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# Assessment of lipolytic activities of bacteria isolated from palm oil processing cottage industries in Ekiti State, Nigeria

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## Abstract

Palm oil industry is currently a world leader in the supply of oils and fats which constitutes one of the major sectors of the highest economic importance in Nigeria. This study investigates the lipolytic activity of microorganisms isolated from palm oil processing cottage industries in Ekiti State. Soil samples were taken from a depth of 10 – 15 cm in six different locations within Ekiti State, Nigeria. Microorganisms were isolated from the effluents and identified using standard microbiological techniques and molecular characterization. The microbial isolates were screened for lipase production using modified mineral salt medium in submerged fermentation. Lipase production by the isolates was assessed by halo zone of clearance on nutrient agar plates after incubation at 37°C for 24 hours. The strains of molecularly identified bacteria were *Pseudomonas aeruginosa* AE016853.1; *P. syringae* CP019871.1 and *P. putida* JQ782512.1. From this study, the microorganisms *(P. aeruginosa, P. syringae* and *P. putida*) isolated from the selected palm oil processing sites display high potential of lipase production. The lipase produced from the *Pseudomonas aeruginosa* exhibited high lypolytic activities. The POMEs could serve as source of bacteria for the production of lipases of commercial uses.

**Keywords:** Assessment; Lipolytic activities; Microorganisms; Palm oil

## 1. Introduction

Palm oil industry is currently a world leader in the supply of oils and fats which constitutes one of the major sectors of the highest economic importance in Nigeria. The importance of Palm oil in the country is due to the versatility of applications of their by-products, such as cooking oil, special fats, margarines, soaps, detergents, cosmetics, toothpastes, candles, lubricants, biofuels and electric power, among many others [1]. According to USDA [2], there are about five million hectares of palm planted in the world, representing 16 million tons of annual production. Colombia is the first country that produces palm oil in North America and the fourth largest in the world after Malaysia, Indonesia and Nigeria [2].

Most palm oil was obtained from the African oil palm (*Elaeis guineensis* J acq.) and hybrids with other species as well. In developing countries like Nigeria, about 80% of traditional palm oil processing is mostly carried out manually in home and cottage industries, using local equipment and mechanized processors thereby making the process labour intensive [3]. Palm oil processing stand as a major agricultural practice by some individuals in the coastal region. Indiscriminate discharge of many agricultural wastes has resulted in the pollution of environment, affecting aquatic lives and other living organisms. Waste discharged to the environment can be recycled using various biological process. The biological processes in our industries involving the use of microorganisms in the biotransformation of wastes has been extensively used in the production of many products [4].

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Palm oil mill effluent is the final liquid discharge after extracting oil from the mashed, fresh fruit bunch. It is a mixture of water, oil residues, crushed shells, proteins and suspended solids which are composed of plant tissues [5]. One of the main wastes derived from palm oil processing are the palm oil mill effluents (POMEs), an oily wastewater generated from milling activities. Waste discharged to the environment can be recycle using various biological process. The biological processes in our industries involving the use of microorganisms in the biotransformation of wastes has been extensively used in the production of many products [6].

Several microbial species with the ability to remediate palm oil mill effluents (POMEs) have been identified. These include species of *Pseudomonas, Bacillus, Alcaligenes, Candida*, *Saccharomyces*, *Pichia* and *Yarrowia* have been identified [7]. 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 [1].

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 [8]. Apart from hydrolysis, lipases are also involved in a wide range of reversible conversion reactions. Lipases are used in the production of food, detergent, pharmaceutical, leather, textile, cosmetic and paper industries [9]. Lipases occur widely in nature and have been found in many species of animals [10], plants [11], bacteria and fungi [12]. 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 [13].

Bioremediation of POMEs has been demonstrated to be an efficient method for the degradation of organic pollutants, enhancing the overall degradative performance by using microorganisms with high degradation ability of specific environmental pollutants [10]. Biological treatment has been found to be the most efficient method for removing fat, oil and grease by degrading them into miscible molecules, therefore, manipulation of microorganisms for treatment and bioremediation purposes afford a very efficient tool for purifying contaminated effluents and natural water. The use of lipases (enzymes) that are produced by all organisms may solve the problem, where they catalyze the synthesis or hydrolysis of fat [8]. Therefore, the aim of this study is to assess the lipolytic activities of bacterial isolates 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. 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°25'18.25N 6°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

Fifty grams (50 g) of samples were taken from a depth of 10 – 15 cm with the aid of soil auger, placed in a sterile polythene bags with appropriate labeling and immediately transported 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 [14]. The media used include nutrient agar and MacConkey agar. These media 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 milliliters (10 mL) each of the palm oil mill effluents (POME) samples was collected with 100mL sterile distilled water and serially diluted up to the appropriate dilutions ranging from $10^{-1}$ – $10^{-5}$. From the diluents, 0.1 mL of the culture was taken from $10^{-3}$, $10^{-4}$ – $10^{-5}$ dilutions, it was dispensed into Petri dishes containing nutrient agar and McConkey agar for incubation at 37 °C for 24 hours.

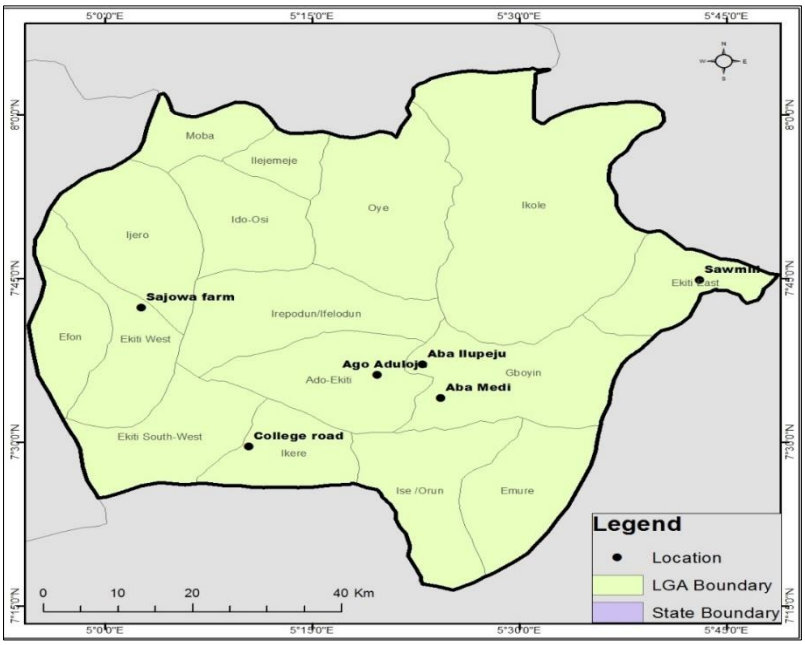



**Figure 1** Map of Ekiti- State showing location of sample collection

### 2.4. Pure Culture Preparation

After incubation, the distinct colonies formed on the nutrient agar and McConkey agar 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 for bacteria and 30°C for 48 – 72 hours for fungi. The pure isolates were stored inside Bijou 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. Biochemical tests and bacterial identification

The bacterial isolates were presumptively identified by means of morphological characteristics, cellular and biochemical tests. Morphological characteristics were observed for each bacterial colony after 24 hours of growth. The colony of each isolate on the nutrient agar media were observed for identification of shape, appearance and colour, colony size, margin and emulsification. The biochemical tests carried out include; catalase test, indole test, methyl red, voges proskauer, citrate and oxidase. The isolates were identified using Bergey's Manual of Determinative Bacteriology [15].

### 2.6. Molecular identification of Isolates

The bacterial isolates that had the highest lipase activity production were subjected to molecular identification using 16S rRNA. DNA was extracted from single colony by alkaline lysis [16]. Extracted DNA was stored at −20 $^{o}$C for further molecular analyses. 16S rDNA amplification and sequencing was performed as described by Rahman *et al.* [16]. Primers used to amplify 16S rDNA sequence were forward: 63F 5CAGGCCTAACACATGCAAGTC and reverse: 1389R 5ACGGGCGGTGTGTACAAG in a PCR thermal cycler (ICycler 170-8740, USA). The amplified DNA was visualized by gel electrophoresis and sequenced. The 16S rDNA sequence was analyzed using Chromas LITE (Version 2.01); The most similar bacterial species was found in the GenBank by using BLAST search (http://www.ncbi.nlm.nih.gov/). The phylogenetic reconstruction was accomplished using the neighbor-joining (NJ) algorithm, with bootstrap values calculated from 1000 replicate runs.

### 2.7. Primary screening of lipase-producing bacteria

The microorganisms were screened for lipase production using the modified methods of Gutarra *et al*. [17]. A small standardized strain was inoculated in Petri dishes containing 0.5% peptone, 0.3% yeast extract, 2% agar and 0.1% tributyrin. The pH of the medium was adjusted to pH 6.0. The plates were incubated at 30°C for 48 hours and examined for halo zones. The halo zones exhibited by each strains of the microorganisms showed their lipase activity with halo radius (R)/colony radius (r) ratio [18].

### 2.8. Secondary screening of bacteria-producing lipase in submerged state fermentation

The microorganisms showing the higher halo zones were selected for further studies and subjected to submerged fermentation. Nutrient broth was used to grow the bacterial isolates. The bacterial inoculum from nutrient broth culture were then transferred to 1000 ml of freshly prepared mineral salts medium (2.75g/l of $K_2HPO_4$, 2.225g/l of $KH_2PO_4$, 1.0g/l of $(NH_4)_2SO_4$, 0.2g/l of $MgCl_2.6H_2O$, 0.1g/l of NaCl, 0.02g/l of $FeCl_3.6H_2O$ and 0.01g/l of $CaCl_2$) pH 7.0, supplemented with 1% w/v POME. The medium were incubated at 30 °C on a rotary shaker at 200 rpm. Growth of bacteria was monitored at 600 nm.

### 2.9. Assay for lipase

Lipase activity of the isolate was quantified as described by Cho *et al*. [19]. The lipase activity was assayed in the reaction mixture containing 180µL of solution A (0:062g 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 200µl enzyme sample. The mixture and the control tubes were incubated at 37 $^oC$ for 15 minutes at room temperature 28 ± 2 $^oC$. 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 410nm 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 [20].

### 2.10. Statistical analysis of data

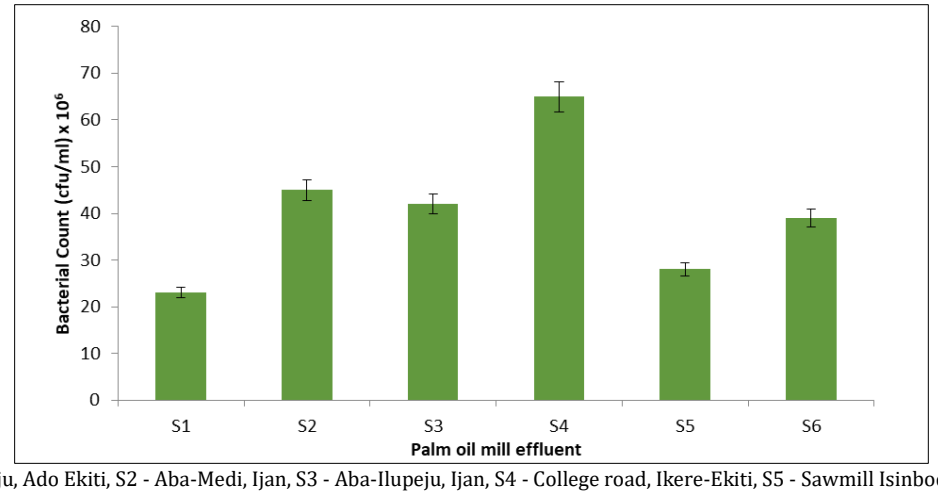
*2.10.1. Statistical analysis*

All the analysis was carried out by using the statistical software package SPSS (Statistical Package for Social Sciences) version 27.0 software.

## 3. Results

The total bacterial count obtained from POME is presented in Figure 2. Sample obtained from College road, Ikere Ekiti recorded high bacterial counts (6.50 x $10^6$cfu/mL), followed by sample from Aba-Medi, Ijan (4.50 x $10^6$cfu/mL), while the least counts was obtained from the sample from Ago Aduloju, Ado Ekiti (2.30 x $10^6$cfu/mL).

The biochemical and morphological characteristics of the bacterial isolates is shown on Table 1. The four bacteria isolated included *Bacillus licheniformis*, *Citrobacter freundii*, *Bacillus cereus* and *Pseudomonas aeruginosa*. All the isolates were catalase and citrate positive, motile. *Bacillus lichinformis* and *Bacillus cereus* were methyl red negative, while *Citrobacter freundii* was methyl red positive. *Citrobacter freundii* was indole, oxidase and Voges proskauer negative, but urease positive, while *Bacillus lichinformis* and *Bacillus cereus* were Voges proskauer positive.




Key: S1 - Ago Aduloju, Ado Ekiti, S2 - Aba-Medi, Ijan, S3 - Aba-Ilupeju, Ijan, S4 - College road, Ikere-Ekiti, S5 - Sawmill Isinbode, S6 - Sajowa farm, Aramoko-Ekiti

**Figure 2** Total bacterial counts from palm oil mill effluent (POME) from sample locations

The frequency of occurrence of bacteria isolated from the POME is represented on Table 2. Total of six strains of *Bacillus cereus* with (6) 42.9% occurrence, two *Bacillus lichenformis* with (2) 14.3% occurrence, one *Citrobacter frendii* with (1) 7.1 % and five *Pseudomonas aeruginosa* with (5) 35.7% were obtained from the POME sample. *Bacillus cereus* had the highest frequency of occurrence 6 (42.9%), while *Citrobacter freundii* had the lowest frequency of occurrence 1(7.1%).

The diameter zone of inhibition of the isolates is also shown in Table 3. The findings revealed that *Pseudomonas aeruginosa* from Aba Medi had the highest halo zone 38 mm while *Bacillus cereus* from College road had the least halo zone 18 mm.

The quantitative lipase activities screening of bacteria associated with POME is illustrated in Figure 3. The bacterial isolates displayed lipase activity that ranged from 75.33µmol/min to 22.44 µmol/min with the highest enzyme activity exhibited by the *P. aeruginosa* and the least *B. cereus* (22.11 µmol/min) from College road had the least zone.

**Table 1** Biochemical and Morphological Characteristics of Bacteria Isolates from Palm Oil Mill Effluent (POME) Samples

| Isolates' code | Gram's Rtn | Cat | Mot | Ind | Cit | Oxi | Ure | MR | VP | Colour | Edges | Surface | Suspected Organisms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BAC1 | GPB | + | + | | + | | + | – | + | Cream | Rough | Segmented | *Bacillus licheniformis* |
| BAC2 | GPB | + | + | | + | | + | – | + | Cream | Rough | Segmented | *Bacillus cereus* |
| BAC3 | GPB | + | + | | + | | + | – | + | Cream | Rough | Segmented | *Bacillus cereus* |
| BAC4 | GNB | + | + | – | + | – | + | + | _ | Cream | Rough | Segmented | *Citrobacter freundii* |
| BAC5 | GNB | + | + | | + | + | + | – | + | Cream | Rough | Segmented | *Bacillus licheniformis* |
| BAC6 | GPB | + | + | | + | | + | | + | Cream | Rough | Segmented | *Pseudomonas aeruginosa* |
| BAC7 | GNB | + | + | – | + | + | + | – | + | Green | Smooth | Smooth | *P. aeruginosa* |
| BAC8 | GNB | + | + | – | + | + | + | – | + | Green | Smooth | Smooth | *P. aeruginosa* |
| BAC9 | GPB | + | + | | + | | + | | + | Green | Smooth | Smooth | *Bacillus cereus* |
| BAC10 | GPB | + | + | | + | | + | | + | Green | Smooth | Flat | *Bacillus cereus* |
| BAC11 | GNB | + | + | – | + | + | + | – | + | Green | Smooth | Flat | *P. aeruginosa* |
| BAC12 | G NB | + | + | – | + | + | + | – | + | Green | Smooth | Smooth | *P. aeruginosa* |
| BAC13 | GNB | + | + | – | + | + | + | – | + | Green | Smooth | Smooth | *P. aeruginosa* |
| BAC14 | GPB | + | + | | + | | + | – | + | Green | Smooth | Smooth | *Bacillus cereus* |

Key: Cat = Catalase; Mot = Motility; Ind = Indole; Cit = Citrate; Oxi = Oxidase; Ure = Urease; MR = Methyl red; VP = Voges Proskauer; GNB = Gram Negative Bacilli; GPB = Gram Positive Bacilli; Positive = +; Negative = – BAC1-14= GPB=

**Table 2** Frequency of Occurrence of Bacterial Isolates from Palm Oil Mill Effluent (POME) from Sample Locations

| Isolates | No of occurrence | Frequency | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| *Bacillus cereus* | 6 | 42.9 | 2 | 4 | – | – | – | – |
| *Bacillus licheniformis* | 2 | 14.3 | 1 | – | 1 | – | – | – |
| *Citrobacter freundii* | 1 | 7.1 | – | 1 | – | – | – | – |
| *Pseudomonas aeruginosa* | 5 | 35.7 | – | – | – | 5 | – | – |
| Total | 14 | 100.0 | 3 | 5 | 1 | 5 | 0 | 0 |

**Key**: S1 - Ago Aduloju, Ado Ekiti, S2 - Aba-Medi, Ijan, S3 - Aba-Ilupeju, Ijan, S4 - College road, Ikere-Ekiti, S5 - Sawmill Isinbode, S6 - Sajowa farm, Aramoko-Ekiti

**Table 3** Primary Screening of Bacteria from Sample Locations for Lipase Production

| Isolate code | Organisms | Diameter of Zone of intensification (cm) |
|---|---|---|
| $S_1\ 10^4$ | *Bacillus licheniformis* | 2.3 |
| $S_1\ 10^5$ | *Bacillus cereus* | 2.8 |
| $S_3 10^5$ | *Bacillus licheniformis* | 3.4 |
| $S_3\ 10^4$ | *Pseudomonas aeruginosa* | 3.8 |
| $S_4 10^4$ | *P. aeruginosa* | 2.9 |
| $S_4\ 10^5$ | *Bacillus cereus* | 1.8 |
| $S_6 10^4$ | *P. aeruginosa* | 3.9 |
| $S_6 10^5$ | *Bacillus cereus* | 2.0 |

**Key**: S1 - Ago Aduloju, Ado Ekiti, S2 - Aba-Medi, Ijan, S3 - Aba-Ilupeju, Ijan, S4 - College road, Ikere-Ekiti, S5 - Sawmill Isinbode, S6 - Sajowa farm, Aramoko-Ekiti

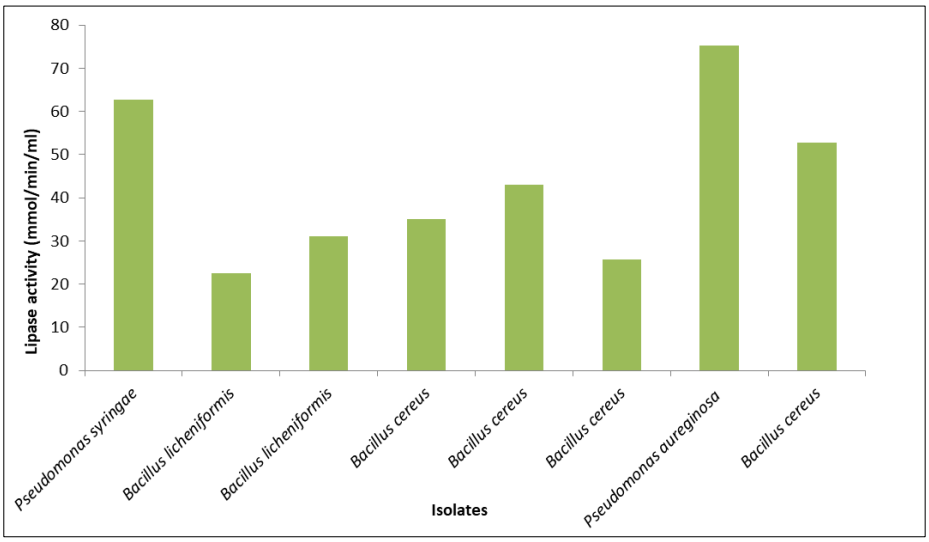



**Figure 3** Lipase activity of bacterial isolates

## 4. Discussion

The bacteria isolated in this study were *Bacillus licheniformis*, *B. cereus*, *Citrobacter freundii*, and *Pseudomonas aeruginosa*. This finding is in line with report of Odeyemi *et al*. [21]; Ohimain *et al*. [22]; Izah and Ohimain [23] who reported similar bacteria from palm oil mill effluent. However, the high microbial load obtained from College road, Ikere-Ekiti in this study might be due to the ability of the bacterial to utilize the substrate more speedily than the other, the type of microorganisms associated with the wastes, suitable environmental factors and various activities exposing the wastes to more contamination [24]. Also, 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 harbours microorganism and human activities. The variation observed in the microbial loads may be due to location, exhaustion of available nutrients in the substrate and the prevailing environmental conditions [25].

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. The zone of clearance around the isolates on the plates could be attributed to the ability of the bacteria to metabolize the substrate in the medium and secretion of active enzymes. Findings on the lipase-producing bacteria have been reported by different researchers [26-28]. The ability of these bacteria to secrets considerable amount of lipolytic enzyme into the culture medium suggests that it can be harnessed for various use both for biotechnological and industrial processes. The production of lipase in

the culture medium in this study is an indication that the enzyme is secreted outside cells [29]; thus easy for extraction during production.

The bacteria 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 bacteria might be attributed to the source of isolation and genetic make-up [28]. Also, the variation observed in the protein content by each of the isolate in submerged state fermentation could be attributed to the production of variety of hydrolytic enzymes in addition to the enzyme of study [6].

## 5. Conclusion

The bacterial isolates from palm oil mill effluent (POME) are capable of producing lipases that enhance the growth and survival of the bacteria. Therefore, these bacteria could serve as viable sources for lipases of commercial value.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

Authors have declared that no conflict of interests exists.

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