SCREENING, *lip*A GENE AMPLIFICATION AND MOLECULAR IDENTIFICATION OF LIPOLYTIC FUNGI FROM CONTAMINATED SOILS.

ABSTRACT

Lipase is a glycerol ester hydrolase that hydrolyzes esters of triglycerides. This study aimed to isolate, screen and characterize lipolytic fungi with *lip*A gene from soil environments. Fungi [molds and yeasts] were isolated from contaminated soil samples randomly collected from Palm oil mills, Abattoirs and Automobile servicing workshops in Eleme and Obio Akpor local governments in Rivers State, Nigeria using standard microbiological techniques. Qualitative Lipase screening assay was carried out on the fungal isolates through gel diffusion assay using Tributryin, Tween 80 and Olive oil respectively. Isolates with maximum zones of clearance were selected and screened for Lipase gene for hydrolysis of triacylglyceride [*lip*A gene] through Lipase gene amplification and were identified by Internal Transcribed Spacer characterization.

A total of 96 fungal isolates were obtained, lipolytic screening showed 57 lipolytic strains in which 21 lipolytic fungal strains were screened from contaminated soil from palm oil mills, 17 lipolytic strains from abattoirs and 19 lipolytic strains from automobile workshops respectively. Seven isolates with maximum zones of clearance showed *lip*A gene in their genomic DNA and were identified as *Candida orthopsilosis* PMS3 [Accession No: OL546803], *Fusarium proliferatum* PMS4 [Accession No: OL546804], *Saccharomyces cerevisiae* PMS8 [Accession No: OL546805], *Penicillium paxilli* ABS8 [Accession No: OL546799], *Pichia kudriavzevii* ENS1 [Accession No: OL546800] *Aspergillus niger* ENS3 [Accession No: OL546801] and *Trichoderma viride* ENS5 [Accession No: OL546802].
This study showed that soil fungi are potential producers of biotechnologically important lipase with diverse industrial applications.

Keywords: Amplification, Electrophoresis, *lipA* gene, Lipolytic fungi, Tributryin.

1 INTRODUCTION

Enzymes are considered as nature's catalysts with unique characteristics of specificity, mild conditions of use and reduced waste generation [1]. In comparison to a chemical process, enzymatic processes present less energy requirements and higher quality of the finished product [2]. One of the prominent enzymes with tremendous biotechnological potentials is Lipase. Lipases are triacylglycerol acylhydrolases [E.C.3.1.1.3] that catalyze the

hydrolysis of triacylglycerol to glycerol and fatty acids [3].These enzymes can also catalyze esterification, transesterification, acidolysis, alcoholysis and aminolysis [4, 5].The number of available industrial lipases has increased considerably since the 1980's in response to an increasing demand for these biocatalysts [6]. Lipases occur widely in nature and have been found in many species of animals, plants and microorganisms [7].

However, microbial lipases are preferred because they are stable, safe and more useful than those derived from plant and animals. This is because of the great variety of catalytic activities available, ease of genetic manipulation and regular supply due to absence of seasonal fluctuations [8].The interest in microbial lipase production has increased in the last decade, because of its large potential in manufacturing applications [9] such as food additives [flavor modification], fine chemicals [synthesis of esters], waste water treatment [decomposition and removal of oil substances], cosmetics [removal of lipids], pharmaceutical [digestion of oils and fats in drugs], leather [removal of lipids from animal skins] medicine [blood triglyceride assay], textile, paper and synthesis of biopolymers [7,10] , biofuels [biodiesel and biolubricant] production[11] . Many industrial lipases have been produced, purified and cloned from fungi and bacteria [12] but fungi are recognized as the best lipase producers and mostly preferred sources due to their ability to produce copious amounts of extracellular lipases, the low cost of extraction, thermal and pH stability as well as activity in organic solvents [13]. Soil contaminated with spillage from the products of oil, dairy wastes and deteriorated food harbors fungal species which have the potential to secrete lipases to degrade fats and oils [14]. The aim of this study is to isolate, screen and carry out lipase gene amplification and molecular identification of lipolytic fungal strains from palm oil mill, automobile servicing workshop and abattoir contaminated soil samples.

2 MATERIALS AND METHODS

2.1 Sample collection

Contaminated soil samples from Automobile servicing workshops at Elijiji [4.8355642°N and 7.0581128°E] and Alesa-eleme [4.7760502°N and 7.1302336°E]; Palm oil mills at Rumuekini [4.9256957°N and 6.9429731°E] and Ebubu-eleme [4.7756238°N and 7.1456339°E] and Abattoirs at Rumu-ahunwo [4.9319067°N and 6.9388067°E] and Aleto-eleme [4.8086852°N and 7.1001792°E] communities within Rivers State in Nigeria were collected randomly using a soil auger from a depth of 30cm in sterile bags and transported to the Industrial Microbiology Laboratory in the University of Port Harcourt, Nigeria for processing.

2.2 Isolation of fungi from contaminated soil samples

The soil samples were mixed together according to the collection sites to obtain the representative of the homogenous mixture, 1g of each sample was weighed, dispensed into test tubes containing 9ml of sterilized normal saline water, followed by centrifugation for 10minutes at 120 round per minute (rpm) to dislodge soil clumps and

allowed to settle. The supernatants were decanted, serially diluted and dispensed on freshly prepared potato dextrose agar plates. They were seeded with 1% lactic acid to prevent bacterial contamination using spread plate technique and subsequently incubated at 30°C for 96hours. Discrete fungal colonies were picked and sub-cultured using conventional streak plate method for yeast and pin point method for mold in duplicates. The sub-cultured plates were incubated at 30°C for 72hours for yeasts and 96hours for molds. Pure fungal colonies were transferred to potato dextrose agar slants and stored at 4°C for further screening.

2.3 Screening of fungal isolates for lipase production

Screening of the fungal isolates for lipase production was carried out using gel diffusion assay method with Tween 80, Olive oil and Tributryin respectively.

2.3.1 Tween 80 Assay

Tween 80 medium was prepared by adding [peptone - 100g, Sodium chloride - 5g, $CaCl_2 \cdot 5H_2O$ - 0.1g and agar-20g into 1litre of distilled water], the pH was adjusted to 7.0 with 1M sodium hydroxide. The medium was sterilized at 121°C for 15minutes, and 1% of sterilized Tween 80 was added to the sterilized medium before plating. Inoculation of 0.2ml of 1.5×10^8 cfu/ml of pure fungal stock solution into 1cm diameter agar wells was carried out and the inoculated plates were set aside for 30minutes for the fungal stock solution to diffuse into the medium. The plates were inverted and incubated at 30°C for 48hours and lipolytic activity was indicated by the appearance of an opaque zone around the well which was measured in millimeter.

2.3.2 Olive oil Assay

Olive oil medium agar [Phenol red - 0.1g, Calcium chloride - 1.47g, olive oil - 1% and agar - 2%] at pH 7 was prepared, 0.2ml of 1.5×10^8 cfu/ml of stock solution of the pure fungal isolates was inoculated into 1cm diameter agar wells and incubated at 30°C for 24hours. Lipolytic activity was indicated by the changing of the red colour of the medium to yellow as a result of increase in acidity due to the release of fatty acids following lipolysis. The zone of clearance was measured in millimeter.

2.3.3 Tributryin Assay

Inoculation of 0.2ml of 1.5×10^8 cfu/ml of the fungal stock solution into 1cm diameter agar wells on tributryin medium agar [peptone - 0.5%, yeast extract - 0.3%, agar - 2% supplemented with 1% tributryin at pH 7] was carried out and was incubated for 48hours at 30°C. Lipolytic activity was indicated by appearance of clear halo zone around the agar well and was measured in millimeter.

2.4 Lipase Gene Amplification

Further lipase screening was carried out through functional lipase gene [*lip*A gene] amplification of selected highly potent lipase producing fungal isolates with maximum zones in the tween 80, Olive oil and tributryin assays and those isolates with *lip*A functional gene in their genomic DNA were selected for molecular identification.

2.4.1 DNA extraction using ZR fungal DNA miniprep [Zymo Research]

The fungal cells broth was prepared with de-deionised water and 2ml of the broth was transferred to a ZR Bashing TM lysis tube and 750µl Lysis Solution was added. The tube content was centrifuged in a microcentrifuge at 10,000rpm for 1minute, 400µl of the supernantant was transferred to a Zymo-spinTM IV spin filter in a collection tube and was centrifuged at 7,000rpm for 1minute. Fungal DNA Binding buffer of 1200µl was added to the supernantant, 800 µl of the mixture was transferred to a Zymo – spin TM IIC column in a collection tube and centrifuge at 10000rpm for 1minute followed by the addition of 200µl DNA pre-wash Buffer to the mixture and centrifuged at 10000rpm for 1minute. About 500µl of Fungal DNA wash buffer was added to the Zymo-spin TM IIC column mixture and centrifuged at 10000rpm for 1minute. It was transferred to a clean 1.5ml micro-centrifuge tube; 100µl of DNA elution buffer was added to the column matrix and centrifuged at 10000rpm for 30seconds to elute the DNA. The DNA supernatant was stored at -20°C for further analysis.

2.4.2 DNA Quantification

The extracted DNA was quantified using the Nanodrop 1000 spectrophotometer and 2µl of sterile distilled water was used to initialize the equipment. 2µl of the extracted DNA was loaded on the lower pedestal and the upper pedestal was lowered to contact the extracted DNA and the DNA concentration was measured.

2.4.3 Amplification of 18S rRNA gene by Polymerase Chain Reaction

The 18S rRNA region of the extracted DNA was amplified by Polymerase Chain reaction using LIPASEF: ATGGTTCACGGTATTGGAGG and LIPASER: CTGCTGTAAATGGATGTGTA as the forward and backward primers.

The PCR mix was made up of 12.5µl of Taq 2X master mix from New England Biolabs [M0270]; 1µl each of 10µM of the forward and backward primer, 2µl of the DNA template were made up with 8.5µl of nuclease free water. The initial denaturation was done at 95°C for 5minutes, followed by 35cycles of denaturation at 95°C for 30seconds, annealing at 55°C for 30seconds and elongation at 72°C for 7minutes and finally held at 10°C.

2.4.4 Gel Electrophoresis

A gram of agarose was mixed with 100ml of 1xTAE in a microwavable flask. The mixture was micro-waved for 1 to 3minutes until the agarose was completely dissolved. The agarose solution was allowed to cool to 50°C for 5minutes,10µl of EZ vision DNA stain was added to aid visualization under ultraviolet [UV] light. The agarose gel was poured into a gel tray with the well comb in place and allowed to set at room temperature for 30minutes until it

was completely solidified. The gel was placed in the electrophoresis gel box and the box was filled with 1xTAE until the gel is covered. Loading buffer was added to the amplicons. The molecular weight ladder was carefully loaded into the first lane of the gel, followed by the amplicons into the additional wells of the gel. The agarose gel electrophoresis was allowed to run for 1hour at 150V, the gel was removed and visualized under UV transilluminator.

2.5 Molecular Identification of lipolytic fungal isolates

The lipolytic fungal isolates that were positive to *lip*A gene amplification were further identified by Molecular identification.

2.5.1 Internal Transcribed Spacer [ITS] Amplification using Polymerase Chain Reaction

The Internal Transcribed Spacer region of the extracted DNA was amplified using Polymerase chain reaction. The PCR mixture using ITS1: 5'TCCGTAGGTGAACCTGCGG3' and ITS4:5'TCCTCCGCTTATTGACATGS3' as forward and backward primers. The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs [M0270]; 1µL of 10µM forward and reverse primer; 2µL of DNA template and the mixture was made up with 8.5µL Nuclease free water. Initial denaturation was carried out at 94˚C for 5minutes, followed by 35cycles of denaturation at 94˚C for 30seconds, annealing at 54˚C for 30seconds ,initial elongation at 72˚C for 45seconds,final elongation step at 72˚C for 7minutes and hold at 10˚C.

2.5.2 Gel Electrophoresis

Loading buffer was added to the amplicons. The molecular weight ladder was carefully loaded into the first lane of the gel and the amplicons into the additional wells of the gel. The agarose gel electrophoresis was allowed to run for 1hour at 150V and then the gel was removed and visualized under UV transilluminator.

2.5.3 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual. Sequencing of the amplicons was carried out using the Big Dye terminator kit at 96˚C for 4minutes for 32cycles on an ABI Genetic Analyzer 3130xl sequencer.

2.5.4 DNA sequence and phylogenetic analysis

The sequence obtained were edited by the Bio - Edit [Bioinformatic Alogarithm Trace Edit] software and analyzed by comparism with closely related sequences retrieved from National Center of Biotechnology Information [NCBI] database using BLAST. The evolutionary history was inferred using the UPGMA method. Using the neighbor joining method, the evolutionary analyses were conducted in MEGA11. The evolutionary distances were computed using the Maximum Composite Likelihood method in the units of the number of base substitutions per site.

3 RESULTS

3.1 Isolation and Screening of lipolytic fungi

A total of ninety six fungal [96] isolates were obtained from all the soil samples, Thirty six [36] fungal isolates [13yeasts and 23molds] from palm oil mills soil samples [PMS], 33 isolates [8 yeasts and 25molds] from automobile servicing workshops soil samples [ENS] and 27 isolates [10 yeasts and 17molds] from Abattoirs soil samples [ABS]. Lipase screening through gel diffusion assay using olive oil, tributryin and tween 80 showed a total of 57 lipolytic strains and 39 non lipolytic strains; 21 lipolytic fungal strains and 15 non lipolytic fungal strains were screened from palm oil mill contaminated soil as presented in the table 1. A total of 17 lipolytic strains and 10 non lipolytic strains were screened from abattoir waste contaminated soil samples as shown in Table 2 and 19 lipolytic strains and 14 non lipolytic strains were screened from automobile servicing workshop soil samples as shown in Table 3 respectively.

Table 1: Lipase screening of fungal isolates from palm oil mill contaminated soil through gel diffusion assay

ISOLATE CODE	ISOLATE IDENTITY	OLIVE OIL ASSAY	TWEEN 80 ASSAY	TRIBUTRYIN ASSAY
PMS1	MOLD	16.000±1.000^{b}	13.000±1.000^{b}	10.667±1.528^{d}
PMS2	MOLD	11.000±1.000^{c}	10.333±0.577^{d}	14.000±1.000^{b}
PMS3	YEAST	31.667±1.528^{a}	28.333±0.577^{a}	28.000±1.000^{a}
PMS4	MOLD	30.667±1.528^{a}	26.333±1.155^{a}	27.667±1.528^{a}
PMS5	YEAST	-ve	-ve	-ve
PMS6	YEAST	-ve	-ve	-ve
PMS7	MOLD	-ve	-ve	-ve
PMS8	YEAST	30.333±0.577^{a}	24.667±1.528^{b}	26.333±1.155^{a}
PMS9	MOLD	10.333±1.155^{c}	9.333±1.155^{e}	13.333±0.577^{b}
PMS10	MOLD	-ve	-ve	-ve
PMS11	MOLD	13.333±1.528^{b}	10.667±1.528^{d}	11.333±0.577^{b}
PMS12	MOLD	-ve	-ve	-ve
PMS13	YEAST	15.333±0.577^{b}	11.000±1.000^{d}	13.667±1.528^{b}
PMS14	MOLD	12.000±1.000^{c}	15.500±0.707^{c}	9.000±1.000^{e}
PMS15	MOLD	9.333±1.155^{d}	11.333±0.577^{d}	6.333±1.155^{g}
PMS16	YEAST	-ve	-ve	-ve

PMS17	MOLD	11.667±1.528[c]	8.333±0.577[g]	10.000±1.000[d]
PMS18	YEAST	-ve	-ve	-ve
PMS19	MOLD	6.667±1.528[g]	4.000±1.000[i]	4.333±0.577[h]
PMS20	YEAST	8.000±1.000[f]	8.667±1.528[f]	11.000±1.000[d]
PMS21	MOLD	6.667±1.528[g]	4.333±1.155[i]	11.000±1.000[d]
PMS22	MOLD	-ve	-ve	-ve
PMS23	MOLD	7.333±0.577[g]	3.333±0.577[j]	5.667±1.528[g]
PMS24	MOLD	-ve	-ve	-ve
PMS25	MOLD	5.667±1.528[h]	4.333±1.155[i]	3.333±0.577[i]
PMS26	MOLD	5.500±1.378[j]	4.167±1.169[j]	2.833±0.753[i]
PMS27	YEAST	-ve	-ve	-ve
PMS28	MOLD	-ve	-ve	-ve
PMS29	YEAST	8.333±0.577[e]	6.333±0.577[h]	6.000±1.000[g]
PMS30	MOLD	13.000±1.000[c]	9.667±1.528[e]	11.667±1.528[c]
PMS31	YEAST	-ve	-ve	-ve -
PMS32	YEAST	-ve	-ve	-ve
PMS33	MOLD	5.667±1.155[h]	3.333±0.577[j]	2.333±0.577[i]
PMS34	MOLD	-ve	-ve	-ve
PMS35	MOLD	9.333±0.577[d]	5.333±0.577[i]	7.333±1.155[f]
PMS36	YEAST	-ve	-ve	-ve

KEY: PMS= isolates from palm oil mill contaminated soil samples; -ve = No zone of clearance

Values are means of triplicate ± Standard deviations *Means that do not share the same letter are significantly different.

Table 2: Lipase screening of fungal isolates from Abattoir soil through gel diffusion assay

ISOLATE CODE	ISOLATE IDENTITY	OLIVE OIL ASSAY	TWEEN 80 ASSAY	TRIBUTRYIN ASSAY
ABS1	YEAST	18.667±1.528[b]	14.000±1.000[b]	15.333±1.155[b]
ABS2	MOLD	5.333±0.577[i]	4.000±1.000[f]	2.667±1.528[g]
ABS3	MOLD	-ve	-ve	-ve

ABS4	YEAST	7.667±1.528 [f]	11.667±1.528 [c]	7.000±1.000[e]
ABS5	YEAST	8.333±0.577[e]	10.000±1.000[c]	11.000±1.000[c]
ABS6	YEAST	6.000±1.000[h]	4.333±0.577[f]	2.333±0.577[h]
ABS7	MOLD	18.333±1.155[b]	17.000±1.000[b]	17.000±1.000[b]
ABS8	MOLD	28.333±0.577[a]	22.667±1.528[a]	25.000±1.000[a]
ABS9	MOLD	-ve	-ve	-ve
ABS10	MOLD	10.333±0.577[c]	4.333±0.577[f]	6.333±0.577[f]
ABS11	YEAST	-ve	-ve	-ve
ABS12	YEAST	7.000±1.000[g]	6.333±1.155[e]	4.667±1.528[f]
ABS13	MOLD	-ve	-ve	-ve
ABS14	YEAST	12.000±1.000[c]	11.000±1.000[c]	13.000±1.000[c]
ABS15	MOLD	-ve	-ve	-ve
ABS16	MOLD	-ve	-ve	-ve
ABS17	MOLD	-ve	-ve	-ve
ABS18	YEAST	6.667±1.528[g]	5.667±1.528[f]	3.333±0.577[g]
ABS19	MOLD	11.000±1.000[c]	8.000±1.000[d]	9.000±1.000[d]
ABS20	MOLD	7.333±1.155[f]	5.333±0.577[f]	3.333±0.577[g]
ABS21	YEAST	8.667±1.528[e]	4.000±1.000[g]	5.000±1.000[f]
ABS22	MOLD	-ve	-ve	-ve
ABS23	MOLD	5.000±1.000[I]	3.667±1.528[g]	2.333±1.155[h]
ABS24	MOLD	9.333±0.577[d]	8.667±1.528[c]	6.333±0.577[f]
ABS25	MOLD	-ve	-ve	-ve
ABS26	YEAST	-ve	-ve	-ve
ABS27	MOLD	4.000±1.000[k]	3.333±0.577[g]	2.667±1.528[g]

KEY: ABS= Isolates from Abattoir contaminated soil samples -ve = No zone of clearance

Values are means of triplicate ± Standard deviations *Means that do not share the same letter are significantly different.

Table 3: Lipase screening of fungal isolates from Automobile servicing workshop soil through gel diffusion assay

ISOLATE	ISOLATE IDENTITY	OLIVE OIL ASSAY	TWEEN 80 ASSAY	TRIBUTRYIN ASSAY

CODE				
ENS1	YEAST	29.333±0.577[a]	21.333±0.577 [a]	22.667±1.528[b]
ENS2	YEAST	-ve	-ve	-ve
ENS3	MOLD	19.000±1.000[b]	18.000±1.000[b]	18.333±0.577[c]
ENS4	YEAST	4.000±1.000[i]	3.667±1.528[i]	2.333±0.577[f]
ENS5	MOLD	28.667±1.528[a]	20.333±0.577 [a]	26.000±1.000[a]
ENS6	YEAST	9.000±1.000[f]	8.667±1.528[f]	12.000±1.000[d]
ENS7	MOLD	11.667±1.528[d]	11.000±1.000[d]	10.333±0.577[d]
ENS8	MOLD	14.000±1.000[c]	7.333±0.577[h]	11.333±1.155[d]
ENS9	MOLD	-ve	-ve	-ve
ENS10	YEAST	18.000±1.000[b]	16.667±1.528[b]	17.333±0.577[c]
ENS11	MOLD	9.000±1.000[f]	10.333±0.577[d]	12.667±1.528[d]
ENS12	MOLD	-ve	-ve	-ve
ENS13	MOLD	14.333±1.528[C]	12.000±1.000[c]	13.000±1.000[d]
ENS14	MOLD	14.333±0.577[g]	13.333±0.577[c]	11.333±1.155[d]
ENS15	YEAST	-ve	-ve	-ve
ENS16	MOLD	-ve	-ve	-ve
ENS17	MOLD	12.000±1.000[c]	11.333±1.155[c]	11.000±1.000[d]
ENS19	MOLD	8.667±1.528[f]	7.667±1.528[g]	11.333±1.155[d]
ENSS20	YEAST	6.000±1.000[h]	4.667±1.528[i]	2.667±1.528[f]
ENS21	MOLD	-ve	-ve	-ve
ENS22	MOLD	-ve	-ve	-ve
ENS23	MOLD	11.000±1.000[e]	8.667±1.528[f]	7.333±0.577[e]
ENS24	MOLD	-ve	-ve	-ve
ENS25	MOLD	-ve	-ve	-ve
ENS26	MOLD	6.667±0.577[g]	3.333±0.577[i]	5.000±1.000[e]
ENS27	MOLD	14.000±1.000[c]	9.667±1.528[e]	11.333±0.577[d]
ENS28	MOLD	-ve	-ve	-ve
ENS29	MOLD	5.667±1.155[h]	4.333±1.155[i]	3.333±1.155[f]
ENS30	MOLD	-ve	-ve	-ve

ENS31	MOLD	-ve	-ve	-ve
ENS32	YEAST	4.000±1.000[l]	3.333±0.577[l]	2.333±0.577[l]
ENS33	MOLD	-ve	-ve	-ve

KEY: ENS= Isolates from Automobile servicing workshop soil; -ve=No zone of clearance

Values are means of triplicate ± Standard deviations *Means that do not share the same letter are significantly different.

3.2 Lipase Gene Amplification

Ten fungal isolates with maximum zones of clearance designated as PMS3, PMS4, PMS8 from palm oil mill contaminated soil samples; ABS1, ABS7, ABS 8 from Abattoir contaminated soil samples and ENS1, ENS3, ENS5 and ENS10 from Automobile servicing workshop soil samples were selected and further screened for the presence of lipA gene in which seven isolates [PMS3, PMS4, PMS8, ABS 8 ENS1, ENS3 and ENS5] showed the presence of lipA gene in their genomic DNA. The gel electrophoresis image showed the amplification of the gene at 300 base pairs as shown in Figure1.

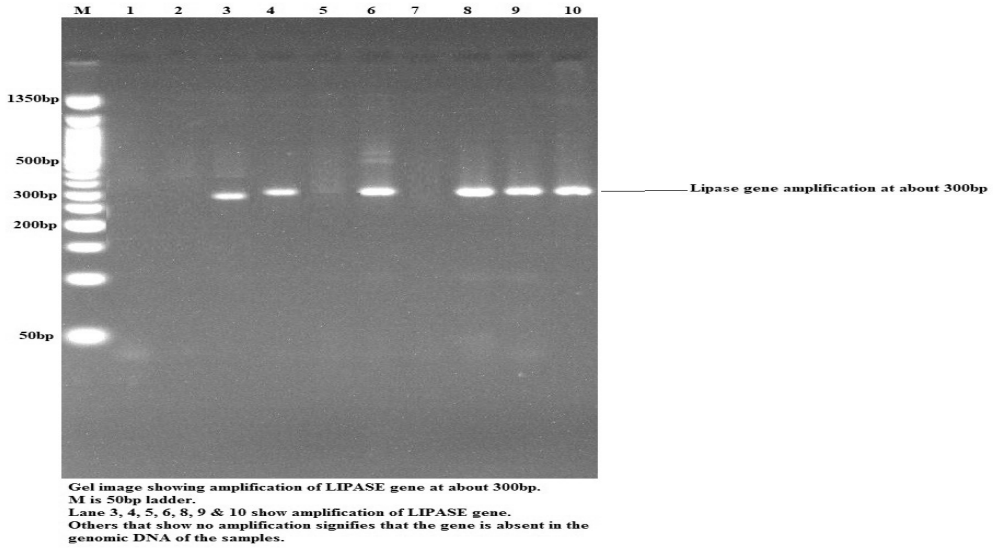
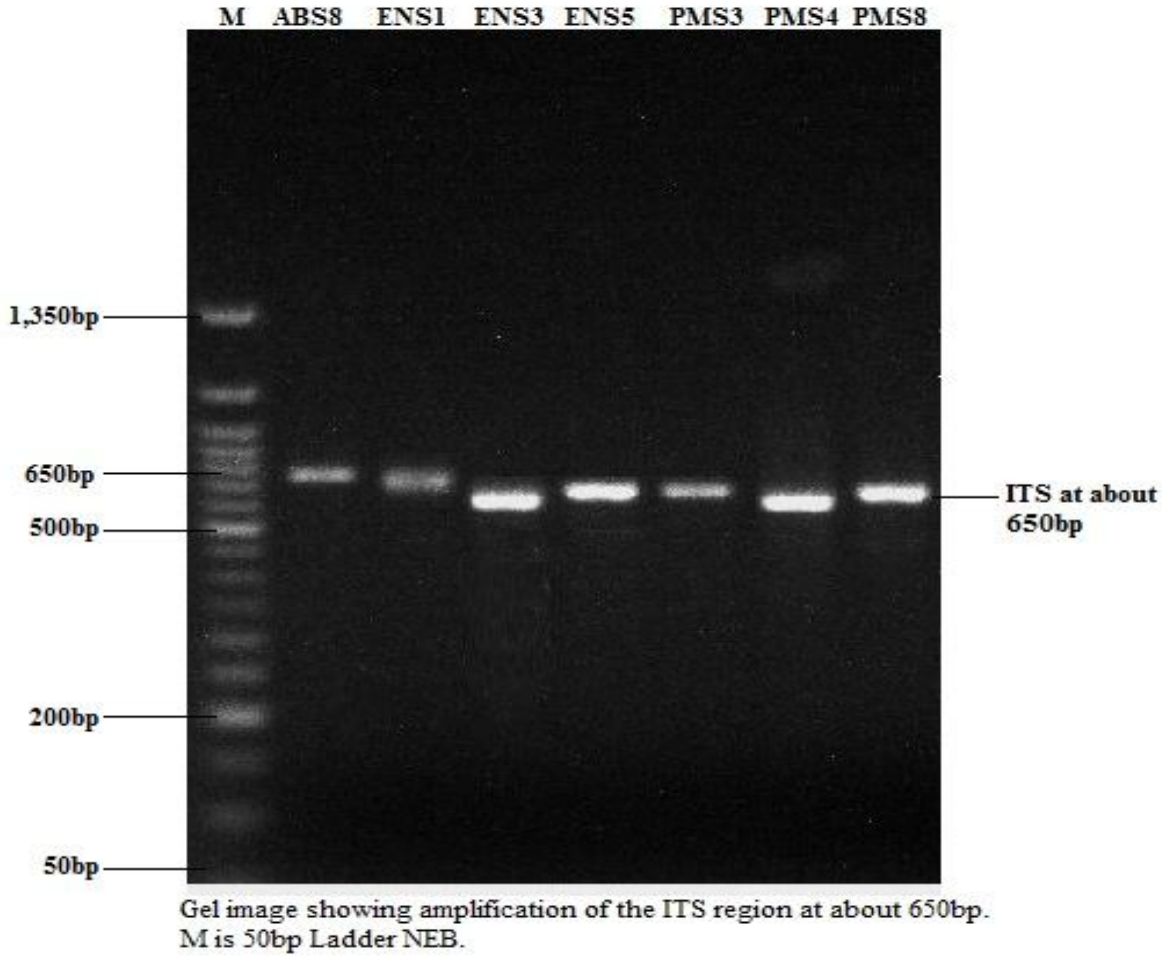


Figure 1: Lipase gene amplification

3.3 Molecular Identification

The seven lipolytic fungal genomic DNA were further sequenced using Internal Transcribed Spacer [ITS] rDNA sequencing for molecular identification. The gel electrophoresis of the PCR products showed distinct band of the marker [Lane 1] and base pairs of the seven fungal strains as depicted in figure 2.The sequencing and phylogenetic results showed maximum resemblance of the ITS rDNA sequences of the strains acquired during the mega blast

search for those sequences that are closely related from the NCBI nucleotide database. The phylogenetic placement of the strains when the evolutionary distances were computed using the Maximum Composite Likelihood method and the lipolytic fungi were inferred as *Candida orthopsilosis* PMS3[Accession No: OL546803], *Fusarium proliferatum* PMS4[Accession No: OL546804],*Saccharomyces cerevisiae* PMS8 [Accession No: OL546805], *Penicillium paxilli* ABS8[Accession No: OL546799], *Pichia kudriavzevii* ENS1[Accession No: OL546800], *Aspergillus niger* ENS3[Accession No: OL546801] and *Trichoderma viride* ENS5[Accession No: OL546802].The phylogenetic representation of the most similarly matched results of the ITS sequences obtained was shown in Figure 3



Gel image showing amplification of the ITS region at about 650bp. M is 50bp Ladder NEB.

Figure 2: ITS PCR gel electrophoresis image of the lipolytic fungal strains.

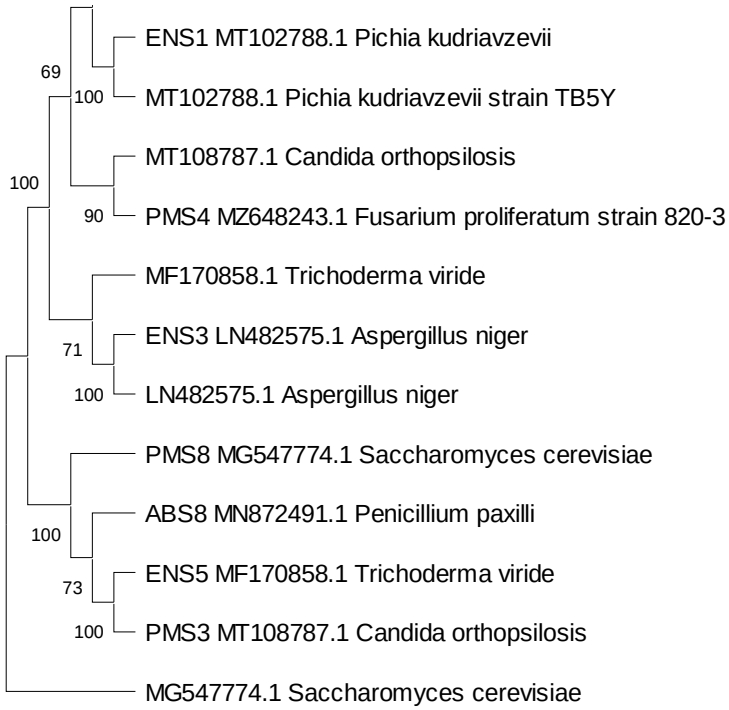


Figure3: Neighbor-joining phylogenetic tree of the lipolytic fungal isolates showing their relatedness based on their sequences.

4 DISCUSSION

Fungal lipase belongs to the most important group of commercial enzymes which act as catalyst in different industrial sectors such as food, waste water treatment, cosmetics, oleo-chemical, pharmaceutics, detergents and biofuel sector [15]. The interest in lipase production has greatly increased in the last decades due to their versatile features, catalytic properties and potential in biotechnological application. Lipase-producing fungi are ubiquitous [16], they can be found in several habitats such as soils contaminated with spillage from oils and dairy products, oily waste water, oil seeds, compost heaps, and deteriorated food [9]. Therefore soil is a biological reservoir for variety of fungal species that have the potential to secrete lipases to degrade fats and oils [17]. This mechanism helps them to adapt to the environmental extremes. This was confirmed by the results obtained with wide range of potential lipase producing fungi from palm oil mill, automobile workshop and abattoir contaminated soil samples in this study, similar studies carried out by [18] revealed twelve fungal strains from palm oil mill dumping site. Lipase producing fungi were also isolated from soil collected from mechanic workshop [19] indicated that *Aspergillus* sp. was identified as the prominent lipase producing strain. However, no work has been reported on lipase producing fungi from abattoir contaminated soil thereby making the result obtained from this study one of its kind.

Several methods can be used for lipase screening based on the determination of the presence of extracellular lipases. The use of a solid medium [gel diffusion method] with inducer substrates such as olive oils, tributyrin and Tween 80 have been widely described in literatures [20, 21 and 22]. The result from this study showed fifty one lipolytic fungal strains. However, some of these substrates may not be adequate for lipase detection because of its similarities with esterase. Both lipase and esterase enzymes catalyze the hydrolysis and synthesis of bound esters [23]. The esterases hydrolyze esters with carboxylic acids of short chain, while lipases prefer long-chain fatty acids [24]. This necessitated the need for the amplification of lipase gene [*lip*A gene] in the selected lipolytic strains and the results obtained from this study were in agreement with a recent study [25] which aimed to isolate and identify *Pseudomonas aeruginosa* from industrial waste water [vegetable oils] based on PCR targeted *lipA* gene which encode the lipase producing protein in the genome sequence.

5 CONCLUSION

Based on the findings from this study, it has been confirmed that contaminated soils are good habitats for novel lipolytic fungi with functional lipase gene of interest [*lipA* gene] with numerous industrial applications. Further studies would be carried out on the production of lipase enzymes using the selected lipolytic fungi and their applications as biocatalysts for biolubricant production.

ACKNOWLEDGEMENTS

The authors are grateful to United Nations Educational, Scientific and Cultural Organization-International Centre for Biotechnology [UNESCO-ICB] for their financial support towards this research.

COMPETING INTEREST

No competing interest exists between the authors.

AUTHOR'S CONTRIBUTION

This research was done in collaboration among all the authors. The final manuscript was read and approved by the authors.

REFERENCES

1. Hollmann F, Fernandez-Lafuente R. Grand Challenges in Biocatalysis. *Frontiers in Catalysis*. 2021; 22.doi.org/10.3389/fctls.2021.633893.

2. Chandra, P, Enespa, Rajan S, Pankaj KA. Microbial lipases and their industrial applications: a comprehensive review. *Microbial Cell Factories.* 2020;19:169.doi.org/10.1186/s12934-020-01428-8.

3. Chapman J, Ahmed EI, Cerasela ZD. Industrial Applications of enzymes: Recent advances, Techniques and outlooks. *Catalysts*. 2018; 8:238-243.doi: 10.3390/catal8060238.

4. Rajendra A, Palanisamy A, Thangavelu V. Lipase - catalyzed ester synthesis for food Processing Industries. *Brazilian Archives of Biology and Technology*. 2009; 52[1]:207-219.ISSN 1516-8913.

5. Duarte GJ, Leone-Ignacio K, Da Silva JC, Fernandez-Lafuente R, Freire DMG. Rapid Determination of synthetic activity of lipases/esterases via transesterification and esterification zymography. *Fuel*.2016; 177[1]:123-129.doi.org/10.1016/j.fuel.2016.02.079.

6. Sumita T. Lipases, its sources, properties and applications: a review. *International Journal of Scientific and Engineering Research*. 2012; 3[7]:1-29.ISSN 2229-5518.

7. Patel N, Dhananjai R, Shivam, Shahane S, Mishra U. Lipases: Sources, Production, Purification and Applications. *Recent Patents in Biotechnology*. 2019; 13[1]: 45-56.doi: 10.2174/1872208312666181029093333.

8. Fariha H, Aamer AA, Abdul H. Industrial applications of microbial lipases. *Enzyme and Microbial Technology*. 2006; 39[2]:235 – 251.doi:10.1016/j.enzmictec.2005.10.016.

9. Ndubuisi GE, Sunday SI, Chikwendu EU, Evans CE, Justice OO. Production and Characterization of Lipase Enzyme Expressed by Crude Oil Contaminated Soil Isolates .*Universal Journal of Microbiology Research* .2019; 7[1]:1-6.http://www.hrpub.org DOI: 10.13189/ujmr.2019.070101.

10. Imandi SB, Garapati H. Lipase production by *Yarrowia lipolytica* NCIM 3589 in Solid state Fermentation using mixed substrate. *Research Journal of Microbiology.*2007;2[5]:469-474.DOI:10.17311/jm.2007.469.474

11. Iara CAB, Raphael ABG, Adriano AM. Biolubricant production from several oleaginous feedstocks using lipases as catalysts: Current scenario and future perspectives. *Bioenergy Research*. 2021; 14[4]: 1039-1057.doi.org/10.1007/s12155-020-10242-4.

12. Treichel HD, Oliveira MA, Di Luccio M, Oliveira JV .A review on microbial lipases production. *Food Bioprocessing Technology.* 2012; 3: 182–196.DOI:10.1007/s11947-009-0202-2.

13. Nasaruddin RR, Zahangir MA, Mohammed SJ. Enzymatic biodiesel production from Sludge Palm oil [SPO] using locally produced *Candida cylindracea* lipase*, African journal of Biotechnology.* 2013; 12[31]:4966-4974.doi:10.5897/AJB2013.12075.

14. Burcu Bakir Z, Metin K. Production and characterization of an alkaline lipase from *Thermophilic anoxybacillus* sp. HBB16, *Chemistry and Biochemical Engineering Quarterly.* 2017; 31[3]:303-312.doi:10-15255/CABEQ.2016.99.

15. Reshma CH, Shanmugam P. Isolation and Characterization of the Lipase from *Aspergillus brasiliensis. International Journal of Biotechnology and Bioengineering Research.*2013;4 [5]:481-486.ISSN 2231-1238.

16. Roy M, Kumar R, Ramteke A, Nandan S. Identification of lipase producing fungus isolated from diary waste contaminated soil and optimization of culture conditions for lipase production by the isolated fungus. *Journal of Microbiology, Biotechnology and Food sciences*. 2018; 8[1]:698-704.doi:10.15414/jmbfs.2018.8.1.698-704.

17. Toshi W, Sudhir KJ. Isolation, Screening and Identification of Lipase Producing Fungi from Oil Contaminated Soil of Shani Mandir Ujjain. *International Journal of Current Microbiology and Applied Sciences.*2017; 6[7]:1872-1878.doi.org/10.20546/ijcmas.2017;607:223.

18. Nwuche, C.O. & Ogbonna, J.C. Isolation of lipase producing fungi from palm oil Mill effluent [POME] dump sites at Nsukka*. Brazilian Archives of Biology and Technology*.2011; 54[1]:10-12.doi.org/10.1590/S1516-89132011000100015.

19. Ibrahim S.I. & Lubabatu, B.S. Screening of lipase producing fungi from groundnut and engine oil contaminated soil. *Dutse Journal of pure and applied Science*.2019; 5[2b]:180-184.ISSN [Online] 2635-3490.

20. Alima, R., Lazhari, T. & Boudjema, S. Isolation and identification of lipase producing fungi from local olive oil manufacture in East of Algeria. *Scientific Study and research*.2018;19[1]:13-22.ISSN 1582-540X.

21. Narasimhan V, Bhimba VB. Screening of extracellular lipase releasing microorganisms isolated from sunflower vegetable oil contaminated soil for biodiesel production. *Asian Journal of Pharmaceutical and clinical research*. 2015; 8[2]:427-430.ISSN-0974-2441.

22. Ire FS, Ike VC. Screening and Optimization of process parameters for the production of lipase in Submerged Fermentation by *Aspergillus carbonarius* [Bainer] IMI366159.*Annual Research and Review in Biology*. 2014; 4[16]:2587-2602.

23. Christopher S, William F, Michail NI, Mark L, Serve WMK, John van der O, Nicholas JH, Jennifer AL. Structural and biochemical characterization of *Archaeoglobus fulgidus* esterase reveals a bound CoA molecule in the vicinity of the active site. *Scientific Reports*. 2016; 6:25542.doi:10.1038/srep25542.

24. Lopez DB, Laira PF, Luciana FF, Gabriella AM. Lipase and esterase: to what extent can this classification be applied accurately? *Food Science and Technology.*2011;31[3]:608-613.doi.org/10.1590/S0101-20612011000300009.

25. Wathiq A, Intissar TL, Farhan HA. Isolation and Identification of *lipA* Gene producing *Pseudomonas aeruginosa* from Industrial Wastewater. *Iraqi Journal of Biotechnology*. 2019; 18[1]: 77-82.