Lipolytic Activities of Bacteria and Fungi Isolated from Soil Samples

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors ROF and TF-O designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BA and VM managed the analyses of the study. Author VM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT
This study was carried out at the Department of Microbiology, Microbiology Laboratory, Ado-Ekiti State University, Ekiti State, Nigeria between July, 2018 to March, 2019. Due to the diverse biotechnological importance of lipases as a biocatalytic enzyme, extracellular production of microbial lipases has to gain lots of interest. This study, therefore, focused on the physicochemical parameters of lipase producing microorganisms from different soil samples. Microorganisms were isolated from four different soil samples using Nutrient Agar (NA) and Potato Dextrose Agar (PDA). The isolates were identified and characterized. Production, an assay for Lipase enzymes, purification, the effect of pH, Temperature and metal ion was investigated. The isolates were culturally, morphologically and biochemically characterized. Two of the bacteria strains (Bacillus sp. and Staphylococcus sp.) and four fungi (Fusarium sp., Aspergillus fumigatus, Aspergillus niger, and Trichophyton sp.) isolates were able to produce lipid using Sudan Black B fat staining techniques. Fusarium sp. isolated from dumpsite soil had the highest specific lipase activity (21.16

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1. INTRODUCTION

Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids and other invertebrates as well as plants and algae [1]. Soil aggregates are the basis of all soil biological processes because they determine the pore size for water and air movement, which in turn controls microbial activity and soil organic matter turnover [2]. In the world today, over 4000 enzymes have been identified and only 200 enzymes are commercially been used [3]. Animals, plants, and microorganisms which include bacteria, yeast and fungi are known to produce lipases. Lipases are also referred to as triacylglycerides acyl hydrolases (EC 3.1.1.3). They belong to the hydrolase group of enzymes that can act on carboxylic ester bonds. They are a special enzyme that hydrolyzes glyceraldehydes to free fatty acids and glycerol. They also catalyzed ester synthesis in liquid media [4-5]. Productions of lipases by microorganisms have gained lots of attention from industries due to their broad substrate specificity, stability, selectivity, shorter generation time and ease of bulk production [6]. Lipid producing microorganisms have been known for many years and their potential as alternative sources of vegetable oils has been periodically assessed. Microbial lipids also known as single-cell oils (SCO), are produced by oleaginous microorganisms such as algae, fungi and yeast, and are considered to be promising candidates for biodiesel production as their oil properties are similar to vegetable oils [7]. Production of lipases does not require huge space and can be produced in a much shorter time than vegetable oils. Also, they are less affected by seasons and the climate [8]. Bacteria lipase is usually extracellular and is greatly influenced by nutritional and physicochemical parameters such as temperature, pH etc [9-10]. Lipases of microbial origin are more stable than plant and animal enzymes and their production is more easy, convenient, safer and cheaper [11].

Bacteria lipases are used extensively in the food and dairy industry for the hydrolysis of cheese ripening, flavor enhancer, milk fat and butterfat. Fungi such as Penicillium sp., Rhizopus sp., Mucor sp., Aspergillus sp. and Fusarium sp. are among the well-known lipase producers [12]. Several microorganisms associated with lipase production are known to inhabit different habitat which include oil contaminated soil and seeds, industrial wastes, vegetable oil factories etc. Microbes can naturally synthesize lipids for maintenance of cell membranes, storage of energy and communication. However, only a certain group of microorganisms can accumulate lipids of more than 20% of their biomass and store them as triacylglycerol molecules [13]. Almost all microbial lipases can be regarded as acid lipases or neutral lipases if they are classified by their optimum pH value for the lipolytic activity [14]. It would be important to identify microorganisms with high lipid-producing ability from the soil. This present study is aimed at isolation, identification and the physicochemical parameters of lipase producing microorganisms from different soil samples.

2. MATERIALS AND METHODS

2.1 Isolation of Lipolytic Microorganisms

Samples were collected from different locations (palm oil mill soil, cultivated soil sample, uncultivated soil sample on a dumpsite and soil from kitchen effluents) at Ekiti State University Teaching and Research Farm. They were cultivated on Nutrient Agar (Biolab Budapest, Hungary) and Potato Dextrose Agar (Biolab Budapest, Hungary) following the method of Patel et al. [15]. All incubation for isolation was done at 37°C for 48 hours.

2.2 Identification and Characterization of Isolates

The selected isolates were identified based on their cultural, morphological and biochemical
2.3 Production of Lipase by a Microorganism

Lipase production was done according to the method of Lotrakul et al. [17] with some modification. The isolates were grown in a basal medium (MSM) containing (g/L): K_2HPO_4, 9.0; MgSO_4.7H_2O, 0.25; KCl, 0.2; NH_4NO_3, 1.0; FeSO_4.7H_2O, 0.05; MnSO_4, 0.002; ZnSO_4, and Olive oil, 1.0; incubated at 35°C for 24 hours. The enzyme produced was stored at 4°C. All chemicals and reagents used were of analytical grade (ANALAR).

2.4 Assay of Lipase Activity

Lipase assay was done using the spectrophotometric method to measure the amount of p-nitrophenol (p-NP) produced at pH 8.0 following the method of Winckler and Stuckmann, [18] with some modification. Briefly, the reaction mixture contained 180μL of 0.062 g of p-NPP in 10 mL of 2-propanol (sonicated for 2 minutes before use), 1620μL of 0.4% Triton X-100 and 0.1 % gum Arabic in 50mM Tris-HCl, pH 8.0 and 200μL of properly diluted enzyme sample. The product was detected at 410 nm wavelength after incubation for 15 minutes at 37°C. Under this condition, the molar extinction coefficient (410 nm) of p-nitrophenol (p-NP) released from p-NPP was 15000 M⁻¹. One unit of lipase activity was defined as 1μmol of p-nitrophenol (p-NP) released per minute by 1 mL of an enzyme.

2.5 Protein Determination

Protein concentration was determined using the Lowry method Lowry and Tinsel, [19].

2.6 Ammonium Sulphate Precipitation

The addition of Ammonium sulphate to the crude enzyme allowed the precipitation of the crude enzyme to 60% saturation. The mixture was centrifuged at 10,000 g for 15 min at 4 °C. The precipitates were then re-suspended in 50mM Tris-HCl, pH 8.0 and dialyzed against the same buffer overnight at 4 °C with three buffer changes. In order to obtain a concentrated enzyme devoid of metal ions and salts, the dialyzed enzyme was concentrated with 4M sucrose solution.

2.7 Purification of Lipase

The concentrated enzyme solution was then applied on a DEAE Sephadex A50 column (1.5cm diameter × 50cm length) pre-equilibrated with 50mM Tris-HCl, pH 8.0. The elution was carried out by 0-0.5 M NaCl in the same buffer at a flow rate of 5 ml/30 min at room temperature and 5 ml fractions were collected. The optical density was measured at 280nm so as to obtain the protein content of fractions. The protein-containing fractions were assayed for lipase activity. Fractions containing lipase activity were pooled, concentrated and applied on a Sephadex G-100 column (1.5cm diameter × 75cm length) pre-equilibrated with 50mM Tris-HCl, pH 8.0. 5ml fractions were collected at a flow rate of 20 ml / h at room temperature. 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 [20].

2.7 Effect of Temperature on Lipase Activity and Stability

The temperature optimum for the enzyme was determined in the range 30 to 80°C, at pH 7.4, as above. The enzyme stability at different temperatures is studied by incubating the enzyme in 50mM Tris-HCl, pH 8.0 at different temperatures for 2 h, followed by the activity estimation at 37°C [21].

2.8 Effect of pH on Lipase Activity and Stability

The effect of pH on enzyme activity is studied by incubating the enzyme with p-nitrophenyl acetate substrate, prepared in different buffers in the pH range 3 to 9. The buffers used are, Sodium acetate (pH 3-5), Sodium phosphate (pH 6-7) and Tris-HCl (pH 9) [21].

2.9 Effect of Metal Ions and EDTA on Lipase Activity

The effect of metal ions and EDTA respectively were determined by estimation of the activity in
presence of 10mM solution of metal salts. The enzyme was incubated in presence of metal ions and EDTA for 15 min followed by estimation of activity by p-nitrophenol liberation [22].

3. RESULTS AND DISCUSSION

A total of twenty-five microbial isolates (fourteen bacteria and eleven fungi) were isolated from four different soil samples. The twenty-five isolates showed good cultural characteristics on nutrient agar and Potato dextrose agar respectively as shown in Table 1 and 2. The bacterial isolates showed various colonial appearances on nutrient agar ranging from smooth surfaces, raised elevation, circular-shaped, mucoid colony, pigmented, translucent, opaque, shiny colony, large, medium colonies, eight of the bacterial isolates were Gram-positive bacilli, two appeared as Gram-positive cocci, while the remaining five were Gram-negative bacilli. The bacterial isolates were identified as Enterobacter sp., Bacillus sp., Escherichia sp., Staphylococcus sp. and Pseudomonas sp. The fungal isolates showed different cultural appearances such as a velvety and flaky surface with grey to black coloration, White and green variants powdery surface growth, dust-like sporulating surface light brown with smooth border, abundant mycelium with pale brown and dark zonation, white air mycelium with quick differentiation, upper side white color with irregularly smooth and fringed. The fungal isolates were identified as Fusarium sp., Aspergillus fumigatus, Scopulariopsis sp., Verticillium sp., Aspergillus sp., Geotrichum sp., and Trichophyton sp. The major constituent of some group of the biocatalyst is lipase and it is known to have lots of biotechnological applications. In this present study, different microorganisms were isolated from four different soil samples. The microorganisms identified include bacteria and fungi. From this research work, both Gram-positive and Gram-negative bacteria were isolated. The fungal isolates showed different microscopic and macroscopic characteristics. Some researchers isolated both Gram-positive and Gram-negative bacteria. Fungi were also isolated from palm oil-contaminated soil and kitchen effluent soil [23-25]. The fungi and bacteria isolated from the different soil samples are dominating species of soil [26].

The result showing the bacterial and fungal isolates that were positive for Sudan B black fat stain is shown in Table 3 and 4. Among the fourteen bacterial isolates obtained from the soil samples, eight isolates (3 Bacillus cereus, 3 Bacillus sp. and 2 Staphylococcus aureus) were positive to sudan black staining reaction. However, only eight fungal isolates (3 Fusarium sp., 1 strain of Aspergillus fumigatus, 3 Aspergillus niger and 1 Trichophyton sp.) were positive to sudan black stain. Hartman, [27] reported that Bacillus cereus, Bacillus mycoides, Azotobacter beijerinckii, Rhizobium leguminosarum, Mycobacterium avium, Mycobacterium leprae, Oospora lactis, Bacillus tenuiscens, water spirilla, and some fungi such as yeast, Aspergillus sp. and Fusarium sp. gave positive fat tests with Sudan Black B. The Sudan Black B stained only the lipidic matter surrounding the cytoplasmic membrane. The modified Sudan Black B method can be applied for primary screening of oleaginous bacteria, fungi and algae.

The rate at which the bacteria and fungi attained peak for lipase production are shown in Table 5. About 57% of the fungal isolates reached their peak for lipase production within 40-60 hours of incubation. Bacterial isolates reached their peak for lipase production at 100% within 30-40 hours of incubation. The result obtains from specific lipase activity is similar to the work of Chiang and Demirkan, [28] who reported that Aspergillus sp. showed the highest lipase activity. Staphylococcus sp. and Bacillus sp. release extracellular lipase in a fermentation medium.

The lipase produced by fungi ranged from 9.04 to 21.15 µmol/min/ml with specific activities ranging from 0.18 to 0.57 µmol/min/mg. Fusarium sp. isolated from Red oil spill soil (ROS11 Fusarium sp.) had the highest specific lipase activity (0.57 µmol/min/mg) while the least was observed in Aspergillus niger isolated from cultivated soil (CS5 Aspergillus niger) with lipase activity of 0.18 µmol/min/mg as shown in Table 6. However, Table 7 shows that the lipase activities for the bacterial isolates ranged from 16.38 to 21.24 µmol/min/ml with specific activities ranging from 0.49 to 0.59 µmol/min/mg. Bacillus sp. isolated from Red oil spill (ROS11 Bacillus sp.) soil had the highest specific lipase activity (0.59 µmol/min/mg) while KESS Staphylococcus sp. had the least activity (0.49 µmol/min/mg). The specific lipase activity was determined and the result from this study is in support of the work of Fleuri et al. [29] who reported that Aspergillus sp. had 15.0 µmol/min/mg lipase activity while the lipase activity of Fusarium sp. had 13.5 µmol/min/mg.
The result showing the lipolytic activity of Bacillus sp. isolated from Red Oil Spill Soil is shown in Table 8. The lipolytic activity for crude extract and Ammonium precipitate concentration is 2.82 µmol/min/ml and 2.57 µmol/min/ml with a total protein of 4330 mg and 244.1 mg respectively. However, the specific activity for both steps is 0.33 µmol/min/mg and 0.48 µmol/min/mg with percentage yield of 100% and 0.48% respectively. For ion-exchange chromatography and gel filtration, their lipolytic activity was 3.43 µmol/min/mg and 4.18 µmol/min/mg. Total protein for both the ion-exchange chromatography and gel filtration is 47.77 mg and 15.93 mg with a specific activity of 1.21 µmol/min/mg and 2.39 µmol/min/mg. The percentage yield therefore, is 4.11% and 2.69% respectively. The purification process at gel filtration had the highest specific activity and percentage yield. Lipase purification was done to get a protein of interest and to remove unnecessary ones [30]. The produced enzyme was purified by Ammonium sulphate precipitation for salting out the proteins. Desalting was performed for removing the traces of salt to increase the enzymatic activity. Pabai et al. [31] reported that increase in lipase activity depends mostly on the concentration of Ammonium sulphate. According to Pabai et al. [31], increased lipase activity depends on the concentration of Ammonium sulphate. Separation using Gel Filtration made the enzymes from large aggregate which was easily separated from other protein [32].

The result showing the lipolytic activity of Fusarium sp. isolated from soil from dumpsites is shown in Table 9. The lipolytic activity for crude extract and Ammonium precipitate concentration is 2.05 µmol/min/ml and 2.75 µmol/min/ml with a total protein of 5705 mg and 176.21 mg respectively. However, the specific activity for both steps is 0.18 µmol/min/mg and 0.41 µmol/min/mg with percentage yield of 1.00% and 6.92% respectively. For ion-exchange chromatography and gel filtration, their lipolytic activity was 3.37 µmol/min/mg and 4.51 µmol/min/mg. Total protein for both the ion-exchange chromatography and gel filtration is 55.27 mg and 18.67 mg with a specific activity of 0.91 µmol/min/mg and 2.46 µmol/min/mg. The percentage yield therefore, is 4.96% and 4.49% respectively. The purification process at gel filtration had the highest specific activity and percentage yield. However, Bacillus sp. produced the highest amount of lipase compared to the Fusarium sp. The aggregation caused a slight increase in specific lipolytic activity of both the Bacillus sp. and Fusarium sp. during purification steps. This is in contrast with the work of Bhosal et al. [10] in which the aggregation caused a slight reduction in lipolytic activity during the purification step. Bhosal et al. [10] reported an optimum temperature of 30°C and pH 8 lipase production by the bacterial strain isolated from oil-contaminated soil [33].

The effect of temperature on Bacillus sp. lipase activity is shown in Fig. 1. The enzyme was optimally active at 50°C (100% relative activity). However, there was an increase in the lipase activity of Bacillus sp. as the temperature increases from 30°C to 50°C. Beyond 50°C, there was a decrease in enzyme activity. At 80°C, a nearly three folds decrease in Bacillus sp. lipolytic activity was observed.

The results of the thermostability of purified enzyme at different temperatures are shown in Fig. 2. Significant differences were observed in the stability of the purified lipase at 40°C, 50°C, 60°C and 70°C. The purified lipase retained 97.9% residual activity at 40°C within 20 minutes. On incubation at 70°C, the lipase activity decreased to about 38% to 35% within 100 to 120 minutes respectively. Temperature is one of the vital environmental factors that influence enzyme production and activity. The optimum temperature for lipase production was found to be 30°C showing lipase units of 0.62 Uml⁻¹. Subsequently, Fusarium sp. (SDS 10) was also capable of producing lipase in the range of 30°C – 80°C with maximum production at 60°C (100% relative activity) as shown in Fig. 3. There was an increase in the lipolytic activity of Fusarium sp. as the temperature increase from 40°C to 60°C. However, the enzyme decreased after 60°C. Then, at 80°C nearly two folds decrease in lipase activity was observed. The thermostability of the purified enzyme at different temperatures is shown in Fig. 2. The purified lipase showed significant difference at 40°C, 50°C, 60°C and 70°C. The purified lipase retained 98.8% residual activity at 40°C at 20 hours. At 60°C and 70°C, the lipase activity decreased to 20% within 120 hours respectively. Subsequently, Fusarium sp. isolated from soil from dumpsite was also capable of producing lipase in the range of 30°C – 80°C with maximum production at 30°C (100% relative activity). Then, at 80°C nearly two folds decrease in lipase yield was observed. This result is also in line with the work of Collae et al. [34] in which his results shows that lipases retained 80% of their activity at 25-30°C. The thermostability of the purified enzyme at different temperatures is shown in Fig. 3.
| Isolate code | Grams reaction | Cultural Characteristics | Catalase Test | Nitrate reduction Test | Motility Test | Indole Test | Citrate Test | Oxidase Test | MR VP Urease Test | Probable Microorganisms |
|--------------|----------------|--------------------------|---------------|------------------------|---------------|-------------|-------------|-------------|------------------|------------------------|
| CS 1         | -ve, bacilli   | Circular, translucent, smooth surface, moisten colony. | +ve           | +ve                    | Motile        | -ve         | +ve         | -ve         | -ve              | +ve +ve +ve Enterobacter sp. |
| CS 2         | +ve, bacilli   | Filamentous, rough edges, translucent, dry colony. | +ve           | -ve                    | Non motile    | -ve         | -ve         | -ve         | -ve              | +ve +ve +ve Bacillus sp.     |
| KES 3        | +ve, bacilli   | Creamy white colony, irregular edges, dry, opaque. | +ve           | -ve                    | Motile        | -ve         | +ve         | -ve         | -ve              | +ve +ve +ve Bacillus sp.     |
| KES 4        | -ve, bacilli   | Smooth, thick, translucent, moist, large colony. | +ve           | +ve                    | Motile        | +ve         | -ve         | -ve         | +ve              | -ve -ve -ve Escherichia coli |
| KES 5        | +ve, cocci in clusters | White, translucent, dry, medium-size, raised elevation colony. | +ve           | -ve                    | Non motile    | -ve         | -ve         | -ve         | -ve              | -ve +ve Staphylococcus aureus |
| UC 6         | +ve, bacilli   | Whitish, irregular, milky colony. | +ve           | -ve                    | Non motile    | -ve         | +ve         | -ve         | -ve              | +ve +ve +ve Bacillus sp.     |
| UC 7         | -ve, bacillus  | Circular shaped, mucoid colony, red-pigmented. | +ve           | +ve                    | Motile        | -ve         | +ve         | +ve         | -ve              | -ve -ve -ve Pseudomonas aeruginosa |
| UC 8         | -ve, bacilli   | Shiny colony, moist, smooth, convex elevation | +ve           | +ve                    | Motile        | -ve         | +ve         | -ve         | -ve              | +ve +ve +ve Enterobacter sp. |
| SDS 9        | -ve, bacilli   | Slightly whitish, round colony, smooth surface, convex elevation, moist, opaque. | +ve           | +ve                    | Motile        | +ve         | -ve         | -ve         | +ve              | -ve +ve +ve Escherichia coli |

Table 1. Morphological and biochemical characterization of bacterial isolates from soil sample
| Isolate code | Grams reaction | Cultural Characteristics | Catalase Test | Nitrate reduction | Motility Test | Indole Test | Citrate Test | Oxidase Test | MR | VP | Urease Test | Probable Microorganisms |
|--------------|----------------|--------------------------|---------------|------------------|--------------|-------------|-------------|--------------|-----|-----|------------|------------------------|
| SDS 10       | +ve, bacilli    | Filamentous, rough edges, translucent, dry colony. | +ve           | -ve              | Motile       | -ve         | +ve         | -ve          | -ve | +ve | +ve        | Bacillus sp.            |
| ROS 11       | +ve, bacilli    | Creamy white, irregular edge, dry, opaque. | +ve           | -ve              | Motile       | -ve         | +ve         | -ve          | -ve | +ve | +ve        | Bacillus sp.            |
| ROS 12       | +ve, cocci in clusters | Whitish, translucent, dry, medium-size colony, raised. | +ve           | -ve              | Non motile   | -ve         | -ve         | -ve          | -ve | -ve | +ve        | Staphylococcus sp.      |
| ROS 13       | -ve, bacilli    | Slightly whitish, round colony, smooth surface, convex elevation, moist, opaque. | +ve           | +ve              | Motile       | +ve         | -ve         | -ve          | +ve | -ve | -ve        | Escherichia coli        |
| ROS 14       | +ve, bacilli    | Creamy white colony, irregular edges, dry, opaque. | +ve           | -ve              | Non motile   | -ve         | -ve         | -ve          | -ve | +ve | +ve        | Bacillus sp.            |

**KEYS:** CS- Cultivated soil, KES- Kitchen effluent soil, UCS- Uncultivated soil, SDS- Soil from the dumpsite, ROS- Red oil spill soil, MR- Methyl red test, VP- Voges Proskauer.
Table 2. Cultural and morphological characteristics of fungal isolates

| Isolates | Macroscopy                                                                 | Microscopy                                                                 | Organism (s)          |
|----------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|-----------------------|
| CS 1     | Abundant mycelium with pale brown and dark zonation                       | Macroconidia septate of about 3-5, very slender with tapered and curved apical cell | *Fusarium* sp.        |
| CS 2     | Velvety and flaky surface due to marked sporulation with grey to green coloration. | Septate hyphae with borne laterally conidiophores and conidia borne in the chain on sterigma. Conidiophores smooth-walled. | *Aspergillus niger*   |
| CS 3     | Dust like sporulating surface, light brown with smooth border periphery | Septate hyphae with rough spiky, borne in simple chain conidiospores       | *Scopulariopsis* sp   |
| CS 4     | Dust like sporulating surface, light brown with rough spiky borne in simple chain conidiospores. Light green and powdery light | Septate hyphae, borne vertically, conidiospores with unicellular elliptical conidia. | *Verticillium* sp     |
| UCS 6    | Velvety and flaky surface due to marked sporulation with grey to black coloration. | Septate hyphae with borne laterally conidiophores and conidia borne in the chain on the sterigma | *Aspergillus niger*   |
| UCS 7    | Velvety and flaky surface due to marked sporulation with grey to black coloration. | Septate hyphae with borne laterally conidiophores and conidia borne in the chain on the sterigma | *Aspergillus niger*   |
| SDS 8    | White air mycelium with quick differentiation.                           | Septate hyphae with dichotomous ramification with no blastospores.         | *Geotrichum* sp.      |
| SDS 9    | Upper-side white color with irregularly smooth and fringed.              | Very coarse, ramified and septate hyphae with roundish microconidia without macroconidia. Numerous chlamydospores in the vegetative mycelium. | *Trichophyton* sp     |
| SDS 10   | Abundant mycelium with pale brown and dark zonation                      | Macroconidia septate of about 3-5, very slender with tapered and curved apical cell. | *Fusarium* sp.        |

**KEYS:** CS - Cultivated soil, KES- Kitchen effluent soil, UCS- Uncultivated soil, SDS- Soil from dumpsite and ROS- Red oil spill soil
### Table 3. Sudan black staining reaction on bacteria

| Isolates | Suspected Microorganism          | Sudan black staining reaction |
|----------|----------------------------------|------------------------------|
| CS 1     | Enterobacter sp.                 | Negative                     |
| CS 2     | Bacillus cereus                  | Positive                     |
| KES 3    | Bacillus sp.                     | Positive                     |
| KES 4    | Escherichia coli                 | Negative                     |
| KES 5    | Staphylococcus aureus            | Positive                     |
| UCS 6    | Bacillus cereus                  | Positive                     |
| UCS 7    | Pseudomonas aeruginosa           | Negative                     |
| UCS 8    | Enterobacter sp.                 | Negative                     |
| SDS 9    | Escherichia coli                 | Negative                     |
| SDS 10   | Bacillus cereus                  | Positive                     |
| ROS 11   | Bacillus sp.                     | Positive                     |
| ROS 12   | Staphylococcus aureus            | Positive                     |
| ROS 13   | Escherichia coli                 | Negative                     |
| ROS 14   | Bacillus sp.                     | Positive                     |

**KEYS:** CS - Cultivated soil, KES - Kitchen effluent soil, UCS - Uncultivated soil, SDS - Soil from the dumpsite, ROS - Red oil spill soil.

### Table 4. Sudan black staining reaction on fungi

| Isolates | Isolate name          | Sudan black staining reaction |
|----------|------------------------|------------------------------|
| CS1      | Fusarium sp.          | Positive                     |
| CS 2     | Aspergillus fumigatus  | Positive                     |
| CS 3     | Scopulariopsis sp.     | Negative                     |
| CS 4     | Verticillium sp.       | Negative                     |
| CS 5     | Aspergillus niger      | Positive                     |
| UCS 6    | Aspergillus niger      | Positive                     |
| UCS 7    | Aspergillus niger      | Positive                     |
| SDS 8    | Geotrichum sp.         | Negative                     |
| SDS 9    | Trichophyton sp.       | Positive                     |
| SDS 10   | Fusarium sp.           | Positive                     |
| ROS 11   | Fusarium sp.           | Positive                     |

**KEYS:** CS - Cultivated soil, KES - Kitchen effluent soil, UCS - Uncultivated soil, SDS - Soil from the dumpsite, ROS - Red oil spill soil.

### Table 5. Rate of attaining peak of lipase production for fungal and bacterial isolate

| Incubation period (Hours) | Number (Percentage %) |
|---------------------------|------------------------|
| **Fungal Isolates**       |                        |
| 20-40                     | 2 (28.57%)             |
| 40-60                     | 4 (57.14%)             |
| 60-80                     | 1 (14.28%)             |
| **Bacterial Isolate**     |                        |
| 30-40                     | 3 (100%)               |

### Table 6. Lipolytic activities of selected fungal isolates from soil

| Isolates     | Protein (mg/ml) | Lipase (µmol/min/ml) | Specific Lipase Activity (µmol/min/mg) |
|--------------|-----------------|----------------------|---------------------------------------|
| CS1 Fusarium sp. | 30.07           | 13.22                | 0.44                                  |
| CS2 Aspergillus fumigatus | 46.34           | 14.84                | 0.30                                  |
| CS5 Aspergillus niger | 43.03           | 11.93                | 0.18                                  |
| UCS6 Aspergillus niger | 66.14           | 21.15                | 0.49                                  |
| UCS7 Aspergillus niger | 46.34           | 11.49                | 0.25                                  |
| SDS10 Fusarium sp. | 49.93           | 19.22                | 0.41                                  |
| ROS11 Fusarium sp | 16.00           | 9.04                 | 0.57                                  |

**Specific Lipase Activity = Lipase Activity (µmol/min/ml) / Protein (µmol/min/ml)**
### Table 7. Lipase activity of selected bacterial isolates

| Isolates           | Protein (mg/ml) | Lipase (µmol/min/ml) | Specific activity (µmol/min/mg) |
|--------------------|-----------------|----------------------|---------------------------------|
| KES5 *Staphylococcus* sp. | 34.76           | 16.38                | 0.49                            |
| UCS6 *Bacillus* sp.     | 34.76           | 20.08                | 0.58                            |
| ROS11 *Bacillus* sp.     | 43.03           | 21.24                | 0.59                            |

Specific Lipase Activity = Lipase Activity (µmol/min/ml) / Protein (µmol/min/ml)

### Table 8. Summary of purification for lipolytic activity of *Bacillus* sp. isolated from Red oil spill soil

| Step                        | Vol. (mL) | Lipolytic Activity (µmol/min/ml) | Protein Conc. (mg/mL) | Total Activity (µmol/min/ml) | Total Protein (mg) | Specific Activity (µmol/min/mg) | Yield (%) | Fold  |
|-----------------------------|-----------|----------------------------------|-----------------------|-----------------------------|-------------------|---------------------------------|-----------|-------|
| Crude Extract               | 500       | 2.82                             | 8.66                  | 1410                        | 4330              | 0.33                            | 100       | 1     |
| Ammonium Precipitate/Conc.  | 45.8      | 2.57                             | 5.33                  | 117.71                      | 244.11            | 0.48                            | 8.35      | 1.48  |
| Ion Exchange Chromatography | 16.88     | 3.43                             | 2.83                  | 57.89                       | 47.77             | 0.91                            | 4.11      | 3.72  |
| Gel Filtration              | 9.1       | 4.18                             | 1.75                  | 38.04                       | 15.93             | 2.39                            | 2.69      | 7.34  |

### Table 9. Summary of purification for Lipolytic activity of *Fusarium* sp. isolated from soil from dumpsites

| Step                        | Vol. (mL) | Lipolytic Activity (µmol/min/ml) | Protein Conc. (mg/mL) | Total Activity (µmol/min/ml) | Total Protein (mg) | Specific Activity (µmol/min/mg) | Yield (%) | Fold  |
|-----------------------------|-----------|----------------------------------|-----------------------|-----------------------------|-------------------|---------------------------------|-----------|-------|
| Crude Extract               | 500       | 2.05                             | 11.41                 | 1025                        | 5705              | 0.18                            | 100       | 1     |
| Ammonium Precipitate/Conc.  | 25.80     | 2.75                             | 6.83                  | 70.95                       | 176.21            | 0.41                            | 6.92      | 2.24  |
| Ion Exchange Chromatography | 15.10     | 3.37                             | 3.66                  | 57.89                       | 55.27             | 0.91                            | 4.96      | 5.19  |
| Gel Filtration              | 10.20     | 4.51                             | 1.83                  | 46.00                       | 18.67             | 2.46                            | 4.49      | 13.72 |
The purified lipase retained 80.0% residual activity at 60°C at 120 minutes. Also, at 70°C, the lipase activity decreased to about 40% within 120 minutes of incubation. This was similar to the work of Sidhu et al. [35] in which lipase production showed maximum activity at 50°C in olive oil-based medium. Oliveira et al. [36] also reported that the enzyme activity declined as the temperature was gradually increased from 30°C. This is because temperature affects the metabolic activity of microbial cells. Etabilli and Barratti, [37] and Salihu et al. [38] also reported that denaturation of enzymes occurs when the temperature is high. Bacterial lipases have a neutral or alkaline optimum pH except lipase from *P. fluorescens* SIK W1 with acidic pH 4.8 [9,39].

3.1 Effect of pH on Activity and Stability of Lipase

The effect of pH over purified lipase of *Bacillus* sp. isolated from red oil spill soil was studied between pH 3.0 to 9.0 and the results are shown in Fig. 5. Significant differences were observed in the activity of purified lipase at different pH. The maximum activity of lipase was obtained in pH values ranging between pH 7.0 and 9.0 with optimum activity at pH 8.0. The pH stability profile of the enzyme ranged between pH 4.0 to 9.0 for 3 minutes is shown in Fig 6. The purified enzyme showed good stability at pH range 7.0 to 9.0. The enzyme retained 98.42% of residual activity at pH 8.0 for 3 hours. The residual activity of 96.36% at pH 8.0 and 88.79% at pH 8.0 was also recorded.

Subsequently, the effect of pH over purified lipase of *Fusarium* sp. isolated from dumpsite soil was studied between pH 3.0 to 9.0 and the results are shown in Fig. 7. Some significant changes were observed in the activity of purified lipase at different pH. The maximum pH activity ranged between pH 6.0 to 8.0 with the optimum activity of pH 7.0.

The pH stability profile of the enzyme ranged between pH 4.0 to 9.0 for 3 min is shown in Fig. 8. The purified enzyme showed a good stability pH range (7.0 to 9.0). The enzyme retained 86.11% of residual activity at pH 9.0 for 3 hours. The residual activity of 84.14% at pH 7.0 and 81.77% at pH 8.0 was recorded.

![Fig. 1. Effect of temperature on the lipolytic activity of *Bacillus* sp. (ROS 11) grown in production media](image1)

![Fig. 2. Thermal stability of lipase produced by *Bacillus* sp. (ROS 11) in a growth medium](image2)
Fig. 3: Effect of temperature on the lipolytic activity of *Fusarium* sp. (SDS 10) grown in production media

Fig. 4. Thermal stability of lipase produced by *Fusarium* sp. (SDS 10) in a growth medium

### 3.2 Effect of Salts (metal ions) on Lipolytic Activity

The effect of different metal ions on the activity of purified lipase with different metal ions such as ZnCl₂, MnCl₂, CaCl₂, KCl₂, KCl₂ increased the enzyme activity by 139.6% while CaCl₂ and ZnCl₂ slightly inhibited lipase activity. MnCl₂ was a strong inhibitor with 35.6% lipase activity. Also, it was observed that KCl₂ enhanced the activity of lipase produced by *Fusarium* sp. Its activity was slightly inhibited by CaCl₂, ZnCl₂, and MnCl₂. In conclusion, fungi and bacteria isolated from Red oil spill soil had the highest specific lipase activity. Lipase activity of *Bacillus* sp. increased as temperature increases from 30°C to 50°C and from 40°C to 60°C for *Fusarium* sp. The maximum pH for lipase production by *Bacillus* sp. is 8, while for *Fusarium* sp is 7. Sidhu et al. [35] reported a similar effect on lipase activity with CaCl₂ for *Bacillus* species. Bhosal et al. [10] reported that CaCl₂ inhibited the production of lipase while and HgCl₂ supported high lipase production. Lipase activity of *Fusarium* sp. increased in the presence of KCl₂ while CaCl₂ enhanced lipase activity by *Bacillus* sp. Microbial enzymes are more stable and safer than plant and animal enzymes. Microbial lipases are employed in wastewater treatment, pharmaceutical (degrading of lipid clogged drains), dairy (hydrolysis of milk and fat, leather (removal of lipids from hides and skins), etc. The demand for microbial lipase is enormous and increasing due
to the generation of frequent generation of waste. If the discharged waste is not treated, it may cause a serious problem and deteriorates the environment. Extensive and persistent screening for new microorganisms and their lipolytic activities will help to provide faster ways to solve most environmental soil pollution including kitchen wastewater.

**Fig. 5.** Effect of pH on the lipolytic activity of *Bacillus* sp. (ROS 11)

**Fig. 6.** pH stability of lipase produced by *Bacillus* sp. (ROS 11)

**Fig. 7.** Effect of pH on the lipolytic activity of *Fusarium* sp. (SDS 10)
4. CONCLUSION

Fungi and bacteria isolated from Red oil spill soil had the highest specific lipase activity. Bacteria isolates reached their peak for lipase production at 100% within 30-40 hours of incubation. Lipase activity of *Bacillus* sp. increased from 30°C to 50°C and from 40°C to 60°C for *Fusarium* sp. The maximum pH for lipase production by *Bacillus* sp. is 8, while for *Fusarium* sp. is 7. Lipase activity of *Fusarium* sp. increased in the presence of KCl₂, while CaCl₂ enhanced lipase activity by *Bacillus* sp.

5. SIGNIFICANCE STATEMENT

This study discovered the lipolytic activities of bacteria and fungi isolated from cultivated soil, kitchen effluent soil, uncultivated soil, dumpsites and red oil spill soil. *Bacillus* sp. isolated from red oil spill soil and *Fusarium* sp. isolated from dumpsites soil produced the highest lipase activity. Lipases of microbial origin are a class of industrial enzymes ideal as biocatalysts. This study will help the researcher to uncover the critical areas of the best lipase purification method, thermal stability, temperature, pH, and the effect of metal ions on the lipolytic activity of lipase obtained from the microbial origin which many researchers were not able to explore. Thus, a new theory on lipase production by *Bacillus* sp. and *Fusarium* sp. using different carbon sources, nitrogen sources and amino acids which are of considerable importance in optimizing lipase production may be arrived at.
COMPEING INTERESTS

Authors have declared that no competing interests exist.

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