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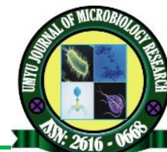

Enhancement of Lipase Production using Bacteria Isolates from Meat Samples within Sokoto Metropolis

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Abstract

The need for biological enzyme production by microbes, such as lipase from bacteria, to replace chemical usage in the food and cosmetic industries will assist in achieving non-toxicity and biodegradability. This study aimed to produce and optimize process conditions of lipase using bacteria isolated from meat samples obtained around Sokoto Modern Abattoir of Sokoto metropolis, Sokoto State Nigeria. Bacteria were isolated and screened using sterile olive oil with phenol red agar. Temperature, substrate concentration, pH, and incubation time were optimized using one factor at a time (OFAT) analyses. The most potent bacteria in the lipase yield production were molecularly characterized. The identified isolates include Bacillus spp., Streptococcus sp., Acinetobacter baumanii, Lactobacillus sp., and Klebsiella pneumoniae. From the screening results, three (3) isolates were positive for lipase production, as evidenced by visible precipitates resulting from the degradation of fatty acids. Out of the three (3) isolates, two (2) exhibit more obvious precipitates from the calcium salt that the fatty acid generated during the hydrolysis reaction. Temperature optimization indicates 40˚C for Bacillus sp. and 50˚C for Acinetobacter baumanii were ideal for lipase synthesis. Acinetobacter baumanii showed more lipase producing activity than Bacillus sp. at pH 8, which was found to be the optimal pH for optimizing lipase synthesis for both isolates. Optimized incubation time also revealed that 48 hours was the ideal duration for the highest yield, while investigations on substrate concentration showed that 2.0% of substrate was ideal for lipase production. The results of the titrimetric assay showed an average of 13.93±8.00U/ml of lipase activity after 24 hours of incubation at 37˚C, with the highest activity recorded from Bacillus sp. (25.00±0.10U/ml). This research revealed Bacillus sp. and Acinetobacter baumanii to be potential candidates in producing lipase, which could serve as a promising biocatalyzing agent for large-scale industrial applications such as food and cosmetic productions.

Keywords: Lipase, production, optimization, meat, bacteria

INTRODUCTION

In recent years, chemical industries have been more interested in creating processes that adhere to the principles of green chemistry because of environmental preservation concerns (Ogodo and Abosede, 2025). Aiming to reduce unwanted byproducts during the reaction process, the use of enzymatic methods has been emphasized as an alternative to traditional chemical processes (Fasim *et al.*, 2021). According to Jothyswarupha *et al.* (2025), the use of catalysts, such as immobilized enzymes, makes it easier to separate and purify the products, lessens environmental issues, and permits catalyst reuse, making them safe, affordable, and environmentally beneficial. All kingdoms of life, including prokaryotes like bacteria and archaea and eukaryotes like plants, animals, and fungi, contain lipases (Verma *et al.*, 2021). Microbial lipases are more stable than those derived from plants and animals, and they are easier, safer, and less expensive to produce in large quantities (Ali *et al.*, 2023).

Although both Gram-positive and Gram-negative bacteria can create a variety of lipases, Gram-negative bacteria produce a larger proportion of bacterial lipases. *Pseudomonas* is the most significant genus of Gram-negative bacteria. It includes at least seven species that produce lipase: *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas fragi*, *Pseudomonas glumae*, *Pseudomonas cepacia*, *Pseudomonas*

fluorescens, and *Pseudomonas putida* (Ullah *et al.,* 2016). The most prevalent Gram-negative bacteria that produce lipase, aside from *Pseudomonas* species, are *Achromobacter, Alcaligenes, Burkholderia,* and *Chromobacterium* strains (Regassa *et al.,* 2021). Microbes that produce lipase have been discovered in a variety of environments, including industrial waste, vegetables, dairy products, oil-contaminated soil, oil-seeds, and decomposing food (Basha *et al.,* 2021).

The market for lipase has grown significantly as a result of growing consumer awareness of animal health and food quality, as well as rising consumption of enzyme-modified cheese (EMC) and enzyme-modified dairy ingredients (EMDI) (Kaya and Özatay, 2024). The market is growing because microbial lipases are more advantageous than plant and animal lipases. Because of their multipurpose advantages in food processing, microbial sources are expected to see a sharp increase in demand in the near future (Adrio and Demain, 2014). Throughout the projection period, the cleaning agent segment is expected to drive the microbial lipase market (Ali *et al.,* 2023).

The development of industrial microbial lipases in the detergent sector is the creative element that allowed lipase to replace harsh chlorine bleach and lessen freshwater pollution from both industry and sewage (Elbrahim and Ma, 2017). Due to its stability, ease of handling, ease of packaging, and consumer preference for transportation, powdered microbial lipases are expected to dominate the microbial lipase markets (Bano *et al.,* 2017). Dairy, food and beverage, animal feed, cleaning, biofuel, pharmaceuticals, textile cosmetics, perfumery, flavor industry, biocatalytic resolution, esters and amino acid derivatives, fine chemicals production, agrochemicals, biosensors, and bioremediation are just a few of the other industries that can benefit greatly from these (Vanleeuw *et al.,* 2019).

Lipases have become more necessary since the 1980s. Because of their advantageous qualities, such as high catalytic efficiency, biodegradability, and high specificity, their application as an industrial catalyst is growing daily (Arife *et al.,* 2015). The demand for lipase in the food business is primarily driven by its unique characteristics, which include temperature (the ideal temperature is 55°C), specificity, non-toxicity, pH dependence, and activity in organic solvents (Mokrani and Nabti, 2024). Both synthetic and hydrolytic lipases with various extraction sources are being studied. The most sought-after characteristics are low product inhibition, reduced reaction time, high yield or activity in non-aqueous media, resistance to temperature changes (Vardar-Yel *et al.,* 2024), the use of mono-, di-, triglycerides, and free fatty acids during trans-esterification, and pH 8 (Khan and Rashid, 2024). Additionally, lipases can react under mild pH and temperature circumstances. This property of lipases aids in lowering the energy required to drive reactions at sporadic pressures and temperatures. Because it alters the kinetics of the reactions, it is therefore possible to protect against the destruction of reactants and products that remain unstable during the reaction. Lipases exhibit stability in organic solvents and can function with their substrate without a co-factor. These characteristics are the primary cause of the rise in microbial lipase demand in biotechnology (Kadam *et al.,* 2024). The aim of this research was to produce and optimize the process conditions of lipase using bacteria isolated from meat.

MATERIALS AND METHODS

Collection of Samples and Processing

Samples of fresh meat were collected from Sokoto Modern Abattoir (latitude 13°0209'N and longitude 005°13.416'E) from different meat sellers for the isolation of lipase-producing bacteria. Two hundred grams (200g) of the samples were aseptically collected in a clean polythene bags and transported to the Microbiology Research Laboratory of the Department of Microbiology, Usmanu Danfodiyo University, Sokoto. The samples were aseptically cut into smaller, thinner pieces using a sterile knife. The analytical portions were placed in separate clean plastic bags to which 200ml of buffered peptone water was added.

Isolation of Lipase-Producing Bacteria

Isolation of lipase-producing bacteria was carried out according to the method described by Mohankumar (2018). One milliliter of each processed meat sample was suspended in 9ml of 0.85% salt (physiological solution). Serial dilution of up to 10^{-4} was made. The diluted solution was spread onto plates of minimum media containing $MgSO_4.7H_2O$ 0.03% (w/v), K_2HPO_4 0.005% (w/v), (NH_4) SO_4 0.5% (w/v), olive oil 2% (v/v), and incubated for 48-72hours at 27°C.

Characterizations of the Bacterial Isolates

The isolates were identified using microscopy, biochemical methods (Urease test, Citrate test, Catalase test, Triple Sugar Iron test), and molecular characterization.

Gram Staining and Microscopy

Gram staining was carried out according to the methods described by Savadori *et al*. (2023). Asmear was made by placing a drop of normal saline on a clean slide. A sterile wire loop was used to pick a colony and emulsified on a clean slide. The smear was heat-fixed by passing the slide over a Bunsen flame. After fixation, the smear was covered with crystal violet for 30 seconds, then rinsed with distilled water. Iodine solution was then applied for 60 seconds and rinsed with water. The slide was decolorized with ethanol and then immediately rinsed with water. The slide was finally counterstained with safranin, allowed to air dry before being examined under a microscope with an oil immersion x100 objective.

Biochemical Characterization of Isolates

The biochemical tests were carried out according to the description of Oyeleke and Manga (2008).

Triple Sugar Iron

With a sterile wire loop, isolates obtained from solid culture media were subcultured. The surface of the test tube containing Triple Sugar Iron (TSI) was streaked, and the bottom was stabbed 2-3 times. The cap was closed loosely and incubated at 37 ˚C for 48 hours. After the overnight incubation, the following readings were taken: Hydrogen sulphide production, glucose production, lactose production, and motility (Savadori *et al*., 2023).

Urease Production

Loopfuls of isolates were streaked into a slant of urease media in universal bottles. After 48 hours of incubation at 37°C with daily inspection, a change in orange to red signifies urease positivity (Oyeleke and Manga, 2008).

Methyl-Red and Voges-Proskauer Test

Five milliliters (5ml) of MR-VP broth was inoculated with the test organism and incubated for 24 hours at 37 ˚C. Then one (1) ml of the broth was transferred to a small serological tube. Two (2) drops of methyl red were added to this quantity. A red colour signifies a positive test, while yellow signifies a negative test. Five (5) drops of 40% potassium hydroxide (KOH) followed by fifteen (15) drops of 5% naphtol in ethanol were loosened and placed in a slanting position. The development of red colour starting from the liquid-air interface within an hour will indicate a VP positive test. No change in colour will indicate a VP negative test (Oyeleke and Manga, 2008).

Indole Production

For twenty-four (24) hours, the organisms were cultivated in 5 milliliters of peptone water. Following a 24-hour incubation period, around three drops of Kovac's Indole reagent were added, and the mixture was gently shaken. A positive reaction was shown by the emergence of a red color in the reagent layer above the soup within 1 minute. The Indole reagent maintains its yellow hue in a negative reaction (Oyeleke and Manga, 2008).

Citrate Utilization Test

Simmons citrate agar comprising ammonium dihydrogen phosphate 1.0g, dipotassium phosphate 1.0 g, sodium chloride 5.0 g, sodium citrate 2.0 g, magnesium sulphate 0.2g, bromothymol blue 0.08 g, and agar 15.0 g in 1000 ml of distilled water was prepared, autoclaved at 121ºC for 15 minutes, and allowed to solidify as slant tubes. Each tube was inoculated with the bacterial culture using a stab-and-streak aseptic technique, and the tubes were then incubated at 37 ˚C for 24 to 48 hours. The slant cultures were checked for growth and any change in color from green to blue (Oyeleke and Manga, 2008).

Hydrogen Sulphide Production

By reducing sulfur from the metabolism of sulfur-containing amino acids, this test determines if bacterial species are capable of producing hydrogen sulfide. Each isolate was inoculated into a speck of triple sugar iron agar, which was then incubated for 48 hours at 37°C. Evolution on blackening of the medium indicates positive, while no evolution resulted in a negative result (Oyeleke and Manga, 2008).

Motility Test

This test determines if bacterial species are capable of producing hydrogen sulfide by reducing sulfur from the metabolism of sulfur-

containing amino acids. Each isolate was inoculated into a speck of triple sugar iron agar, which was then incubated for 48 hours at 37 °C.

Molecular Identification of the Isolates

DNA Extraction/Isolation

Genomic DNA was extracted from the samples using the Quick-DNA Fungal Miniprep Kit (Zymo Research, Catalogue No. D6005). 50-100mg (wet weight) of the fungal cells were added to a ZR BashingBead™ Lysis Tube (0.1 and 0.5 mm). Afterwards, 750 µL of Bashing Bead™ Buffer was added to the tube. The tube was secured in a bead beater (Disruptor Genie) and processed for 20 minutes. The ZR BashingBead™ Lysis Tubes (0.1 and 0.5 mm) were thereafter centrifuged at 10000 x g for 1 minute. 400 µL of the resulting supernatant was transferred into a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8000 x g for 1 minute. Afterwards, the Zymo-Spin™ III-F Filter was discarded. The Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 50µl ofDNA Elution Buffer was added directly to the column matrix. The assembly was thereafter centrifuged at 10,000 x g for 30 seconds to elute the DNA (Zhang *et al.*, 2000).

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was used to amplify the 16S rRNA encoding gene from purified genomic DNA primers using the particular primers 16SF: 5′ GAGTTTGATCCTGGCTTAG-3′ and 16SR: 5′-GGTTACCTTGTTACGACTT-3′. The PCR amplification was carried out in this manner: Using the Qiagen Proof-start Tag Polymerase kit (Qiagen, Hilden, Germany), PCR amplification was carried out. About 2 µl of template DNA (20 ng/µl), 12.5 µl of PCR Master Mix, 20 pmol (2 µl) of each forward and reverse primer, and 8.5 µl of water DNAase-free water completed the reaction volume. All of these substrates were mixed in a total volume of 25 µl, and this was done on ice. At the Biotechnology Research Center at Suez Canal University in Ismalia, the automated thermocycler TC-3000 was used to incubate the entire reaction mixture.

Initial denaturation at 94°C for 5 minutes, 37 cycles of denaturation at 94°C for 30 seconds, annealing at 51°C for 30 seconds, and extension at 72°C for 30 seconds were the reaction conditions. The last extension was carried out for five minutes at 72°C (Qiagen, Hilden, Germany). The amplified 16S rRNA genes were clean using PCR clean up and Gel extraction kit (Thermo Fisher, MA, USA) and sequenced 9ABl 3730xl; Applied Biosystem) and were subjected to a BLAST search (Adeleke, 2024). A BLAST search was performed on the amplified 16S rRNA genes after they were cleaned using a PCR clean up and gel extraction kit (Thermo Fisher, MA, USA) and sequenced using 9ABl 3730xl; Applied Biosystem (Adeleke, 2024).

DNA Sequencing

Dideoxy-mediated chain-termination cycle sequencing was used to sequence the purified PCR product (Sanger *et al.*, 1977). To determine the level of DNA similarity, the obtained 16S rRNA sequence of the bacterial isolate was first examined using the sophisticated BLAST search tool available on the NCBI website: http://www.ncbi.nlm.nih.gov/BLAST/.
Molecular phylogeny and multiple sequence alignment were assessed using the CLUSTALW tool (http://clustalw.ddbj.nig.ac.jp/top-ehtml). The TREE VIEW software was used to illustrate the phylogenetic tree. Using the 16S rRNA gene sequence, a phylogenetic tree was created and compared to the 16S rRNA gene sequences of various standard bacterial strains that were acquired from GenBank.

Screening of Bacteria for Lipase-Producing Bacteria

The identified bacteria were injected onto Tween-20 agar plates and left to incubate for the entire night at 37.0°C. The full breakdown of the fatty acid's salt during the hydrolysis reaction produced a visible precipitate from the calcium salt that the fatty acid created, indicating the presence of lipase activity (Gopinath *et al.*, 2005). The Lipase activity test was conducted using the procedure outlined by Lee *et al.* (2015). Every isolate's pure colony was injected onto sterile olive oil with phenol red agar and incubated for twenty-four hours at 37^0C. Lipase activity was measured by the color shift of phenol red from pink to yellow, which was caused by bacteria that produce lipase.

Through submerged fermentation, lipase was generated from a subset of bacterial isolates (A and D). Peptone 2.0 g, NH4H2PO4 1.0 g, NaCl 2.5 g, MgSO4.7H2O 0.4 g, CaCl2.2H2O 0.4 g, Olive oil 2%(v/v)), Tween 20 (1–2) drop, and distilled water (1000 ml); pH 7.0) comprise the production medium (Composition g/L) (Veerapagu *et al.*, 2013). Following sterilization in the autoclave at 121°C for 15 minutes, the

activated culture of the isolates was inoculated into the production medium (100 ml) contained in Erlenmeyer flasks (500 ml). For 48–72 hours, the flask was kept at 37°C on a metabolic shaker (150 rpm). Following incubation, the crude enzyme extract was extracted using centrifugation for 20 minutes at 10,000 rpm.

Lipase Activity Assessment Using Titrimetric Assay

Each fermentation broth containing *Bacillus* sp. and *Acinetobacter baumanii* was transferred to falcon tubes 24 hours after the inoculation. These falcon tubes were centrifuged at 10,000 rpm for 10 minutes, and the crude enzyme was extracted from the supernatant. After that, the crude enzyme was titrated against 0.05M NaOH to determine its enzyme activity. This was done after each 48 and 72h for each species. The amount of lipase generated was exactly proportional to the amount of acid in the solution, which was determined by the amount of NaOH utilized (Pualsa *et al.*, 2013). Acid value was calculated by the formula:

µmol fatty acid/ml (U) = [(ml NaOH) for sample - ml NaOH for blank x N x 1000]/M

Where U =µmol fatty acid released/ml

N =the normality of NaOH titrant used (0.05 in this case)

M =Total volume of reaction mixture used.

One lipase unit has been defined as the amount of the enzyme that releases one µmol fatty acid per ml under standard assay conditions (U=µmol of fatty acid released/ml) (Karunarathna and Samaraweera, 2024).

Effects of pH, Temperature, and Substrate Concentrations on Lipase Production

The pH range of 4 to 11 was selected to detect the optimum pH where enzymes exhibit maximum activities. Citrate phosphate buffer (4.0 7.0), Glycine-NaOH buffer (7.0 9.0) and Tris-HCl buffers (9.0 11.0) were used for pH adjustments. The pH stability of the enzyme purified was evaluated by incubating the enzyme for about 30 minutes in corresponding buffers of different pH values ranges of 4.0-11.0. To study the effect of temperature on lipase activity, the activity assay was conducted at a temperature range of 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, and 60°C to determine the optimal temperature for maximum enzyme activity. The enzyme stability was assessed by incubating the enzyme with Tris buffer (pH 8) for about 30 min at different specified temperatures, and the remaining enzyme activity was then quantified. Bacterial cells were cultivated on basal media with a variety of carbon sources and Tween 20 to induce lipase synthesis. Fermentation media with the carbon sources at different concentrations of 1, 2, 3, 4, and 5% were inoculated and incubated at 37 °C for 24 h with an agitation speed of 150 rpm. The culture filtrate obtained post-extraction was then employed for quantitative assessment of extracellular lipase. All other variables were kept constant at their optimal settings (Abo-Kamer *et al.*, 2025; Devi *et al.*, 2025).

RESULTS

Microbiological Analysis

Table 1 shows the heterotrophic mean count of Bacteria isolated from meat collected from different meat sellers at Sokoto Modern Abattoir, Sokoto State, Nigeria. The result indicates sample c had the highest colony forming units ($4.53x10^4$±0.025CFU/g) as compared with that from sample a, b, and d, with sample d having the lowest colony forming units ($2.83x10^4$±0.005CFU/g) in Table 1 below.

Table 2 illustrates the morphological and biochemical characterization of the bacterial isolates, in which the samples consist of both Gram-positive and Gram-negative bacteria. Based on morphology, the isolates are rod-shaped (bacilli), cocci, and intermediate cocco-bacilli. A total of seven (7) bacterial isolates were identified from four (4) samples collected from the meat sellers. The isolates identified include *Bacillus* sp., *Streptococcus* sp., *Klebsiella pneumoniae*, *Lactobacillus* sp., and *Acinetobacter baumanii*.

Table 3 presents the extracellular crude lipase activity of *Bacillus* sp. and *Acinetobacter baumanii* after 48 and 72hours of incubation. After 48h, both *Bacillus* sp. and *Acinetobacter baumanii* show activity of 0.505±0.0007U/ml and 0.573±0.0014U/ml. At 72h, 1.329±0.0007U/ml and 1.024±0.0028U/ml were recorded for both species, respectively.

Table 1: Heterotrophic Mean Count of Bacteria Isolated from Meat Collected from Different Meat Sellers at Sokoto Modern Abattoir

Sample	Mean of Bacterial Colony Count (CFU/g)
A	$4.53 \times 10^4 \pm 0.025$
B	$3.90 \times 10^4 \pm 0.015$
C	$5.05 \times 10^4 \pm 0.050$
D	$2.83 \times 10^4 \pm 0.005$

Table 2: Morphological and Biochemical Characterization of the Bacterial Isolates

CFU = Colony forming unit S/No	Gram Reaction	Shape	Catalase	Indole	Citrate	Urease	MR/VP	Glucose	Lactose	Sucrose	Motility	H_2S	Organism
1	+	Rod	+	-	+	-	+/-	+	+	+	+	-	*Bacillus* sp.
2	+	Cocci	-	-	-	-	+/-	+	+	+	-	-	*Streptococcus* sp.
3	-	Coccobacilli	+	-	+	-	-/-	+	-	-	-	-	*Acinetobacter baumanii*
4	-	Rod	+	-	+	+	-/+	+	+	+	-	-	*Klebsiella pneumoniae*
5	+	Rod	-	-	-	-	-/-	+	+	+	-	-	*Lactobacillus* sp
6	-	Rod	+	-	+	-	+/-	+	+	+	+	-	*Bacillus* sp.
7	-	Coccobacilli	+	-	+	-	-/-	+	-	-	-	-	*Acinetobacter baumanii*

Key: + = Positive, - = Negative

Table 3: Extracellular Crude Lipase Activity of *Bacillus* sp. and *Acinetobacter baumanii*

Isolates	Activity after 48 hours incubation (U/ml)	Activity after 72 hours incubation (U/ml)
***Bacillus* sp.**	0.505±0.0007	1.329±0.0007
Acinetobacter baumanii	0.573±0.0014	1.024±0.0028

Screening of Bacteria for Lipase Production

Screening and Identification of Lipase-Producing Bacteria

The screening of bacterial isolates for lipase production is presented in Plates 2 and 3. Out of the seven (7) bacteria isolated, three (3) were positive for lipase production by showing changes in the colour of phenol red dye from pink to yellow on the olive oil-phenol red agar plates and visible precipitates, including a zone of hydrolysis of lipase-producing bacteria on Tween-20 agar. Plate 3 highlights the presence of lipase activities by visible precipitates and a zone of hydrolysis of lipase-producing bacteria on Tween-20 agar. Two (2) of these three (3) Bacterial isolates produced more visible precipitates resulting from the calcium salt formed by the fatty acid from the hydrolysis reaction due to the complete degradation of the salt of the fatty acid.

Molecular Identification

The electrophoretogram of 16S rRNA primer products indicates the amplification based on polymerase chain reaction (PCR), which is presented in Plate 1. The amplification produced 16S ribosomal RNA gene amplicons of approximately 400bp on agarose gel electrophoresis.

Molecular identification of lipase-producing bacteria isolated from meat collected from different meat sellers at Sokoto Modern Abattoir, along Kasuwan Daji, Sokoto. The sequences identified by NCBI BLAST indicates that the two isolates belonged to genera *Bacillus sp.*strain *AT-b3*and *Acinetobacter baumanii*with variation at species level. Their query cover and percentage hit similarity is above 96% as shown in Table 4.

Phylogenetic Analysis of the Bacterial Isolates

Figures 1 and 2 show the phylogenetic tree indicating the evolutionary relationship among the identified species and other species based on similarities and differences in their evolutionary genetic characteristics as compared with their related species from the database of the GenBank NCBI.

Figures 1-2 are the phylogenetic trees of the two isolates constructed with one of their closest

BLAST hits in the NCBI database. The phylogenetic relationship between the nucleotide sequences received from Inqaba Biotech and their closest blast hit from the NCBI database aligned using the Clustal W program in Molecular Evolutionary Genetics Analysis (MEGA), is seen in Figures 1-2. Sample A (*Bacillus* sp.) closest blast includes MG726065.1:701-1026 *Bacillus sp.* strain *XGNQNY17-8* 96%. While for Sample D (*Acinetobacter baumanii*) strain *SL100* closest blast includes MF953991.844-1042 *Acinetobacter* sp. strain *KLP7M1* 96%.

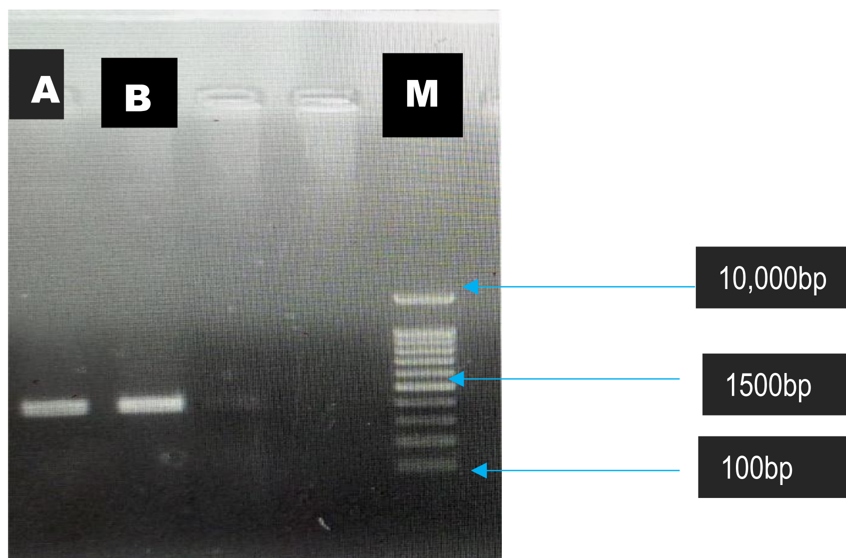
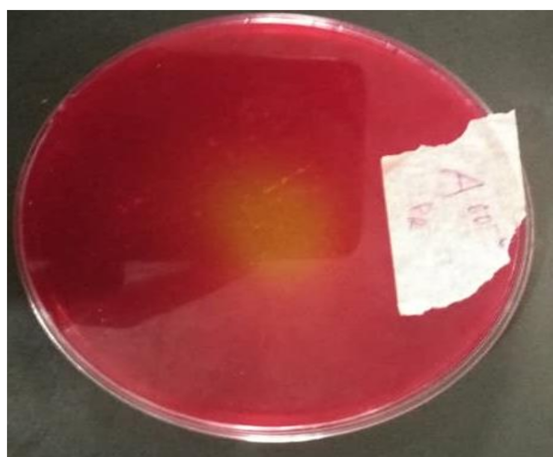


Plate 1: Amplification of 16S rRNA of an average size of 1500bp of Bacterial isolates from meat.
Key:
Lane A=*Bacillus* sp.
Lane B = *Acinetobacter baumanii*
Lane M=DNA ladder



A. *Bacillus* sp.

B. *Acinetobacter baumanii*

Plate 2: Lipase activity indicated by changes of the colour of phenol red dye from pink to yellow on Olive oil-phenol red agar plates by the isolates.

Optimization of Fermentation Conditions for Maximum Lipase-production by the bacterial isolates

One Factor at a Time (OFAT) method was employed in determining the effect of different conditions (Incubation time, pH, Temperature, and Substrate concentration) in Tables 5, 6, 7, and 8 for both *Bacillus* sp. and *Acinetobacter baumanii*.

Effect of Incubation Time on Lipase Production by the Bacteria Isolated from Meat

Table 5 shows the effect of the incubation period of 24 to 96 hours on lipase production for both

Bacillus sp. and *Acinetobacter baumanii*. From the result, the optimal incubation time for both isolates was observed after 48 hours.

Effect of Temperature on lipase-production by the Bacteria isolated from meat

Table 7 presents the effect of the temperature range of 30 to 60 °C on lipase production. The result indicates that for *Bacillus* sp., the highest lipase activity was observed at 40°C, whereas the maximum lipase activity was observed at 50°C for *Acinetobacter baumanii*.

Effect of pH on lipase production by the Bacteria isolated from meat

Table 6 shows the effect of a pH range of 4-10 on lipase production. The result shows that pH significantly affects the lipase production. The maximum pH for both *Bacillus sp.* and *Acinetobacter baumanii* to produce lipase was observed at pH 8.

A. *Bacillus* sp.

B. *Acinetobacter baumanii*

Plate 3: Presence of lipase activities indicated by visible precipitates and zone of hydrolysis of lipase-producing bacteria on Tween-20 agar plates.

Table 4: The BLAST results showing the similarity between the sequence queried and the biological sequences within the NCBI database.

Sample Identity	Predicted Organism	Percentage Similarities	Accession Number
Bacillus sp.	*Bacillus* sp. strain AT-b3	96%	MH348970.1:509-834
Acinetobacter baumanii	*Acinetobacter baumanii* strain SL100	96%	K-1996166.1:789-945

Table 5:Effect of Incubation Time on Lipase Activity (U/ml) for *Bacillus* sp. and *Acinetobacter baumanii*.

Incubation Time (hrs)	Lipase Activity (U/ml)	
	Acinetobacter baumanii	*Bacillus* sp.
24	0.026± 0.001	0.019 ±0.001
48	0.173 ±0.016	0.136 ±0.001
72	0.147 ±0.009	0.119 ±0.001
96	0.109 ±0.015	0.104 ±0.007

Table 6: Effect of pH on Lipase Activity for *Bacillus* sp. and *Acinetobacter baumanii* 37°C

pH	Lipase Activity (U/ml)	
	Acinetobacter baumanii	*Bacillus* sp.
4	0.160±0.011	0.140± 0.003
6	0.164±0.011	0.146±0.006
8	0.276±0.006	0.262± 0.013
10	0.171±0.003	0.212±0.016

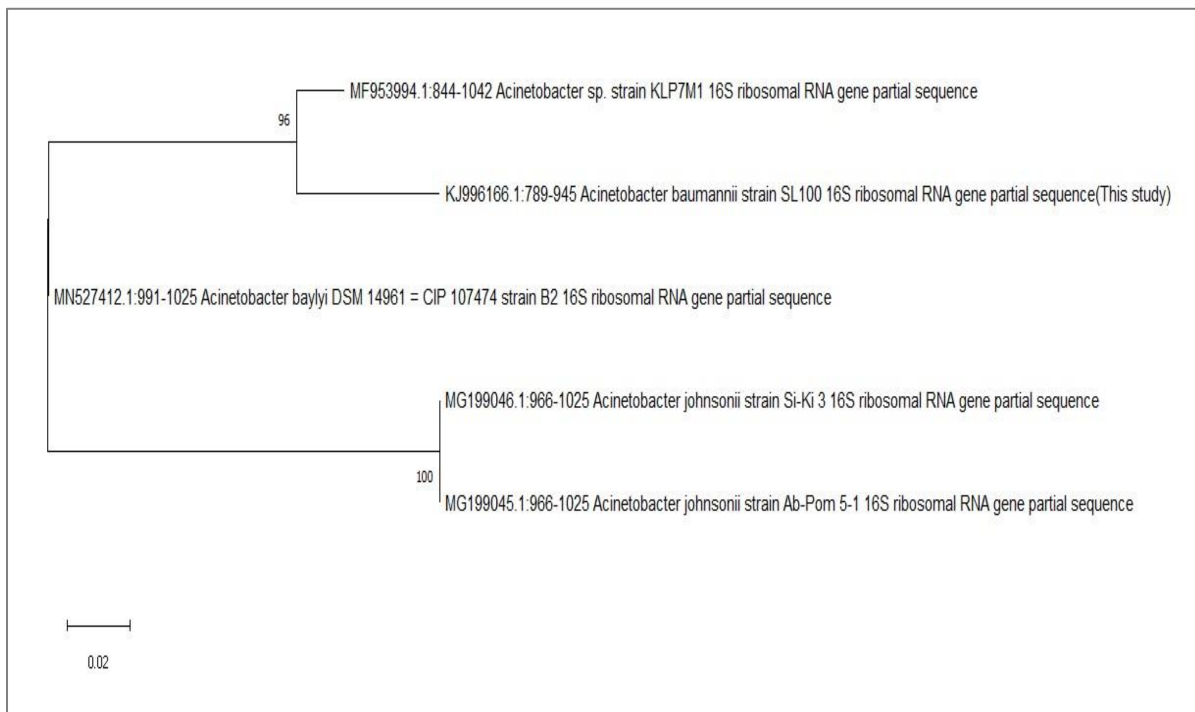


Figure 1: The Neighbour-joining phylogenetic dendrogram based on 16S rRNA gene sequences showing the relationship between the isolate *Acinetobacter baumanii* and closest taxa from NCBI. Bootstrap values are shown at the branching point (greater than 50%).

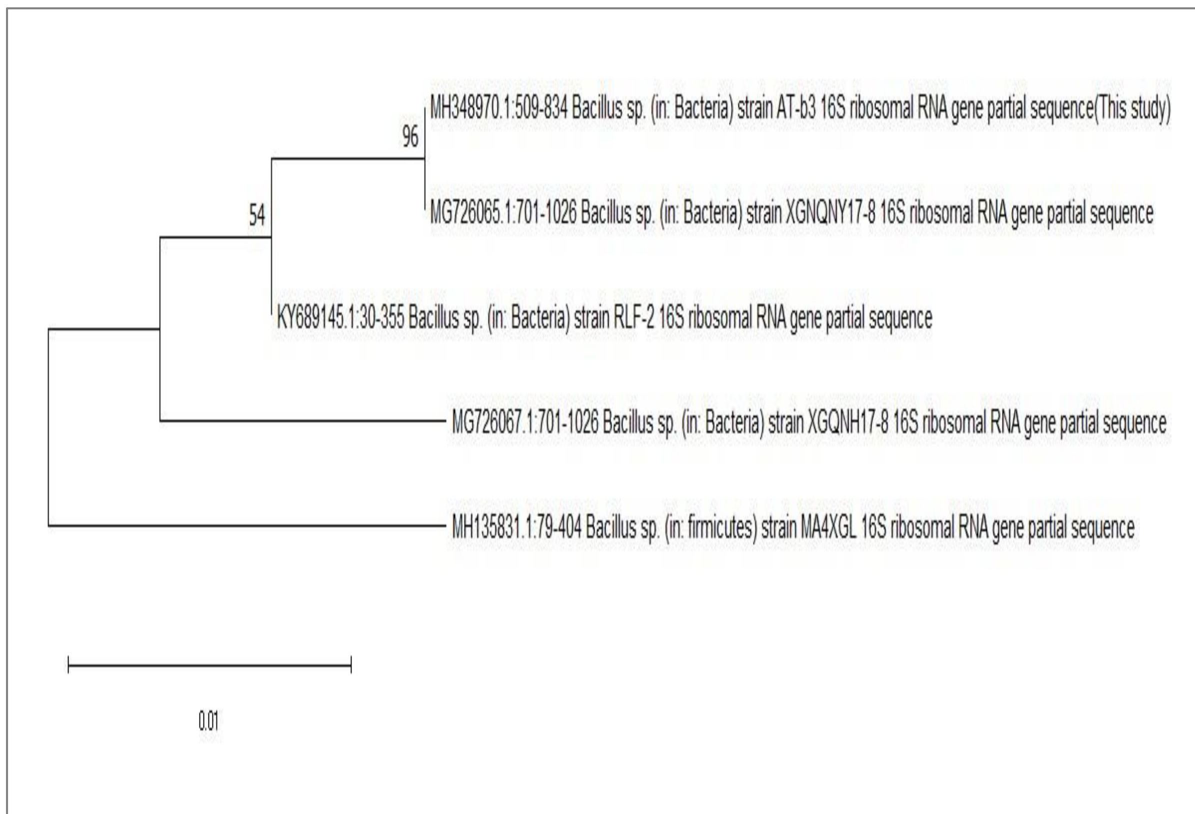


Figure 2: The Neighbour-joining phylogenetic dendrogram based on 16S rRNA gene sequences showing the relationship between the isolate *Bacillus* sp. and closest taxa from NCBI. Bootstrap values are shown at the branching point (greater than 50%).

Table 7: Effect of Temperature on Lipase Activity for *Bacillus* sp. and *Acinetobacter baumanii*.

Temperature	Lipase Activity (U/ml)	
	Acinetobacter baumanii	*Bacillus* sp.
30	0.126 ±0.001	0.061±0.014
40	0.128 ±0.001	0.265±0.017
50	0.361 ±0.015	0.116±0.016
60	0.111 ±0.014	0.150±0.002

Table 8: Effect of Substrate Concentration on Lipase Activity for *Bacillus* sp. and *Acinetobacter baumanii*.

Substrate Conc. (%)	Lipase Activity (U/ml)	
	Acinetobacter baumanii	*Bacillus* sp.
1	0.094±0.016	0.097±0.008
2	0.109±0.008	0.131±0.006
3	0.100±0.004	0.087±0.012
4	0.095±0.017	0.095±0.016
5	0.089±0.004	0.073±0.016

Effect of Substrate Concentration on Lipase Production by the Bacteria Isolated from Meat

Table 8 illustrates the effect of substrate concentration of 1% to 5% on lipase production. The result shows that the optimum lipase activity at a substrate concentration for *Bacillus* sp. and *Acinetobacter baumanii* was observed at 2%.

Titrimetric Assay of lipase activity by the Bacterial isolates from meat

The titrimetric assay showed an average of 13.93±8.00 U/ml of lipase activity after 24 hours of incubation at 37 °C, with the highest activity recorded from Bacillus sp. lipase as 25.00±0.10 U/ml in Tables 9 and 10.

Table 9: Titrimetric lipase activity of *Bacillus* sp.

Time (Min)	Activity (μmol/ml)
0	0.00±0.00
5	7.50±0.01
10	15.2±0.15
15	17.55±0.05
20	20.1±0.05
25	25.0±0.10
30	22.6±0.10

Table 10: Titrimetric lipase activity of *Acinetobacter baumanii*

Time (Min)	Activity (μmol/ml)
0	0.00±0.00
5	5.00±0.10
10	10.0±0.05
15	12.6±0.05
20	17.5±0.05
25	20.0±0.10
30	22.4±0.10

DISCUSSION

In this research, the highest heterotrophic bacterial count (CFU/g) was obtained in meat Sample C with a colony forming unit of 4.53×10^4±0.025CFU/g compared to the other samples (a, b, and d). But sample d was found to possess the least number of colony-forming units of 2.83x104±0.005 CFU/g as provided in Table 1. The presence of bacteria in meat samples is usually attributed to the moisture and blood content present in the meat sample. The presence of these substances makes the proliferation of bacteria possible. This is in conformity with the study carried out by Atlabachew and Mamo (2021) and Uzoigwe *et al*. (2021) on meat samples.

The morphological and biochemical characterization of the bacterial isolates, as presented in Table 2, indicated that isolation of both Gram-positive and Gram-negative bacteria from the meat sample, thereby indicating their presence in meat. From the meat samples collected, a total of 7 bacterial isolates were identified from the 4 samples collected. The isolates identified include *Lactobacillus* sp., *Bacillus* sp., *Streptococcus* sp., *Acinetobacter baumanii*, and *Klebsiella pneumoniae*. This aligns with the study of Mohammad *et al*. (2022).

The extracellular crude lipase activity of *Bacillus* sp. and *Acinetobacter baumanii* after 48 and 72hours of incubation is shown in Table 3. As presented in the Table, after a 48-hour incubation period, both *Bacillus sp.* and *Acinetobacter baumanii* showed varying lipase activity of 0.505±0.0007U/ml and 0.573±0.0014U/ml. While 1.329±0.0007U/ml and 1.024±0.0028U/ml were recorded for both

species after 72h. It showed that Acinetobacter baumanii showed higher activity than Bacillus sp. at 48 hours. Within the next 72 hours, Bacillus sp. was found to show a greater potential by possessing a higher extracellular crude lipase activity than *Acinetobacter baumanii*. This is in line with the research carried out by Musa and Tayo (2012), which showed the lipase activity of both *Bacillus* sp. and *Acinetobacter baumanii*.

From the seven (7) bacteria isolated in this research, 3 species showed lipase-producing activity, which was indicated by the zone of clearance or hydrolysis and also by changes in the colour of phenol dye from red to yellow on the specific agar plates. Two out of the three (3) bacterial isolates, which are *Bacillus sp*. and *Acinetobacter baumanii*, produced more visible precipitates. This is largely attributed to the calcium salt formed by the fatty acid from the hydrolysis reaction due to the complete degradation of the fatty acid salt (Plates 2-3). This is in agreement with the study of Cardenas *et al*. (2001), who observed a clear zone around the colonies that showed lipase-producing activity, as indicated by the zone of clearance or hydrolysis reaction.

Molecular characterization of the two most potent bacterial isolates (*Bacillus* sp. and Acinetobacter baumanii), as presented in Table 4, confirmed that both have the highest similarity index of 96% in the NCBI genebank based on 16S rRNA sequencing after running BLAST, where they were revealed to relate to Bacillus sp. strain AT-b3 and *Acinetobacter baumanii,* respectively. Musa and Tayo (2012), working with various oils, spoiled food, and other residues, identified and characterized 13 genera with lipase activity, including Acinetobacter sp. and *Bacillus sp*. One of the most important factors for the increase in lipase activity by these species is the availability of a carbon source. Several studies confirm a high production of lipase in culture media by these species in the presence of olive oil at a concentration of 0.1% to 3% (Feitosa *et al.*, 2010; Sooch and Kauldhar, 2013; Iqbal and Rehman, 2015), which confirms the results obtained in the present work.

After obtaining the bacterial isolates with the highest lipase activity, different growth parameters that influence lipase production were varied using a one-variable-at-a-time approach for the optimization of lipase production. Temperature optimization indicates that 40°C is the optimal temperature for lipase production for *Bacillus* sp., whereas the maximum lipase activity was observed at 50°C for *Acinetobacter baumanii*. However, *Bacillus* sp. has a higher production efficiency at this temperature, making it potentially more suitable for industrial applications where lipase production is the goal. This isolate shows both higher overall production and a wider range of high-efficiency temperatures.

Temperature is an important factor that affects the growth of microorganisms. Similar observations were reported by Liu *et al*. (2013), who found that thermal stress at higher temperatures led to decreased protein synthesis in *Bacillus subtilis* due to enzyme denaturation and impaired metabolic functions. The maximum lipase activity was observed at 50°C for *Acinetobacter baumanii*which was similar to that of lipase from *Acinetobacter*. *Calcoaceticus* LP009 by Dharmsthiti *et al*. (1998), which demonstrates that the enzyme is thermostable.

The effect of pH on lipase production indicates that there is a significant drop in % lipase production at pH levels lower than 8 and higher than 8. It was observed that the enzyme had no activity at acidic pHs, and the substrate underwent self-hydrolysis at very high pHs. The production is minimal at pH 6, indicating that acidic conditions are less favourable for *Bacillus* sp. For *Acinetobacter baumanii*, the % lipase production also peaks at pH 8, although the peak is higher compared to *Bacillus* sp. Similar to *Bacillus* sp., *Acinetobacter baumanii* also exhibits a drop in % lipase production at pH levels outside the optimal range (5 and 9). Both isolates show minimal % lipase production at pH 6 and 9, indicating that extreme acidic or alkaline conditions are unfavourable for lipase production. The graph indicates that pH 8 is the optimal pH for maximizing % lipase production for both *Bacillus* sp. and *Acinetobacter baumanii*, with *Acinetobacter baumanii* having superior performance at this pH. Maintaining a neutral pH is essential for effective lipase production, with *Bacillus* sp. being the more efficient producer in neutral conditions. These findings are consistent with the study by Liu *et al*. (2013), which demonstrated that neutral pH conditions are generally favorable for optimal bacterial growth and protein synthesis. At neutral pH, enzymes involved in metabolic pathways function optimally, leading to enhanced biomass and protein production.

The effect of substrate concentration on percentage (%) lipase production indicates that the % lipase production by *Bacillus* sp. increases

as the substrate concentration rises from 1% to 2%, peaking at around 0.14 U/ml at 2% substrate concentration. Beyond 2%, the % lipase production begins to decrease, showing a decline at 3% and a significant drop at 5% substrate concentration. The highest efficiency in % lipase production is achieved at a substrate concentration of 2.0%, indicating this as the optimal concentration for *Bacillus* sp., which generally produces a higher % lipase across all substrate concentrations compared to *Acinetobacter baumanii*, except at 5%, where *Acinetobacter baumanii* outperforms *Bacillus* sp. The decline in % lipase production at substrate concentrations above 2% suggests that higher concentrations might lead to substrate inhibition or other metabolic limitations. This finding aligns with previous research by Lyu *et al*. (2025), which demonstrated that an optimal substrate concentration is critical for maximizing microbial growth and protein production, and that excessive substrate can lead to inhibitory effects. At optimal concentrations, the bacteria can efficiently utilize the substrate for growth and protein synthesis. However, beyond the optimal point, excess substrate may cause metabolic imbalances or toxic by-product accumulation, leading to reduced biomass and protein production.

For incubation time optimization, the highest % lipase production for *Bacillus and Acinetobacter* species was observed after 48 hours. *Bacillus* sp. and *Acinetobacter baumanii* lipase production increases steadily, peaking at 48 hours with a yield of around 0.14U/ml and 0.15U/ml. After 48 hours, there is a decline in lipase production, which continues to decrease through 72 and 96 hours. Therefore, for both isolates, the incubation period should be set to around 48 hours to maximize lipase yield before the onset of any decline in production.

Qian and Chun-Yun (2009) in their studies reported a 4% increase in lipase activity with olive oil in the culture medium. Nwachukwu *et al*. (2017) compared several natural oils: olive oil, palm oil, peanut oil, soybean oil, and crude oil. The results with olive oil showed the highest lipase activity compared to other oils, and 0.8% olive oil concentration resulted in an increase in lipase production by 30%. The work of Vishnupriya *et al*. (2010) and Esakkiraj *et al*. (2010) also demonstrated the ability of olive oil as the best source of carbon for bacterial lipase production when compared to other oils. The use of the Tween 20 emulsifier in the orbital shaker showed better growth. The Tween 20 in small amounts acts as a surfactant, homogeneously dispersing the oils through the culture medium, making the lipids easily available to the microorganism. It may also increase cell permeability, thereby increasing the secretion of several molecules across the cell membrane (Silva *et al*., 2005). According to Ramani *et al*. (2010), the addition of surfactants in the culture medium can increase both the activity and the stability of the enzyme. Among bacteria lipases being exploited, those from *Bacillus* species have been reported to exhibit interesting properties that make them potential candidates for biotechnological applications. For example, *Bacillus subtilis, Bacillus pumilis, Bacillus licheniformis, Bacillus coagulans, Bacillus srearothermophilus,* and *Bacillus alcalophilus* are the most common bacterial lipases (Treichel *et al*., 2010).

In the pursuit of understanding the dynamic titrimetric assay of both *Bacillus* sp. and *Acinetobacter baumanii,* the assay was carried out and recorded in Table 5 (for *Bacillus* sp.) and Table 6 (for *Acinetobacter baumanii*). At first, both species were found to possess 0.00±0.00U/ml titrimetric lipase activity. But as the reaction proceeds, their activity increases until Bacillus sp. finally reaches 22.6±0.10U/ml and Acinetobacter baumanii achieves 22.4±0.10U/ml. *Bacillus* sp. showed a slight increase in activity compared to the counterpart *Acinetobacter baumanii*. Furthermore, the titrimetric assay showed an average of 13.93±8.00 U/ml of lipase activity after 24 hours of incubation at 37°C, with the highest activity recorded from *Bacillus* sp. as 25.00±0.10 U/ml. This was similar to the studies carried out by Jaiganesh and Jaganathan (2018) that reported maximum enzyme production at 37°C for 24 hours of incubation. This result highlights the potential of *Bacillus sp.* and *Acinetobacter baumanii* as candidates for lipase production.

CONCLUSION

This research demonstrated the potential of *Bacillus and Acinetobacter* species for the production of lipase using bacteria isolated from meat. The study isolated, screened, and characterized various strains, particularly *Bacillus* sp. and *Acinetobacter baumanii*, which showed remarkable capabilities in lipase production. The optimal conditions for maximizing lipase were identified at a pH of 8, a temperature of 40°C for *Bacillus* sp. *and* 50°C for *Acinetobacter baumanii*, substrate concentration of 2%, and an incubation period of 48 hours at 150rpm. Therefore, these enzymes

(lipases) could serve as a promising biocatalyst in industries.

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