* (Figure 4.2 and Figure 4.3). Although certain Gram-positive lipolytic bacteria, such as *Bacillus* sp., *Staphylococcus* sp., and *Clostridium* sp., have less expressive lipolytic activity than Gram-negative bacteria, their lipolytic activity is less expressive than Gram-negative bacteria (Rousenau and Jaeger, 2000). Nutritional and physiological variables such as temperature, pH, and carbon sources have an impact on bacterial extracellular lipases.

One of the most important factors for the increase in lipolytic activity is the carbon source, because lipases are of the inductive type, and their preferred substrates are long chain monounsaturated fatty acids (more than ten carbons), such as oleic acid (C18:1) found in greater quantity in olive oil (78%). The effect of various carbon sources on enzyme synthesis varies. The greatest lipase activity (65.95^{a} U/mL) was found in the presence of glucose as a carbon source in this research while the lowest lipase activity was (17.07^{g} U/mL) in the presence of starch as the carbon source as reported in (figure 4.1). Ulker *et al.* (2011) reported similar results of maximal

lipase activity (1.25 g/L) when glucose carbon sources. Rehman *et al*. (2019) found similar results using different carbon sources for lipase synthesis by *Pleurotus ostreatus*; when glucose was used as a carbon source, lipase activity reached its peak (2654 U/gds).

Contrary to the results of Ulker *et al.* (2011) which reported results of maximal lipase activity (1.25 g/L) with peptone as nitrogen sources, the highest lipase activity (88.99[a] U/mL) in this study was observed with yeast extract as the nitrogen source (Figure 4.2) and the lowest lipase production (34.48[f] U/mL) was observed. Sharma *et al*. (2001) reported that peptone and yeast extract are the best nitrogen sources for microbial lipases when compared to other nitrogen sources like meat extract, tryptone, or wheat bran, found that. However, Kebabci and Cihangir (2012) reported that adding ammonium compounds increased lipase synthesis the most. Similar to these Lopes *et al.* (2016) reported that when the fermentation medium was added with ammonium sulphate, Lopes *et al.* (2016) found the highest lipase activity (486 U/g). The nitrogen supply utilized in this study, yeast extract, can thus be inferred to be a lipase inducer.

At pH 6.0, the highest lipase production (80.64[a]U/mL) was observed and the lowest was found to be (21.02[h] U/mL) (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. 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 6.0 media by *P. mirabilis.*

The optimal temperature for lipase synthesis was found to be 30℃ in this study. The highest lipase activity in this study was found to be (69.41[a] U/mL) with the temperature at 30℃ followed by (60.48[b] U/mL) at 25℃ and the least lipase synthesis at which lipase grew was (24.19[f] U/mL) at 50℃ as shown in (figure 4.3). According to Ugras and Uzmez (2016), bacterial lipases may have optimum manufacturing activity at low and high temperatures. Similar to results from Gutarra *et al.,* (2009), at 35°C the lipase enzyme showed high activity. Temperature is a vital parameter that must be managed, and it differs amongst organisms. Temperature affects extracellular enzyme secretion by altering the physical characteristics of the cell membrane. Low

temperatures also inhibit microorganism development, resulting in lower enzyme output (Kumar *et al.,* 2011; Oliveira *et al.,* 2016).

5.2 CONCLUSION

In conclusion, soil samples from contaminated areas were collected and tested for the production of bacterial lipase. On solid agar, isolate PSN07 had the highest lipase activity among the 15 isolates tested, as such was selected for further analysis. The results of the morphological and biochemical characterizations of the isolate showed the probable identity to be *Streptomyces* sp. Among the carbon and nitrogen sources studied, glucose, yeast extract and sodium nitrate supported the highest lipase production. *Streptomyces* sp. PSN07 is a good source of lipase production that may be harnessed for further researches and developments.

5.3 RECOMMENDATIONS

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

- Lipase produced by *Streptomyces* PSN07can be used for the industrial production of biotechnologically important products such as food, beverages, leather, textiles, detergents, and soaps.
- Cheaper sources of carbon and nitrogen can be employed for further reduction in production cost.
- Strain improvement can be conducted on *Streptomyces sp.* PSN07 to improve the yield of lipase, as such, increase its industrial value.

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Appendix: Statistical Analysis

Table A: Effect of carbon sources on lipase production by *Streptomyces sp.*

Carbon sources	Lipase production (U/mL)
Galactose	29.95[f]
Glucose	65.95[a]
Fructose	60.77[b]
Maltose	41.76[d]
Mannitol	47.52[c]
Sucrose	34.27[e]
Starch	17.07[g]

Table B: Effect of nitrogen sources on lipase production by *Streptomyces sp.*

Nitrogen Sources	Lipase production (U/mL)
Peptone	56.16[e]
Yeast extract	88.99[a]
Urea	34.48[f]
Ammonium sulphate	64.51[c]
Sodium nitrate	65.66[b]
Potassium nitrate	62.24[d]

Table 3: Effect of incubation temperature on lipase production by *Streptomyces sp.*

Incubation temperature (^{o}C)	Lipase production (U/mL)
25	60.48[b]
30	69.41[a]
35	54.14[c]
40	27.07[d]
45	27.04[e]
50	24.19[f]

Table 4: Effect of initial pH on lipase production by *Streptomyces sp.*

pH	Lipase production (U/mL)
3.0	21.02[h]
4.0	23.62[g]
5.0	51.60[e]
6.0	80.64[a]
7.0	70.61[b]
8.0	61.10[c]
9.0	55.47[d]
10.0	31.68[f]