# Original Research Article
# Optimization, Purification and Characterization of Lipase by *Pseudomonas aeruginosa* Isolated from Palm Oil Processing Cottage Industries in Ekiti State, Nigeria




## ABSTRACT

**Introduction:** The importance of palm oil in the country is due to the versatility of applications of their by-products, such as cooking oil, margarines, soaps, detergents, cosmetics, lubricants, biofuels and electric power, among many others.
**Aims:** This study investigates the optimization, purification and characterization of lipase produced by *Pseudomonas aeruginosa* isolated from palm oil processing cottage industries.
**Methodology:** Effluent samples were taken from a depth of 10-15 cm in six different locations within Ekiti State, Nigeria. Bacteria species isolated from the effluents and identified using standard microbiological techniques and molecular characterization. The physiochemical characterizations of the isolates were carried out and the process parameters (nitrogen, carbon, temperature and pH) were optimized. Partial purification of crude extract from *P. aeruginosa* was carried out by ammonium sulphate precipitation and dialysis.
**Results:** The strains of molecularly identified bacteria were *Pseudomonas aeruginosa* AE016853.1; *P. syringae* CP019871.1 and *P. putida* JQ782512.1. The pH of the inoculum ranged from 6.23 to 6.01 oil and grease contents ranged from 149.6 to 114.2 mg/l while the biochemical oxygen demand ranging from 42760 to 33800 mg/l. The chemical oxygen demand ranged from 1.9 to 1.8 mg/L. *Pseudomonas aeruginosa* exhibited considerable enzyme activity ranging from 75.33 to 22.44 µmol/min/mL. High lipase activity of 2.68 µmol/min from *P. aeruginosa* was recorded from a medium supplemented with palm oil, while the medium supplemented with goya oil had least lipase activity 2.31 µmol/min. The lipase activity increased with increase in incubation temperature at 50$^{o}$C with relative activity of 99.9 % before it declined to 15 % at 80$^{o}$C.
**Conclusion:** The lipase produced from the *P. aeruginosa* exhibited high lypolytic activities. It can therefore, be a biodegradable agent for industrial applications especially in the waste treatment especially of waste rich in oil and fats. Thus, lipase may act as a biodegradable agent for industrial applications.


*Keywords: Characterization, Lipase, Optimization, Palm oil, Pseudomonas aeruginosa*

## 1. INTRODUCTION

Oil palm (*Elaeis guineensis* J acg) belonged to the family of Palmae and it was an indigenous plant in West Africa according to Aghalino [1]. Oil palm trees were originally inter breeding in traditional agricultural production systems along with other annual and perennial crops. Sridhar and AdeOluwa [2] reported that *Elaeis guineensis* J acq have been domesticated for over 5,000 years ago in Nigeria. Oil Palm is often regarded as the most

productive and economic oil crop in the world [3]. The cultivation and processing of oil palm is a source of livelihood for many rural dwellers in Nigeria. A hectare of oil palm typically generates between 10 to 35 tonnes of fresh fruit bunch (FFB) per year [4].

Palm oil is edible oil derived from the fleshy mesocarp of the fruit of oil palm tree [5]. It is an organic liquid that is light-yellow to orange or brownish in colour due to the presences of fat soluble carotenoids [6]. It is a versatile raw material for both food and non-food industries. Direct applications include the use of crude palm oil in the production of biodiesel, cooking ingredient, ingredient for detergents, soaps, lipsticks, waxes and polish bases in a condense form, an ingredient in margarine production and in most confectionaries. It is used to reduce friction during manufacturing of steel [2]. It is also used in the production of pharmaceutical products and engine lubricants [7].

Palm oil mill effluent contains substantial quantities of solids, both suspended solids and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L respectively. These solids are commonly named palm oil mill sludge (POMS). The solid waste that are produced in the process of extraction are the leaves, trunk, decanter cake, empty fruit bunches, seed shells and fiber from the mesocarp [8].

Fresh POME is a hot, acidic (pH between 4 and 5), brownish colloidal suspension containing high concentrations of organic matter, high amounts of total solids (40,500 mg/L), oil and grease (4,000 mg/L) COD (50,000 mg/L) and BOD (25,000 mg/L) [9]. According to Vairappan and Yen [10]. The raw or partially treated POME has an extremely high content of degradable organic matter as no chemicals were added during the oil extraction process.

There are lots of organisms that are associated with palm oil mill effluent POME. This includes *Micrococcus* species, *Bacillus* species, *Pseudomonas* species and *Staphylococcus aureus* [11]. Investigations have been conducted on aerobic digestion process for the treatment of oil and grease present in POME. The major problems lie in the establishment of the most suitable microbial population for POME waste to be treated and some aerobic treatment approaches include: degradation of POME using a tropical marine yeast (*Yarrowia lipolytica*) NCIM 3589 in a lagoon, trickling filter (TF) and rotating biological contactors (RBC) [12].

Microorganisms that produce lipases have been found in diverse habitats such as industrial wastes, oil processing factories, dairies, soils contaminated with oil, decaying oil seeds, decaying foods, compost heaps, coal tips and hot springs [13]. These microorganisms include bacteria, fungi, yeasts and actinomyces [14]. Bacterial lipases are glycoproteins, though some extracellular bacterial lipases are lipoproteins [15]. Among bacteria, the following: *Achromobacter* spp*., Alcaligenes* spp*., Arthrobacter* spp*., Pseudomonas* spp*., Staphylococcus* spp*. and Chromobacterium* spp. have been exploited for lipase production [15].

Several microbial species with the ability to remediate POMEs have been identified. These include species of *Pseudomonas, Bacillus, Alcaligenes, Candida*, *Saccharomyces*, *Pichia* and *Yarrowia* have been identified [16]. There are only few studies on the degradation of these wastewaters using native aerobic microbial consortia consisting of microorganisms isolated from highly polluted wastes. Moreover, the use of native microorganisms for the remediation of POMEs might improve the adaption, survival and degrading ability of microorganisms on effluents containing high amounts of toxic contaminants.

Microbial lipases play a vital role in the hydrolysis of long chain triglycerides to intermediate and short chain di and monoglycerides, free fatty acid and glycerol [17]. Lipases occur

widely in nature and have been found in many species of animals [18], plants [20], bacteria, yeasts and fungi [20]. Microbial lipases are preferred because they are stable, safe and more useful than those derived from plant and animals because of the great variety of catalytic activities available, ease of genetic manipulation and regular supply due to absence of seasonal fluctuations [21]. Therefore, the objective of this study is to investigate the optimization, purification and characterization of lipase by *P. aeruginosa* isolated from palm oil processing cottage industries in Ekiti State, Nigeria.

## 2. MATERIAL AND METHODS

### 2.1 Study Area

The research covered some cottage industries in Ekiti State, Nigeria. The palm oil mill effluents (POME) were collected from six different palm oil processing sites namely (Ago Aduloju, Ado Ekiti (S1), Aba-Medi, Ijan (S2), Aba-Ilupeju, Ijan (S3), College road, Ikere-Ekiti (S4), Sawmill Isinbode (S5) and Sajowa farm, Aramoko-Ekiti (S6)) all in Ekiti State, Nigeria. Ekiti State is located in the tropical belt of South-Western part of Nigeria. The sample site descriptors and GPS coordinates (via Google Earth) were recorded and documented in the sample site data collection sheet as $7^{o}25'18.25N\ 6^{o}2'45.09E$. Ekiti State comprises 16 Local Government areas and 3 Geographical zones. Coordinates of the areas where the samples were collected is represented on Ekiti State map (Figure 1).

### 2.2 Collection of Samples

Samples were taken from a depth of 10-15 cm, placed in a sterile polythene bags with appropriate labeling and then transferred to the Microbiology laboratory, the Federal University of Technology, Akure, Nigeria for further microbiological and chemical analyses. The physiochemical characteristics of the samples were determined in accordance with the standard methods published by American Public Health Association [22].The media used include nutrient agar and MacConkey agar. These were prepared and sterilized according to the manufacturer's specifications. All the media were sterilized in an autoclave 121°C for 15 minutes.

### 2.3 Sample Preparation and Isolation of Bacteria

Ten mL (10 mL) each of the palm oil mill effluents (POME) samples was collected with 100 mL sterile distilled water and serially diluted up to the appropriate dilutions ranging from $10^{-1}$ – $10^{-5}$. From the diluents, 0.1mL of the culture was taken from $10^{-3}$, $10^{-4}$ – $10^{-5}$ dilutions, it was dispensed into different petri dishes for incubation at $37^{o}C$ for 24 hours.

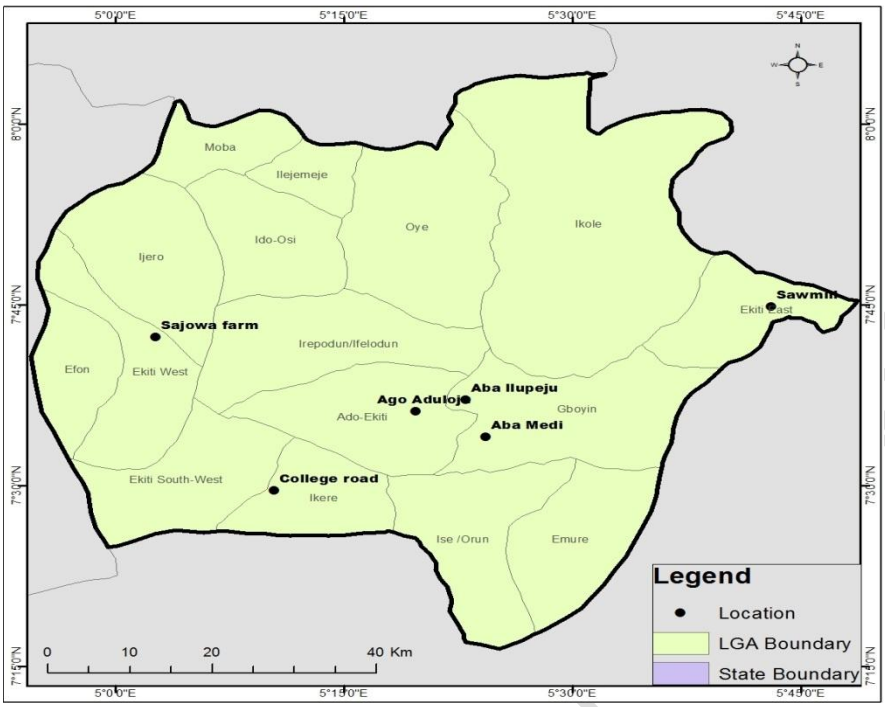


**Figure 1. Map of Ekiti- State showing location of sample collection**

## 2.4 Pure Culture Preparation

After incubation, the distinct colonies formed on the plates were purify by repeated streaking onto plates containing fresh media under aseptic condition using flamed sterilized inoculating loop and inoculating needle. The sub cultured plates were further incubated aerobically at 37°C for 24 hours. The pure isolates were stored inside Bijou bottles slants containing about 5 mL of sterilized double strength media and kept inside refrigerator at $4^{o}$C for further characterization and identification.

## 2.5 Assay for lipase

Lipase activity of the isolate was quantified as described by Cho *et al*. [23]. The lipase activity was assayed in the reaction mixture containing 180 µL of solution A (0:062 g of p-NPP in 10 mL of 2-propanol, sonicated for 2 minutes before use), 1620 µL of solution B (0.4% triton x100 and 0.1 % Arabic gum in 50 mM TrisHCl, pH 8.0) and 200 µL enzyme sample. The mixture and the control tubes were incubated at $37^{o}$C for 15 minutes at room temperature 28±$2^{o}$C. After incubation for 5 minutes in a water bath for colour development, the tubes were removed from the water bath. Changes in colour to pink indicated the release of p-nitrophenol (pNP) and the optical density of the solution was measured against the temperature inactivated enzyme used as blank at 410 nm wavelength (Genesys 20 Spectrophotometer). One unit of lipase activity is equivalent to as the amount of lipase releasing 1 µmol of p-nitrophenol (pNP) per minute by 1 ml of enzyme [24].

## 2.6 Optimization of Cultural Conditions for Lipase Production

### *2.6.1 Effect of Palm oil mill effluent concentration on lipase activity*

The effect of POME concentration on lipase activity was investigated by diluting the POME with distilled water to 25, 50, 75 and 100. Ten milliliter (10 mL) of the inoculum suspension in a 500 ml Erlenmeyer flask containing 90 ml of POME, this was done for various dilutions and undiluted POME maintained at 210 rpm. Incubation was carried out at 30 °C for 7 days on a rotary shaker maintained at 210 rpm. Samples were taken each day and filtered through Whatman No. 1 filter paper to remove mycelial fragments and other suspended solids. The bacteria cell filtrates were evaluated for pH, lipase activity and COD [25] Lipase activity of the supernatant was determined as earlier described in assay of lipase.

### *2.6.2 Effect of nitrogen source on lipase production*

Effect of nitrogen source on the production of lipase was carried by adding different nitrogen sources to the production medium while keeping other component of the basal medium constant. The medium was autoclaved at 121$^{o}$C at 15 atm. for 20 minutes. The production medium was inoculated with 10 mL of bacterial isolate. The culture was incubated in a rotary shaker incubator at 30$^{o}$C for 48 hours at 210 rpm during which 5 mL of the mixture was taken at 12 h intervals kept in the freezer. The samples collected were centrifuged at 5,000 rpm at 4$^{o}$C for 20 min. The progressive growth of cells was determined by measuring the absorbance of the collected fractions at 600 nm. The supernatant collected after centrifugation was preserved at 4$^{o}$C until required, while the residue was discarded. Lipase activity of the supernatant was determined as earlier described in assay of lipase.

### *2.6.3 Effect of carbon source on lipase production*

Effect of carbon source on the production of lipase was carried by adding different carbon sources to the production medium while keeping other component of the basal medium constant. The medium was autoclaved at 121$^{o}$C at 15 atm. for 20 minutes. The production medium was inoculated with bacterial isolate. The culture was incubated in a rotary shaker incubator at 28$^{o}$C for 48 h at 210 rpm during which 5 ml of the mixture was taken at 12 h intervals and kept in the freezer. The samples collected were centrifuged at 5,000 rpm at 4$^{o}$C for 20 min. The progressive growth of cells was determined by measuring the absorbance of the collected fractions at 600 nm. The supernatant collected after centrifugation was preserved at 4$^{o}$C until required, while the residue was discarded. Lipase activity of the supernatant was determined as earlier described in assay of lipase.

### *2.6.4 Effect of temperature on lipase production*

The effect of temperature on the production of the lipase was studied by the method of Kumar *et al*. [25]. One milliliter (1 mL) of inoculum from 18 hour-old seed culture was inoculated into the standard basal medium containing 10% POME at different temperatures varying from 30 to 90$^{o}$C. The culture was incubated in a rotary shaker incubator at 28$^{o}$C for 36 hours at 150 rpm during which 5 mL of the mixture taken at 6 h intervals and was kept in the freezer. The samples collected were centrifuged at 5,000 rpm at 4$^{o}$C for 20 minutes. Lipase activity of the supernatant was determined as earlier described in assay of lipase.

### *2.6.5 Effect of pH on lipase production*

Effect of pH on the lipase production was studied according to the method described by Aderiye *et al*. [26]. One milliliter (1 mL) of inoculum from 18 hour-old culture was inoculated into the standard basal medium containing 10 % POME and the pH of the medium was adjusted to different pH varying from 3 to 9 using the following buffers (all at 50 mM): acetat-acetic acid (3-5), sodium phosphate (pH 6.0 and 7.0), Tris–HCl (pH 8.0 and 9.0). The culture was incubated in a rotary shaker incubator at 28$^{o}$C for 48 hours at 200 rpm during

which 5 mL of the mixture taken at 6 h was kept in the freezer. The samples collected were centrifuged at 5,000 rpm at 4$^{o}$C for 20 minutes. Lipase activity of the supernatant was determined as earlier described in assay of lipase.

## 2.7 Lipase Purification

### *2.7.1 Ammonium sulphate precipitation*

The crude enzyme was precipitated by adding ammonium sulphate to 60% saturation. The mixture was centrifuge at 10,000 g for 15 mins at 4°C. The precipitates were then re-suspended in 50 mM Tris-HCl, pH 8.0 and dialyzed against the same buffer overnight at 4°C with three buffer changes. Dialyzed enzyme solution was concentrated with 4 M sucrose solution to get concentrated enzyme free from salt and metal ions [27].

### *2.7.2 Purification of lipase*

The concentrated enzyme solution was put in a DEAE-sephadex A50 column (1.5 cm diameter × 50 cm length) and pre-equilibrated with 50 mM Tris-HCl, pH 8.0. The elution was carried out by 0-0.5 M NaCl in the buffer at a flow rate of 5 mL/30 minutes at room temperature (28$^{o}$C) and 5 mL fractions were collected. The protein-containing fractions were assayed for lipase activity. Fractions containing lipase activity were pooled, concentrated and applied on a sephadex G-100 column (1.5 cm diameter × 75 cm length) pre-equilibrated with 50 mM Tris-HCl, pH 8.0. Five (5 mL) fractions were collected at a flow rate of 20 mL/hour at room temperature (28$^{o}$C). The protein content of fractions was determined by measuring optical density at 280 nm. The protein-containing fractions were assayed for lipase activity. Fractions containing lipase activity were pooled and concentrated for further analysis [27].

## 2.8 Characterization of Purified Lipase

### *2.8.1 Effect of temperature on activity and stability of purified lipase*

The temperature optimum for the enzyme was determined in the range 30 to 80°C, at pH 7.4. The enzyme stability at different temperatures was studied by incubating the enzyme in 50 mM Tris-HCl, pH 8.0 at different temperatures for 2 hours, followed by the activity estimation at 37 °C [28].

### *2.8.2 Effect of pH on activity and stability of purified lipase*

The effect of pH on enzyme activity was studied by incubating the enzyme for 2 hrs with p-nitrophenyl acetate substrate, prepared in different buffers in the pH range 3.0 to 9.0.The buffers used are sodium acetate (pH 3-5), sodium phosphate (pH 6-7) and Tris-HCl (pH 9) [28].

### *2.8.3 Effect of metal ions on purified lipase activity*

The effect of metal ions was determined by estimation of the activity in presence of 10 mM solution of metal salts. The enzyme was incubated in presence of metal ions for 15 min followed by estimation of activity by p-Nitrophenol liberation [29].

## 2.9 Evaluation of effectiveness of the purified enzyme

A piece of cotton white fabric was cut to equal size (3 inch by 3 inch) and placed in a small beaker, a drop of palm oil was placed on the white cloth. Then few drops of purified enzyme was also placed on the oil. It was agitated using stirring rod and left for one hour (1hr) after which the fabric was rinsed and air dried for 24 hours for assessment of the stain removing ability of the enzyme. The control was also done without the addition of the purified enzyme on the white fabric.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

Figure 2 shows the effect of different incubation periods on the lipase activity, protein content and growth of *Pseudomonas aeruginosa* in solid state fermentation. The lipase activity, protein content and growth increased with increase in fermentation periods and beyond the optimal period a decline was observed. *Pseudomonas aeruginosa* exhibited its highest lipase activity (80.67 U/ml) at 24 hours of incubation, while maximum protein content of 20.38 mg/ml was attained at 18 hours of fermentation. The optimum growth was attained at 30 hours of incubation.

Figure 3 shows the effect of nitrogen source on lipase production from *P. aeruginosa*. Fermentation medium supplemented with ammonium nitrate had the highest lipase activity 2.81 mmol/min, while the medium supplemented with potassium nitrate recorded the least lipase activities 1.50 mmol/min.

Figure 4 shows the effect of carbon source on lipase production from *P. aeruginosa*. High lipase activity of 2.68 mmol/min from *P. aeruginosa* was recorded from a medium supplemented with palm oil, followed by groundnut oil (2.59 mmol/min), while the medium supplemented with goya oil had least lipase activity 2.31 mmol/min.

The effect of incubation temperatures ($30^oC$ - $90^oC$) on the biosynthesis of lipase from *P. aeruginosa* is presented in Figure 5. The lipase activity increased with increase in incubation temperature until maximum activity was attained at $50^oC$ with 3.42 mmol/min at 96.85% relative activity before it decline.

Figure 6 shows the effect of initial pH range 3.0 to 9.0 on the production of lipase by *P. aeruginosa*. Lipase production increased with the increase in pH until the maximum activity of 3.14 U/ml with relative activity 99.99 mmol/min reached at pH 8.0, beyond this, the lipase activity reduced.

The ammonium sulphate-dialysate fraction on Sephadex A-50 produced from ion exchange yielded one prominent activity peak designated A and minor peaks designated with B, C and D, while bound proteins obtained from the minor peaks were eluted with linear salt (NaCl) gradient (Figure 7). Lipase activities were detected between tubes 8.0 to 27.0 with optimum activity 2.5 mmol/min (A), 1.16 mmol/min (B); and 43.0 to 50.0 with optimum activity 1.11 mmil/min (C), 0.89 mmol/min (D). Further fractionation on Sephadex G-150 produced one activity peak (A) with optimum activity (6.84 mmol/min) (Figure 8). The summary of lipase purification processes revealed that the purified lipase was obtained in three folds with approximately total protein recovery of 7% with enzyme activity 129.15 µmol/min/ml and specific activity 0.80 µmol/min/mg. The purification fold increased from 1% to 6% due to desalting of the enzyme.

Figure 9 shows the effect of temperature on activity of purified lipase from *P. aeruginosa*. The lipase activity increased with increase in incubation temperature. The optimum

temperature with relative activity 67% was attained at 50$^{o}$C. Beyond this, the lipase activity decreased. The lipase activity was very stable at 30$^{o}$C and relatively thermostable at temperatures 40$^{o}$C to 60$^{o}$C and retains more than 78% of its residual activity after 2 hours of incubation at 40$^{o}$C. The lipase activity at 70$^{o}$C dropped drastically with 55.48% residual activity at 120 minutes (Figure 10).

The effect of pH on the activity of purified lipase from *P. aeruginosa* is illustrated in Figure 11. The optimum pH was attained at pH 8.0.The enzyme activity was relatively stable at pH 4.0 and 5.0 between 0 and 120 minutes (Figure 12).

All the metal ions exhibited varied activities on the purified lipase from *P. aeruginosa* (Figure 13). The lipase activity was enhanced by $Na^{+}$, $Al^{3+}$, $Ca^{2+}$ $Mg^{2+}$ and $K^{+}$, while varied degree of inhibition was exhibited by $Zn^{2+}$, $Mn^{+}$, $Hg^{+}$ and $Pb^{2+}$. The lipase activity was almost lost with $Hg^{+}$ and $Pb^{2+}$ at approximately 9% and 5% relative activity respectively.

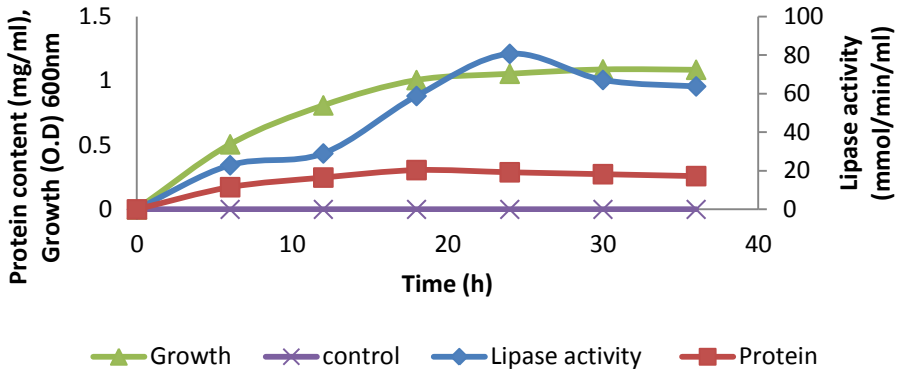


**Figure 2: Production of lipase by isolate (*Pseudomonas aeruginosa*)**

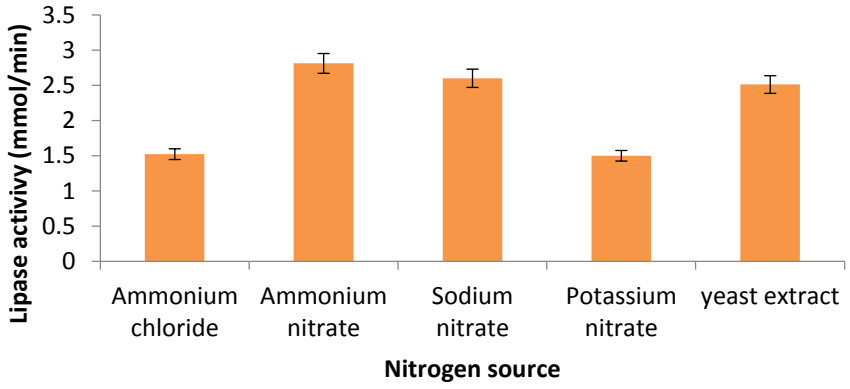


**Figure 3: Effect of nitrogen source on lipase production by the isolate**

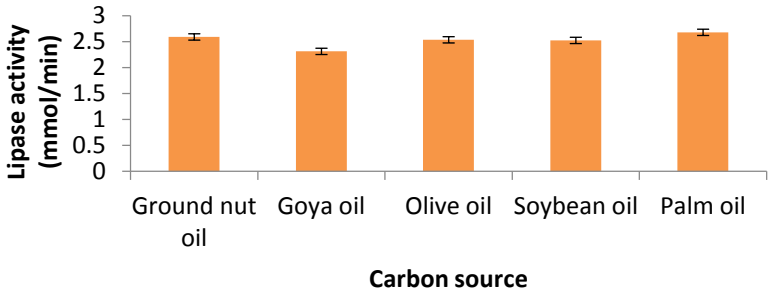


**Figure 4: Effect of carbon source on lipase production**

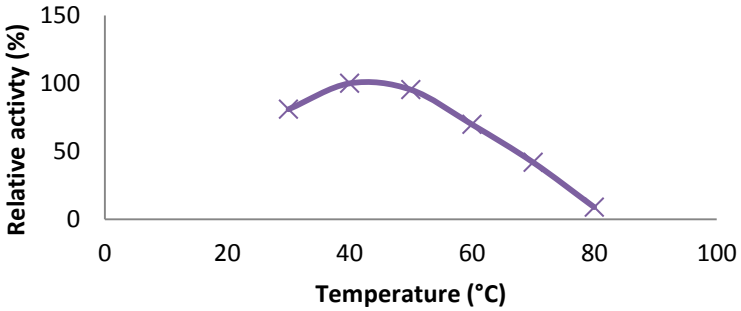


**Figure 5: Effect of temperature on lipase production**

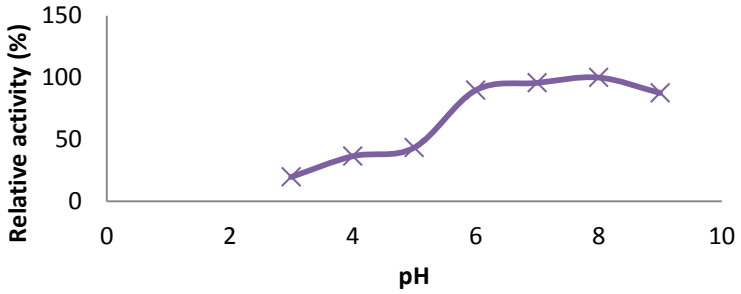


**Figure 6: Effect of pH on lipase production**

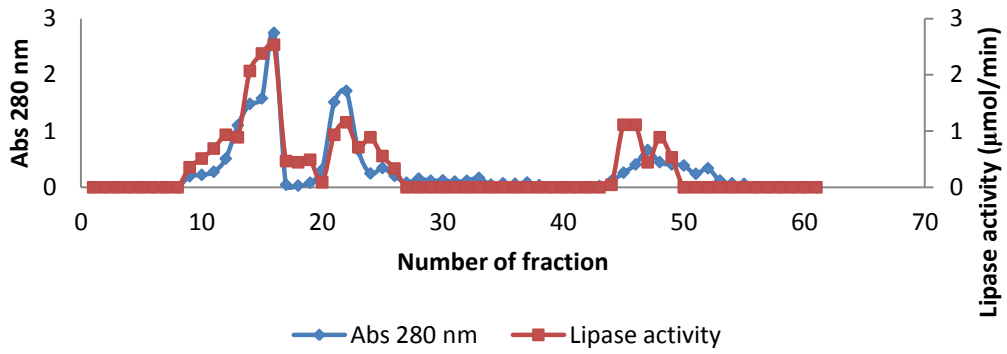


**Figure 7: Purification by ion exchange chromatography of crude lipase from *P. aeruginosa***

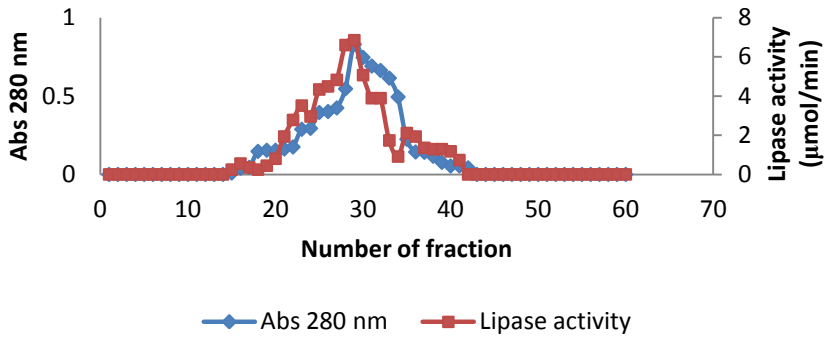


**Figure 8: Purification by gel filtration of crude lipase from *P. aeruginosa***

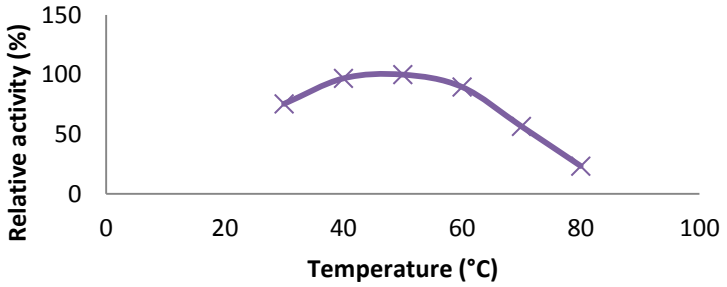


**Figure 9: Effect of temperature on activity of purified lipase from *Pseudomonas aeruginosa***

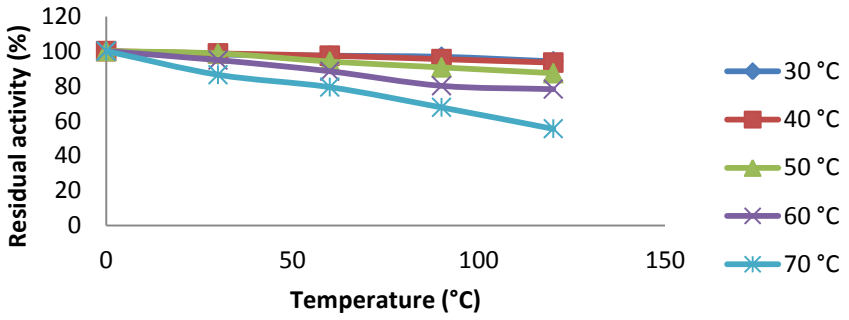


**Figure 10: Effect of temperature on the stability purified lipase from *Pseudomonas aeruginosa***

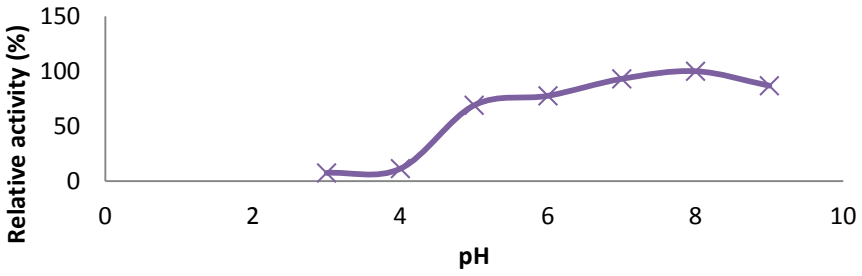


**Figure 11: Effect of pH on activity of purified lipase from *Pseudomonas aeruginosa***

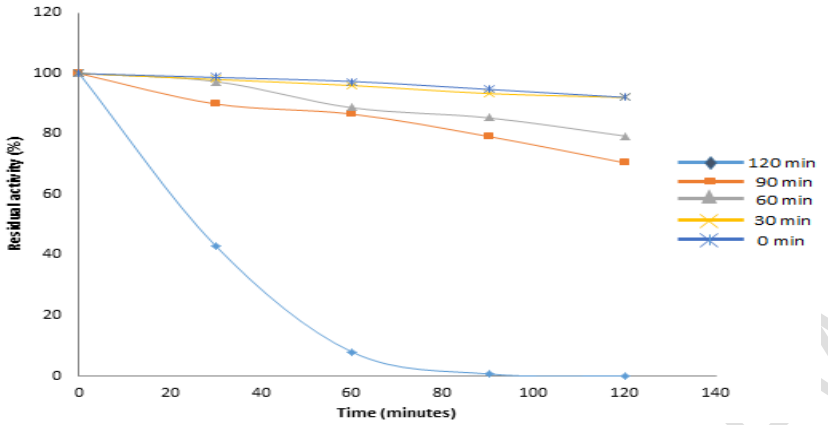


**Figure 12: Effect of time on stability of purified lipase from *Pseudomonas aeruginosa***

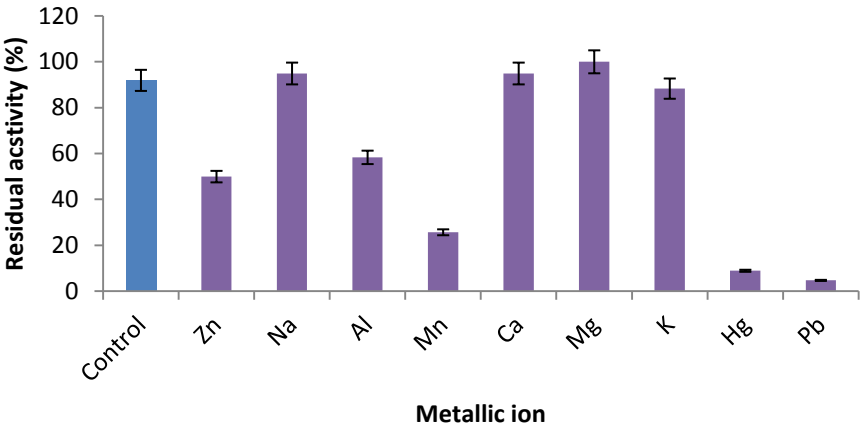


**Figure 13: Effect of metal ion on activity purified lipase from *Pseudomonas aeruginosa***

## 3.2 Discussion

The bacteria isolated in this study include *Bacillus licheniformis*, *Citrobacter freundii*, *Bacillus cereus* and *Pseudomonas aeruginosa*. This findings is in line with report of Odeyemi *et al*. [30]; Ohimain *et al*. [31]; Izah and Ohimain, [32] who reported similar bacteria from palm oil mill effluent in this study. The bacteria isolated from the palm oil mill effluents might probably originate from the palm oil processing site where there is influx of leachates of water, processing materials like woods which harbour microorganisms and human activities [33].

The primary screening of lipase-producing bacteria was based on the halo zones around the colony on the plate containing 0.1% tributyrin. The bacteria isolated exhibited varied lipase activities. Findings on the lipase-producing bacteria have been reported by different researchers [26, 34, 35]. The ability of these bacteria to secrets considerable amount of lipolytic enzyme into the culture medium suggests that it can be harnessed for various industrial processes. The production of lipase in the culture medium in this study is an indication that the enzyme is secreted extracellularly [36].

The *P. aeruginosa* isolated from the pam oil mill effluents exhibited lipase activities in submerged state fermentation with variation in their rate of enzyme production. The variation observed in the enzyme activity of the lipase-producing *P. aeruginosa* might be attributed to the source of isolation and genetic make-up [26]. Also, the variation observed in the protein content by the isolate in submerged state fermentation could be attributed to the production of variety of enzymes (amylases, cellulases, proteases, mannanase, linamarase and xylanases) in addition to the enzyme been examined in this study [37].

Optimization time is one of the major factors for lipase production by most bacteria [36]. Usually, incubation time cause a great effect for enzymes production and other physiological functions of microbial biomass. The optimum incubation time was attained at 24 hours for lipase production by *P. aeruginosa* when monitored for 40 hours with lipase activity (80.67 U/mL). The decrease observed in lipase activity beyond optimum incubation time might be due to the depletion of nutrients and accumulation of other by-products like proteases in the fermentation medium initiating the autolysis of cells and inactivation of secretory machinery of the enzymes [38]. Maximum lipase activity (5.111 Mol) at fifth day of incubation has been reported [38].

Changes in the pH induce morphological changes and direct impact on enzyme biosynthesis. The sensitivity of lipase yield depends on the pH value. Most microorganisms grow and produce enzyme at minimum, optimum or maximum pH range. The pH ranges for enzymes production vary from one microorganism to another [39]. The optimum pH value for lipase production from *P. aeruginosa* was obtained at pH 8.0. pH 7.0 has been reported for lipase production from *B. pumilis* [39]. A pH 6.0 has been reported for lipase produced from *B. pumilis* [40].The varying pH in this study might be due to environmental factors such as time of collection of samples and site of samples collection.

The temperature requirements by most bacterial isolates account for the mesophilic nature of temperature optimal for the lipase production; temperature is one of the major factors that determine the enzyme yield and activity. The optimum temperature for lipase production was attained at 50$^{o}$C. Reduction in lipase activity at higher temperature due to high protease production has been reported [41]. Optimum temperature 30$^{o}$C has been reported for lipase produced from *Pseudomonas* spp. and *Lysinibacillus sphaericus* strain ODE16_Ekiti [24, 26]. The optimum temperature for lipase activity was obtained at 50$^{o}$C. Beyond this optimum temperature the enzyme may be denatured or its activity reduced. The optimum temperature for lipase activity obtained in this study was similar to the findings of Adeleke *et al*. [37] who reported 50 $^{o}$C for linamarase from *Lactobacillus plantarum* which can also displayed high enzyme activity. The varying temperature in this study might be due to environmental factors such as pH, humidity, and so on.

The purification of crude extract on Sephadex A-150 appreciably showed an increase in lipase activity; beyond this a sharp decline in lipase activity was observed when compared to the other minor peaks. The variation observed in the activity peaks might be due to the rate of elution and surrounding conditions. The further purification of the crude extract on sephadex G-150 yielded only one activity peak with lipase activity 6.84 µmol/min/mL at fractionation tube twenty nine. Purification of lipase from *P. aeruginosa* activities increased in this study corroborate with the findings of Sirisha *et al*. [42] who reported similar findings on purified lipase from *Pseudomonas.*

## 4. CONCLUSION

From this study, it was established that the bacteria isolated from the selected palm oil processing sites display high potential of lipase production. The lipase produced from the *P.*

*aeruginosa* exhibited high lipolytic activities. It can therefore, be a biodegradable agent for industrial applications especially in the waste treatment especially of waste rich in oil and fats.

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