Microbiology Research Journal International

31(5): 27-43, 2021; Article no.MRJI.69500
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Lipolytic Activities of Bacteria and Fungi Isolated from Soil Samples

Racheal Oluwayemisi Fashogbon[1*], Bose Adebayo[2], Victoria Musa[2] and Titilayo Femi-Ola[2]

[1]Department of Biological Sciences, Faculty of Natural Sciences, Ajayi Crowther University, Oyo State, Nigeria.
[2]Department of Microbiology, Faculty of Sciences, Ekiti State University, Ado Ekiti, Nigeria.

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.

Article Information

DOI: 10.9734/MRJI/2021/v31i530318
Editor(s):
(1) Prof. Juliano De Dea Lindner, Federal University of Santa Catarina (UFSC), Brazil.
(2) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Selma Soares de Oliveira, Universidade Federal do Rio de Janeiro (UFRJ), Brazil.
(2) Manu Mitra, University of Bridgeport, USA.
Complete Peer review History: https://www.sdiarticle4.com/review-history/69500

Original Research Article

Received 20 April 2021
Accepted 23 June 2021
Published 30 July 2021

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

**Corresponding author: E-mail: rachealyms@yahoo.com;*

µmol/min/ml) while *Bacillus* sp. isolated from red oil spill soil had the highest lipase activity (0.59 µmol/min/mg). The specific activity of partially purified lipase for *Fusarium* sp. was 2.39 µmol/min/mg while *Bacillus* sp. had a specific activity of 2.46 µmol/min/mg. 30°C - 50°C, pH 7.0 to 9.0 and KCl_2 (139.672%) supported the highest production of lipase by the *Bacillus* sp. and *Fusarium* sp. This study demonstrated that the *Bacillus* sp. produced a high amount of lipase activity followed by *Fusarium* sp. Extensive and persistent screening for new microorganisms and their lipolytic activities will help to provide faster ways to solve most environmental soil pollution.

Keywords: Lipolytic activity; soil samples; Sudan black B; purification; lipase production.

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 (SCOs), 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

characteristics (catalase test, coagulase test, indole test, oxidase test, Voges-Proskauer and carbohydrate fermentation). Bacterial isolates were characterized based on Bergy's Manuel of Systematic Bacteriology [16] while pure fungi cultured plates were observed for their colony characteristics (shape, pigmentation, sizes, mycelium, zonation) and microscopically.

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, 0.9; $MgSO_4.7H_2O$, 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℃. 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℃. 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 tumescens*, 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 Cihangir 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 KES5 *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 4.18µmol/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 2.05µmol/min/ml and 2.75µmol/min/ml with a total protein of 5705 mg and 176.21mg respectively. However, the specific activity for both steps is 0.18µmol/min/mg and 0.41µmol/min/mg with percentage yield of 1.00% and 6.92% respectively. For ion-exchange chromatography and gel filtration, their lipolytic activity was 3.37µmol/min/ml and 4.51µmol/min/mg. Total protein for both the ion-exchange chromatography and gel filtration is 55.27mg and 18.67mg 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 50ºC (100% relative activity). However, there was an increase in the lipase activity of *Bacillus* sp. as the temperature increases from 30ºC to 50ºC. Beyond 50ºC, there was a decrease in enzyme activity. At 80ºC, 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 40ºC, 50ºC, 60ºC and 70ºC. The purified lipase retained 97.9% residual activity at 40ºC within 20 minutes. On incubation at 70ºC, 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 30ºC – 80ºC with maximum production at 60ºC (100% relative activity) as shown in Fig. 3. There was an increase in the lipolytic activity of *Fusarium* sp. as the temperature increase from 40ºC to 60ºC, However, the enzyme decreased after 60ºC. Then, at 80ºC nearly two folds decrease in lipase yield was observed. The thermostability of the purified enzyme at different temperatures is shown in Fig. 4. The purified lipase showed significant difference at 40ºC, 50ºC, 60ºC and 70ºC. The purified lipase retained 98.8% residual activity at 40ºC at 20 hours. At 60ºC and 70ºC, 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 30ºC – 80ºC with maximum production at 30ºC (100% relative activity). Then, at 80ºC 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 25-30ºC. The thermostability of the purified enzyme at different temperatures is shown in Fig. 3.

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
CS 1	-ve, bacilli	Circular, translucent, smooth surface, moisten colony.	+ve	+ve	Motile	-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	*Bacillus* sp.
KES 3	+ve, bacilli	Creamy white colony, irregular edges, dry, opaque.	+ve	-ve	Motile	-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	*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	*Bacillus* sp.
UC 7	-ve, bacillus	Circular shaped, mucoid colony, red-pigmented.	+ve	+ve	Motile	-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	*Enterobacter* sp.
SDS 9	-ve, bacilli	Slightly whitish, round colony, smooth surface, convex elevation, moist, opaque.	+ve	+ve	Motile	+ve	-ve	-ve	+ve	-ve	$-_{ve}$	*Escherichia coli*

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	O.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 Filteration	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 60ºC at 120 minutes. Also, at 70ºC, 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.

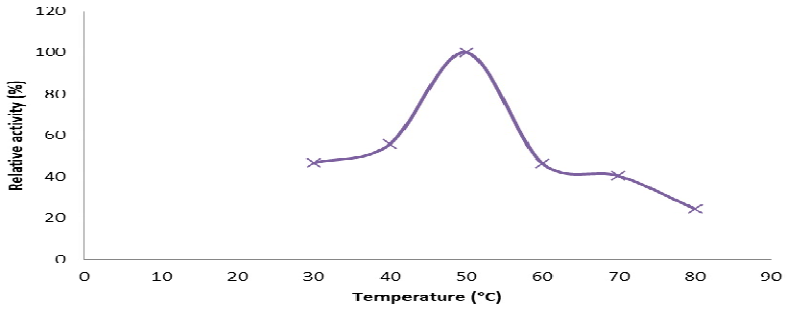

Fig. 1. Effect of temperature on the lipolytic activity of *Bacillus* sp. (ROS 11) grown in production media

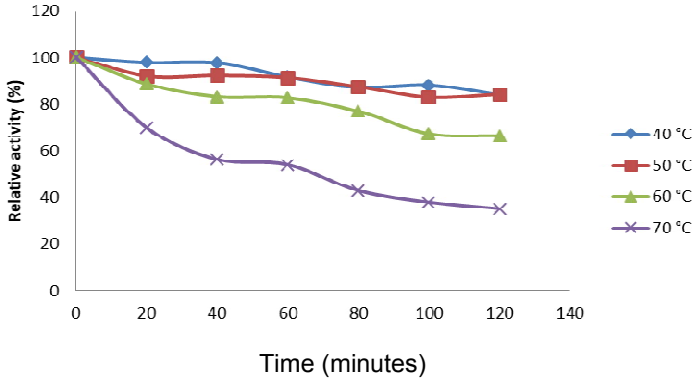

Fig. 2. Thermal stability of lipase produced by *Bacillus* sp. (ROS 11) in a growth medium

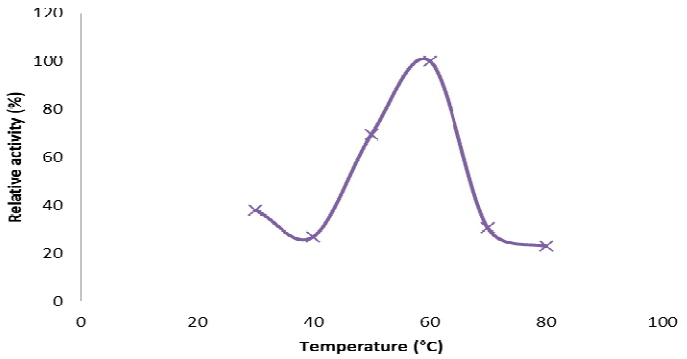

Fig. 3: Effect of temperature on the lipolytic activity of *Fusarium* sp. (SDS 10) grown in production media

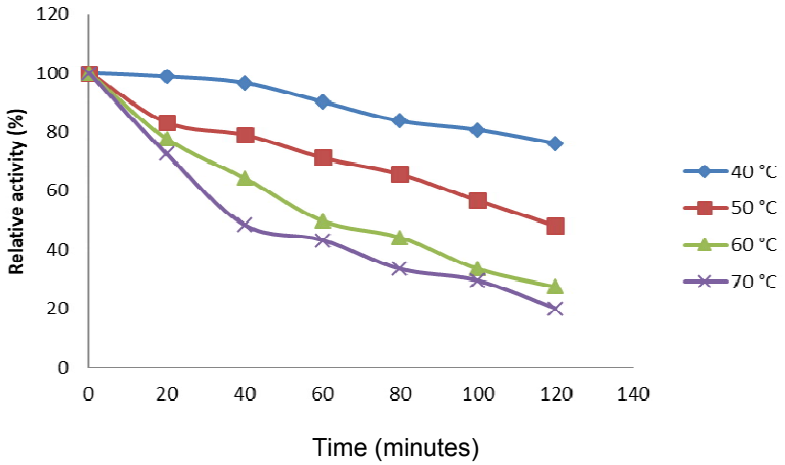

Fig. 4. Thermal stability of lipase produced by *Fusarium* sp. (SDS 10) in a growth medium

3.2 Effect of Salts (metal ions) on Lipolytic Activity

The effect of different metal ions on the activity of purified lipase of *Bacillus* sp. isolated from red oil spill soil and *Fusarium* sp. isolated from soil from dumpsite is shown in Fig 9. $CaCl_2$ increased the enzyme activity by 104%. $MnCl_2$, KCl_2 and $ZnCl_2$ slightly inhibited lipase activity, while $HgCl_2$ was a strong inhibitor with 4.14% lipase activity.

Significant differences were observed in the activity of the purified lipase with different metal ions such as $ZnCl_2$, $MnCl_2$, $CaCl_2$, KCl_2. KCl_2 increased the enzyme activity by 139.6% while $CaCl_2$, and $ZnCl_2$ slightly inhibit lipase activity. $MnCl_2$ was a strong inhibitor with 35.6% lipase activity. Also, it was observed that KCl_2 enhanced the activity of lipase produced by *Fusarium* sp. Its activity was slightly inhibited by $CaCl_2$, $ZnCl_2$, and $MnCl_2$. 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 $CaCl_2$ for *Bacillus* species. Bhosal et al. [10] reported that $CaCl_2$ inhibited the production of lipase while and $HgCl_2$ supported high lipase production. Lipase activity of *Fusarium* sp. increased in the presence of KCl_2, while $CaCl_2$ 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.

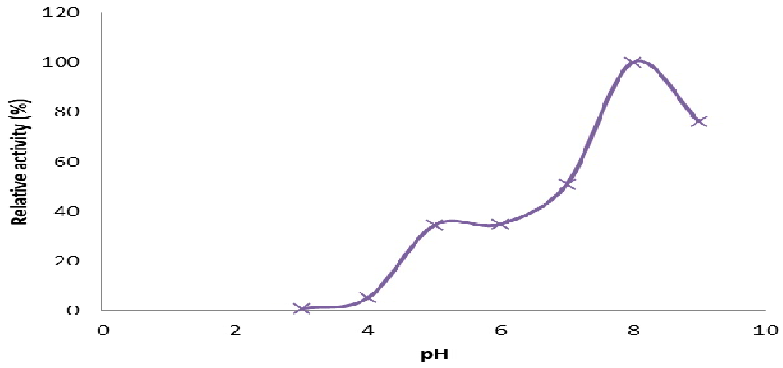

Fig. 5. Effect of pH on the lipolytic activity of *Bacillus* sp. (ROS 11)

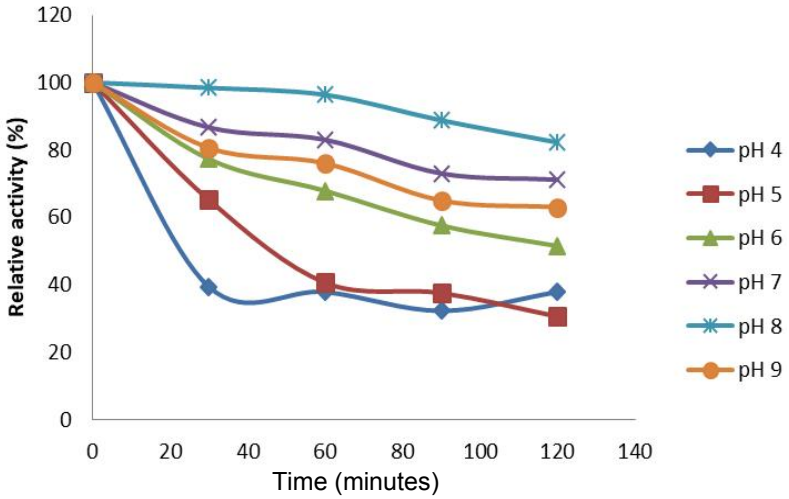

Fig. 6. pH stability of lipase produced by *Bacillus* sp. (ROS 11)

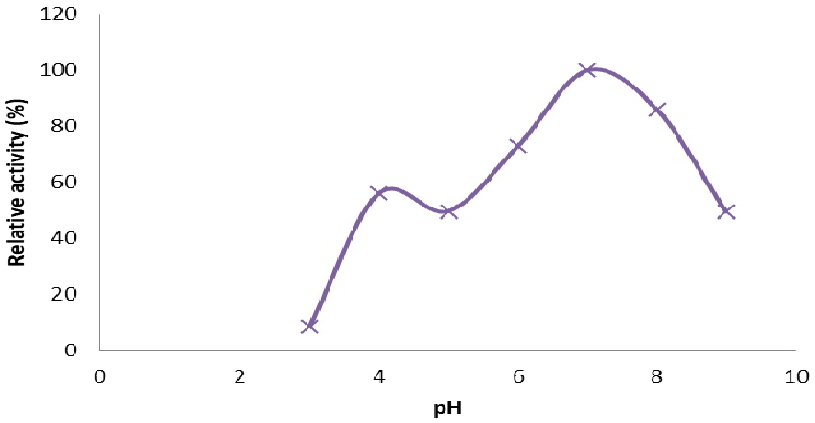

Fig. 7. Effect of pH on the lipolytic activity of *Fusarium* sp. (SDS 10)

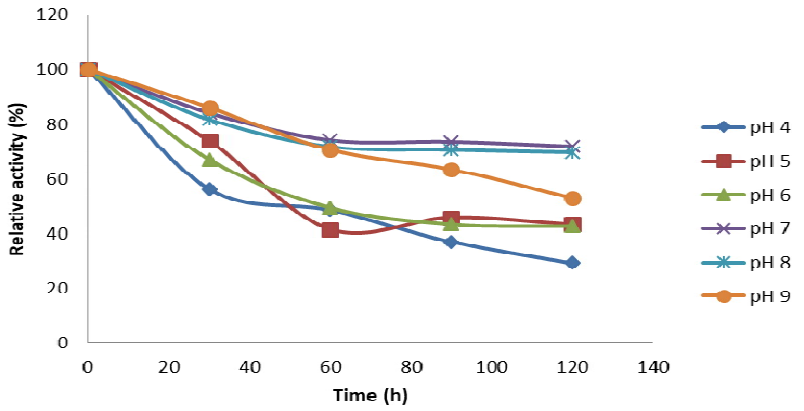

Fig. 8. pH stability of lipase produced by *Fusarium* sp. (SDS 10)

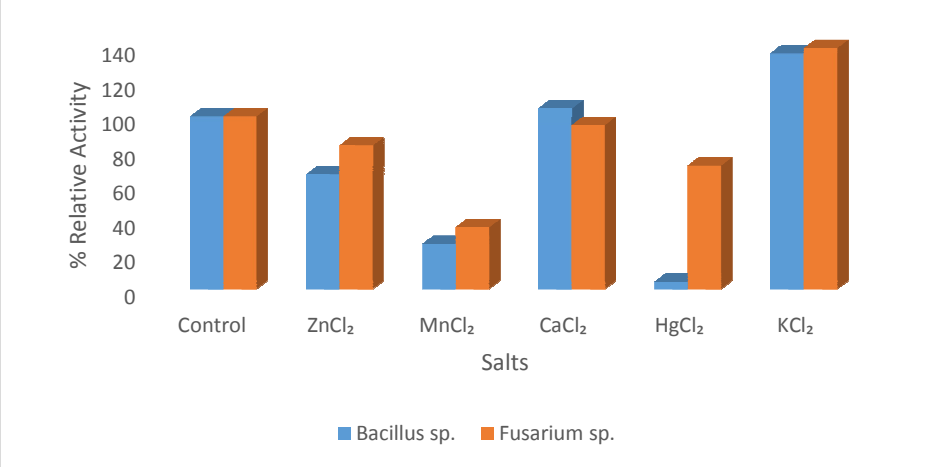

Fig. 9. Effect of soil on Lipolytic activity of *Bacillus* sp. isolated from red oil spill soil and *Fusarium* sp isolated from soil from dumpsite in production media

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 KCl_2, while $CaCl_2$ 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 a 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dominati E, Patterson M, Mackay A. A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecological Economics. 2010;69:1858–1868. Available:https://doi.org/10.1016/j.ecolecon.2010.05.002
2. Munkholm LJ, Heck RJ, Deen B, Zidar T. Relationship between soil aggregate strength, shape and porosity for soils under different long-term management. Geodermatology. 2016;26:852-859. Available:https://doi.org/10.1016/j.geoderma.2016.01.005
3. Sharma R, Chisti Y, Banerjee U. Production, purification, characterization and application of lipases. Biotechnology Advance. 2001;19:627-662. Available:https://doi.org/10.1016/s0734-9750(01)00086-6.
4. Zhang Y, Adams IP, Ratledge C. Malic enzyme: the controlling activity for lipid production? Overexpression of malic enzyme in *Mucor circinelloides* leads to a 2.5-fold increase in lipid accumulation. Microbiology. 2007;153(7):2013-2025. Available:https://doi.org/10.1099/mic.0.2006/002683-0
5. Lacointe C, Dubreucq E, Galzy P. Ester synthesis in aqeous media in the presence of various lipases. Biotechnology Letter. 1996;18:869-874. Available:https://doi.org/10.1007/BF00154612
6. Lomthaisong K, Buranarom A, Niamsup H. Investigation of isolated lipase producing bacteria from oil-contaminated soil with proteomic analysis of its protein responsive to lipase inducer. Journal of Biological Science. 2012;12(3):161-167. Available:https://doi.org/: 10.3923/jbs.2012.161.167
7. Kumar A, Kanwer, SS. Lipase production in solid state fermentation (SSF): Recent developments and biotechnological Application. DYN Biochemical Process of Biotechnological Molecular Biology. 2012;6(1):13-27. Available:https://doi.org/10.1007/s12010-007-8068-0.
8. Du W, Li Q, Liu DH. Perspectives of microbial oils for biodiesel production. Applied Microbiology Biotechnology. 2008;80(5):749-756. Available:https://doi.org/10.1007/s00253-008-1625-9.
9. Markossian S, Berker, Markl H, Antranikian G. Isolation and characterization of lipid degrading *Bacillus thermoleovorans* IHI-91 from an Icelandic hot spring. Extremophiles. 2000;4:365-371. Available:https://doi.org/10.1007/s007920070006.
10. Bhosal H, Zaker US, Bismile PC. Optimization of Lipse production by thermo-alkalophilic *Bacillus* sp. 8C. Research Journal of Microbiology. 2015;19(11):523-532. Available:https://doi.org/10.3923/jm.2015.523.532
11. Vakhlu J, Kour A. Yeast lipase: enzymes purification, biochemical properties and gene cloning. Electrical Journal of Biotechnology. 2006;9(1):69-85. Available:https://doi.org/10.2225/vol9-issue1-fulltext-9
12. Hassim NA, Ismail NH, Dian NL. Enzymtic interesterification of palm fractions for the production of cocoa butter alternatives. Journal of Oil Palm Research. 2018;30. Available:https://doi.org/10.21894/jopr.2018.0038
13. Ratledge C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie. 2004;86(11):807-815. Available:https://doi.org/10.1016/j.biochi.2004.09.017
14. Zaliha RN, Rahman RA, Baharum SN, Basri M, Salleh AB. High-yield purification of an organic solvent-tolerant lipase from *Pseudomonas* sp. strain S5. Analytical Biochemistry. 2005;341(2):267-274. Available:https://doi.org/10.1016/j.ab.2005.03.006.
15. Patel M, Mistry J, Suraj D, Patel S. Isolation and charaacteriztion of lipase producing bacteria from vegetable oil spillage site. International Journal of Current Microbiology and Applied Science. 2016;59:214-232. Available:https://doi.org/10.20546/ijcmas.2016.508.023
16. Buchana RE, Gibbons NE. Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins Co; 1997.

Available:https://doi.org/10.1111/j.1550-7408.1975.tb00935.x.

17. Lotrakul P, Dharmsthiti S. Lipase production by *Aeromonas sobria* LP004 in a medium containing whey and soybean meal. World Journal of Microbiology and Biotechnology. 1997;13:63–166. Available:https://doi.org/10.1023/a:1018581512540
18. Winckler UK, Stuckmann M. Glycogen, hyaluronate, and some other polysaccharide greatly enhance the formation of exolipase by *Serratia marcescen.* Journal of Bacteriology. 1979;138:663-70. Available:https://doi.org/10.1128/JB.138.3.663-670.1979.
19. Lowry Rl, Tinsley IJ, Rapid colorimetric determination of three fatty acids. Journal of America Oil Chemical Society. 1976;53:470-472. Available:https://doi.org/10.1007/BF02636814.
20. Shukla BN, Desai PV. Isolation, characterization and optimization of lipase producing Pseudomonas spp. From oil contaminated site. International Journal of Current Microbiology and Applied science. 2016;5(5):902-909. Available:https://doi.org/10.20546/ijcmas.2016.505.093
21. Sepahy AA, Jabalameli L. Effect of culture conditins on the production od an extracellilar protease by *Bacillus* sp. Isolated from Soil Sample of Lavizan Jungle Park. 2011;1-7. Available:https://doi.org/10.4061/2011/219628
22. Kunamnein A, Poluri E, Davuluri SP. Purification and partial characterization of thermostable serine alkaline protease from a newly isolated *Bacillus subtilis* PE-11. AAPS Pharmacological Science Technology. 2003;4(4):56. Available:https://doi.org/10.1208/pt040456.
23. Williams JO. Assessment of the physicochemical and microbiological quality of palm oil mill effluent (POME) ad soill in Aluu, Rivers State, Nigeria. Asian Journal of Biology. 2018;6(3):1-11. Available:https://doi.org/10.9734/AJOB/2018/41855
24. Mateus ZR, Agualimpa B, Zafra G. Isolation and molecular characterization of microorganisms with potential for the degradation of oil and grease from pal oil refinery waste. Chemical Engineering Transition. 2016;49:517-522. Available:https://doi.org/10.3303/CET1649087
25. Vijayaraghavan K, Ahmad D, Aziz M. Aerobic treatment of palm oil mill effluent. Journal of Environmental Management. 2007;82:24-31. Available:https://doi.org/10.1016/j.jenvman.2005.11.016.
26. Jasuja ND, Saxena R, Chandra S, Joshi SC. Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan. African Journal of Microbiology Research. 2013;7(41):4886-4891. Available:https://doi.org/10.5897/AJMR2012.2413
27. Hartman TL. The use of Sudan Black B as a bacterial fat stain. Journal of Stain Technology. 2009;15:23-29. Available:https://doi.org/10.3109/10520294009110328
28. Cihangir N, Demirkan ES. Investigation of lipase production by a new isolate of *Aspergillus* sp. World Journal of Microbiology Bitechnology. 2004;20:193-197. Available:https://doi.org/10.1023/B:WIBI.0000021781.61031.3a
29. Fleuri LF, De Oliveira MC, Aureuri ML, Capoville BL, Pereira MS, Delgado CH, Novelli PK. Production of Fungal lipases using wheat bran and soybean bran and incorporation of sugarcane bagasse as a co-subsrtate in slid state fermentation. Food Science Biotechnology. 2004;23(4):1199-1205. Available:https://doi.org/10.1007/s10068-014-0164-7
30. Jape A, Harsulkar A, Sapre VR. Modified Sudan Black B staining method for rapid screening of oleaginous marine yeasts. International Journal of Current Microbiology and Applied Science. 2014;3(9):41-46. Available:https://doi.org/10.1023/B:WIBI.0000021781.61031.3a
31. Pabai F, Kermasha S, Morin A. Lipase from *Pseudomonas fragi* CRDA 323: partial purification, characterization and interesterification of butter fat. Applied Microbiology Biotechnology. 1995;43:42–51. Available:https://doi.org/10.1007/BF00170621.

32. Dharmsthiti S, Kuhasuntisuk B. Lipase from *Pseudomonas aeruginosa* LP602: biochemical properties and application for waste water treatment. Journal of Industrial Microbiology and Biotechnology. 1998;21:75-80. Available:https://doi.org/10.1038/sj.jim.2900563
33. Collae LM, Ficanha AM, Rizzardi J, Bertolin TE, Reinehr CO, Costa JAV. Production and characterization of lipase by two new isolates of *Aspergillus* through solid-state and submerged fermentation. Biomedical Research International. 2015;1-9. Available:https://doi.org/10.1155/2015/725959
34. Sidhu P, Sharma R, Soni SK, Gupta JK. Production of extracellular alkaline lipase by a new thermophilic *Bacillus* sp. Folia Microbiology. 1998;43:51-54. Available:https://doi.org/10.1007/BF02815542.
35. Oliveira AC, Fernandes ML, Mariano AB. Production and characterization of an extracellular lipase by *Candida guilliermondii*. Brazilian Journal of Microbiology. 2014;45(4):1503-1511. Available:https://doi.org/10.1590/s1517-83822014000400047
36. Ettallibi M, Baratti JC. Purification, properties and comparison of invertase, exoinolinase and endoinilinase of *Aspergillus flucuum*. Applied Microbiology Biotechnology. 1987;26(1):13-20. Available:https://doi.org/10.1590/S1516-89131998000300003
37. Salihu A, Alam MZ, Abdulkarim ML, Sellah HM. Suitability of using palm oil mill effluent as a medium for production. African Journal of Biotechnology. 2011;10(11):2004-2052. Available:https://doi.org/10.5897/AJB10.1701
38. Gupta RN, Gupta T, Rathi P. Bacterial lipases: an overview of production, purification and biochemical properties. Applied Microbiology Biotechnology. 2004;64:763-781. Available:https://doi.org/10.1007/s00253-004-1568-8
39. Bharathi D, Rajalakshmi G, Komathi, S. Optimization and production of lipase enzyme from bacterial strains isolated from petrol spilled soil. Journal of King Saud University Science. 2019;31(4):898-901. Available:https://doi.org/10.1016/J.JKSUS.2017.12.018

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/69500