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Screening of Selected Soil Environments of Jos North Local Government Area for Lipase-Producing Fungi

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Consequent from increased demands for lipase enzymes for various purposes, the soil environment of Jos North LGA of Plateau State, Nigeria was surveyed for lipase-producing fungi.
Materials and Methods: Soil samples (300 g each) were collected randomly in triplicates from five locations (Terminus, Agwan Rukuba, Gada Biu, Farin Gada and Katako) in Jos North metropolis. Physicochemical properties (soil type, pH, temperature) and fungal counts of the soils were determined. Fungal isolates from the soil samples were preliminarily screened on phenol red agar for lipase production. Lipase activities of isolates with higher lipolytic potentials were determined using the spectrophotometric method with p-nitrophenyl dodecanoate serving as lipid substrate.
Results: The soil samples were mostly of sandy and loamy types. Mean pH and temperature ranges of the soils were 6.7-7.5 and 23.5ºC - 26.8ºC respectively. Total fungal counts of the soil samples ranged between 2.0 x 10^3 cfu/g and 3.9 x 10^4 cfu/g with Angwan Rukuba having the highest

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count. Fungal isolates from the study included *Aspergillus niger* (100%), *Aspergillus ochraceus* (60%), *Fusarium* sp. (20%), *Penicillium* sp. (40%), *Rhizopus stolonifer* (60%) and *Rhizopus* sp (60%). *R. stolonifer* produced the highest lipolysis zone diameter (3.69 mm) on phenol red agar. A lipase activity of 0.183µmol/min/ml was recorded for *R. stolonifer* while *A. niger*, *Fusarium* sp. and *Rhizopus* sp. had equal lipase activities of 0.184µmol/min/ml.
Conclusion: The findings show that the soil environment of Jos North LGA contain lipase-producing fungi which could be harnessed for industrial and environmental purposes after optimising the lipase production process.

Keywords: Environment; fungi; lipase; screening; soil; Aspergillus niger.

1. INTRODUCTION

Lipases (EC 3.1.1.3 triacylglycerol acylhydrolases) are a class of water soluble enzymes that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids [1]. They act at the interface between aqueous and organic phases and they primarily catalyze the hydrolysis of ester bonds in water-insoluble lipid substrates [2].

From the mid-nineteenth century when lipases were first discovered, interest in lipases has continued to increase mainly due to the wide variety of potential applications of these enzymes [3]. Different lipases have been isolated from various sources including plants, animals, and microorganisms. Although lipases can be obtained from different sources, only microbial lipases are of commercial importance [4]. Also, microbial lipases have been found to be more useful than those of plant and animal origin. This is due to a number of factors: microbial lipases have a variety of catalytic activities; microorganisms are easy to manipulate genetically; they are capable of rapid growth on inexpensive media. In addition, microorganisms are less affected by seasonal fluctuations, and as such, can be continuously used for lipase production, resulting in production of high amounts of lipases from the microbial cells [5].

Among the microbial sources of lipases, fungi are reputed to be the best source. Fungal lipases are highly valued in the industries over animal, plant, and bacterial lipases due to substrate specificity and stability of fungal lipases under varied chemical and physical conditions. Fungi naturally produce their enzymes extracellularly, and as such, can be extracted easily from fermentation media, leading to significant reduction in production cost [1].

Filamentous fungi belonging to the genera *Aspergillus, Rhizopus, Mucor, Penicillium, Geotrichum, Fusarium,* and *Trichoderma* are among the most useful microbial sources of lipases for industrial purposes [6,7,2].

Lipases are used in a wide range of applications. They are used in the pharmaceutical, food, detergent, and leather industries among others. The widespread use of lipase enzyme is due to lower energy requirement of enzymatic hydrolysis compared to energy requirement of chemical processes. Enzymatic hydrolysis, not only requires less energy, but also generates products of higher quality [8]. With rapid advances in enzyme application technology, the use of lipases has also extended to other areas including paper manufacturing, organic chemical processing, biosurfactant synthesis, oleochemical, and agrochemical industries [9,10]. Lipases have also found applications in waste management and in improvement of tanning techniques.

A number of authors have reported the isolation of lipase-producing fungi from Nigerian soils. [5] isolated lipase-producing fungi from soils in Keffi metropolis, Nigeria while [11] isolated lipase-producing fungi from soils in Port Harcourt, Nigeria. There is however paucity of information on the lipase-producing ability of diverse microorganisms that inhabit the soil environment of several other parts of Nigeria. Little or no work has been done in Jos North with respect to exploring the soil environment for lipase-producing fungi. The aim of this study therefore was to screen selected soil environments of Jos North Local Government Area of Plateau State, Nigeria for lipase-producing fungi which could be harnessed for industrial and other uses.

2. MATERIALS AND METHODS

2.1 Sample Collection

Three-hundred grams (300 g) each of soil samples were collected from five different locations (Farin Gada Market, Angwan Rukuba,

Gada Biyu, Katako, and Terminus) in Jos North Local Government Area of Plateau State, Nigeria. The sampling sites were selected by random sampling among popular and geographically different parts of Jos North Local Government Area. Six soil samples were collected from each location at a depth of about 4-6 cm with the help of pre-sterilised metal spoons. The soil samples were aseptically collected into plastic containers which had been decontaminated with 70% alcohol and transported to the Microbiology Department Laboratory, University of Jos, for analyses.

2.2 Determination of Physico-Chemical Properties of Soil Samples

2.2.1 Soil type

Soil type of each soil sample was determined using the jar test as described by [12]. Percentage heights of sand, silt, and clay sediments from soil-water suspensions of each soil sample in a jar were determined and subsequently used to estimate the soil type of each sample with the aid of a soil textural triangle.

2.2.2 Soil acidity (pH)

Acidity (pH) of the soil samples was determined with the aid of a digital pH metre using the method employed by [13] with slight modification. Forty grams (40 g) of soil was weighed into a beaker containing 40 ml of sterile distilled water. The mixture was stirred for five (5) seconds and allowed to stand for 10 minutes. The electrode of the pH metre was then inserted into the mixture and three consecutive readings were taken for each sample. The average of the three readings was recorded as the pH of the sample.

2.2.3 Soil temperature

A soil thermometer was used to determine the temperature of the soil at the various locations. The thermometer was inserted into the soil at a depth of about 5cm and allowed to stand for five minutes, after which three consecutive temperature readings were taken. The average of the three readings for each site was recorded as the soil temperature for that site.

2.3 Determination of Fungal Load, Isolation, and Identification of Fungi

Determination of fungal load of the soils and isolation of Fungi were carried out using the dilution plate method with malt extract agar (MEA) being the enumeration and isolation medium. The composition of the MEA was as follows: malt extract – 30 g; mycological peptone – 5 g; agar – 15 g; distilled water – 1000 ml. One gram of soil sample from each site was transferred into a sterile test tube containing 9 ml of sterile distilled water to make a 10^{-1} soil suspension. Ten-fold serial dilutions of the soil suspension was carried out up to the 10^{-3} dilution. One milliliter of the 10^{-3} dilution was pipetted into a sterile Petri-dish. About 15 ml of sterile molten MEA at a temperature of about 45 ^{0}C was poured into the Petri-dish. Two drops of 0.05 mg/ml of chloramphenicol antibiotic was added to prevent growth of bacteria. The Petri-dish was swirled gently to properly mix the content which was then allowed to set. The plates were incubated aerobically at room temperature (26 ± 2 ^{0}C) for 5 days with daily observation for fungal growth. For determination of fungal load, triplicate plates were used. Fungal colonies on the plates were counted and the mean number of colonies per plate determined. Total fungal count was determined using standard formula and the result was expressed in colony forming unit per gramme (cfu/g) of soil sample. For fungal isolation, triplicate plates were also used. Distinct fungal colonies observed on the agar medium were sub-cultured on MEA to obtain morphologically separate cultures. The fungal isolates were identified based on their macroscopic and microscopic morphologies using fungal identification manuals by [14] and [15].

2.4 Preliminary Screening of Fungal Isolates for Lipolytic Activity

Two qualitative tests were carried out to screen the fungal isolates for lipolytic activity. The isolates were assessed on tween-80 agar and phenol red agar in line with the methods adopted by [5] with slight modification. Tween-80 agar was prepared and poured into sterile Petri-dishes in 20 ml volumes and allowed to set. The composition of the medium was as follows: peptone (15 g), sodium chloride (5 g), calcium chloride (1 g), tween-80 (10 ml), agar (15 g), distilled water (1L). The pH of the medium was adjusted to 6.0 using 1M NaOH. The tween-80 agar plates were aseptically inoculated with the fungal isolates and the plates were incubated at 37 ºC for 48 h. Presence of an opaque precipitate of calcium monolaurate around the colonies was an indication of lipolytic activity by the fungi.

In the second qualitative determination, phenol red agar plates were prepared with the following composition: phenol red (0.01% w/v), with olive oil (1% v/v), calcium chloride (0.1% w/v), agar (2% w/v), with pH adjusted to 7.4. Sterile phenol red agar plates were separately inoculated with each of the fungi and the plates incubated at 37ºC for 48 h. A change in the colour of the agar medium from pink red to yellow around the colonies signified lipolysis. The magnitude of lipolysis caused by each of the fungi was assessed on phenol red agar. Five-millimetre mycelial discs were obtained from the margins of 4-day old pure cultures of the fungi with the aid of flame-sterilised cork borers. Sterile phenol red agar in Petri-dishes were centrally inoculated with each of the fungi in triplicates. The inoculated plates were incubated at 37ºC for 48 h after which diameters of yellow-coloured zones of lipolysis were measured with a metre-rule and mean values recorded. A larger zone diameter indicated a higher level of lipolytic activity.

2.5 Determination of Lipase Activity

Four out of the six fungi isolated in this study were selected based on higher lipolytic activity on phenol red agar and used for determination of lipase activity. Lipase activity was assayed using p-nitrophenyl dodecanoate as substrate and measured spectrophotometrically. With the aid of sterile cork borers, 5-mm mycelial discs were obtained in triplicates from the edges of 4-day old actively growing MEA cultures of the fungi on and separately inoculated into 200 ml of sterile Bushnell Haas broth in sterile 500 ml conical flasks. The Bushnell Haas broth used had the following composition: magnesium phosphate (0.20 g), calcium chloride (0.02 g), monopotassium phosphate 1.00) g) dipotassium phosphate (1.00 g), ammonium nitrate (1.00 g), ferric chloride (0.05 g), all dissolved in 1000 ml of distilled water. Triplicate flasks were provided for each fungus. The flasks were corked and incubated at 25 ºC for nine days with periodic hand shaking every 30 minutes. Ten millilitres of broth was harvested each day from each of the flasks with the aid of a sterile Pasteur pipette and transferred into a centrifuge tube and then centrifuged at 5000 rpm for 10 minutes. The supernatant obtained was filtered through sterile muslin cloth. The clear filtrate served as crude enzyme. A volume of 80 µl of the enzyme, 40 µl of p-nitrophenyl dodecanoate, and 980 µl of Tris-HCl buffer was transferred into a sample container with the aid of a micropipette. The resultant mixture (reaction mixture) was incubated for 15 minutes at 37ºC after which, 400 µl of 0.5 mM NaOH (sodium hydroxide) and 500 µl of 0.5M TCA (Trichloroacetic acid) were added to stop the reaction and the absorbance of the mixture was read on a UV-enabled spectrophotometer at a wavelength of 405 nm. This was done on a daily basis for 9 days. Daily absorbance readings were recorded and enzyme activity was calculated. The experiment was carried out in triplicate and mean values were determined. One unit of lipase activity was defined as the amount of enzyme which produces 1µmol of fatty acids per minute per milliliter of substrate under the specified assay conditions.

2.6 Statistical Analysis

Lipolytic abilities of the fungal isolates on phenol red agar and lipase activity of the selected fungal isolates were analysed statistically using one-way analysis of variance (ANOVA) with the aid of Microsoft Excel 2010 software. Means with significant differences were separated using least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Soil Samples

Table 1 shows the physicochemical properties of the soil samples collected from selected locations in Jos North Local Government Area of Plateau State. The soil samples were mainly of sandy and loamy types. The pH values of the soil samples were in the range of 6.7-7.5 while the soils temperatures ranged between 23.5ºC and 26.8ºC. Katako soil recorded the highest mean temperature (26.8±3.03ºC) while Farin-Gada soil had the lowest mean temperature (23.5±1.30ºC). The highest pH was recorded against Terminus soil while Farin-Gada soil had the lowest pH value. Soils of Terminus, Farin-Gada and Katako locations were Loamy while soils of Angwan Rukuba and Gada-Biyu were sandy. Soils from Angwan Rukuba, Farin-Gada and Katako were slightly acidic while those from Terminus and Gada-biyu tended towards neutrality. These findings differ slightly from those of [5] who reported higher values for the analysed parameters in Keffi. The differences were probably due to difference in weather conditions of Keffi and Jos.

Table 1. Physicochemical properties of soil samples from selected locations in Jos north local government area

Location	Soil temperature	Soil type	Soil pH
Terminus	26.6±1.63	Loamy	7.5±0.50
Angwan Rukuba	26.3±1.52	Sandy	6.9±0.48
Gada-Biyu	26.0±3.21	Sandy	7.0±0.27
Farin-Gada	23.5±1.30	Loamy	6.7±0.38
Katako	26.8±3.03	Loamy	6.8±0.31

Table 2. Total fungal Count (TFC/g) of soil samples from selected locations in Jos North LGA, Plateau State

Location	Total Fungal Count (TFC/g)
Terminus	2.0×10^3
Angwan Rukuba	3.9×10^4
Gada-Biyu	6.0×10^3
Farin-Gada	3.7×10^3
Katako	7.0×10^3

3.2 Fungal Load of Soil Samples

The total fungal count of the soil samples from the different locations within Jos North Local Government Area of Plateau State is presented in Table 2. The total fungal counts of the soil samples ranged between 2.0×10^3 cfu/g and 3.9×10^4 cfu/g. Terminus soil recorded the lowest fungal count while soil samples from Angwan Rukuba recorded the highest fungal count.

3.3 Fungal Isolates from Soil Samples

Table 3 shows the frequencies of occurrence of fungal isolates from the soil samples. A total of six fungi were isolated from the soils. The fungal isolates were *Aspergillus niger*, *Aspergillus ochraceus*, *Fusarium* sp., *Penicillium* sp. and *Rhizopus stolonifer. A. niger* had the highest frequency of occurrence (100%) while *Fusarium* sp. had the lowest frequency of occurrence (20%). The different locations recorded varying fungi occurrence frequencies. This variation could have resulted from differences in the composition of the various soils as well as in the physicochemical properties of the soils. *A. niger* had the highest frequency of occurrence (100%) while *Fusarium* sp., had the lowest occurrence frequency (10%). Microorganisms survive under environmental conditions favourable to each species which explains the variation in the occurrences of the isolated fungi. The high occurrence frequency of *A. niger* was probably due to the fact that *A. niger* and other aspergilli have the ability to utilize a wide variety of organic substrates and adapt well to a broad range of environmental conditions [16].

3.4 Lipolytic Abilities of Fungal Isolates

Preliminary screening for lipolytic ability of the isolates was done based on the zone of lipolytic activity expressed around the fungal colonies [17]. All the six fungal isolates exhibited lipolytic ability both on tween-80 agar and on phenol red agar. Table 4 shows the magnitude of lipolytic activity of the fungal isolates as determined by diameter of zone of hydrolysis on phenol red agar. *Rhizopus stolonifer* recorded the highest mean lipolysis zone diameter of 3.69±0.06 cm while *Penicillium* sp. had the lowest diameter of 0.87±0.01cm. The presence of lipolytic microorganisms in the soil is in line with findings of Sharma [4] who reported that lipase-producing microorganisms have been found in different habitats such as industrial wastes, vegetable oil processing factories, dairy plants, and soil contaminated with oil and oilseeds among others. Four out of the six isolates, namely *A. niger, Fusarium* sp., *R. stolonifer*, and *Rhizopus* sp. had higher lipase producing potentials based on magnitude of their zones of lipolysis on phenol red agar. Production of zones of enzyme activity around microbial colonies has been utilized by various authors [17,18,16] for preliminary screening of enzyme production. According to [17], it has a direct correlation of enzyme production by the organism. These fungi have similarly been reported by some authors to possess lipolytic activity [19, 20, 16]. *Rhizopus stolonifer* had the highest lipolytic activity. This

finding is in line with that of [5] who reported that *R. stolonifer* exhibited the highest zone diameter of lipolytic activity among other soil fungi isolates in Keffi, Nigeria. *Penicilium* sp. on the other hand, exhibited the lowest ability to produce lipase. The observed variation in lipolytic activity could be attributed to species differences.

3.5 Lipase Activity of Selected Fungal Isolates

All the four selected fungi (*Rhizopus stolonifer*, *Fusarium* sp., *Aspergillus niger* and *Rhizopus* sp.) were able to produce lipase enzyme in the culture medium. Lipase production by the fungi peaked at Day-3. These implies that a three-day fermentation period is optimum time for lipase enzyme production for each of the fungi. Maximum lipase activities of the fungi ranged between 0.183 µmol/min/ml and 0.184 µmol/min/ml. Details of the lipase activities of the fungi are given in Fig. 1. The lipase activities of the test fungi were generally lower than those reported by a number of authors. [19] reported a lipase activity of 3.75 µmol/min/ml for *Aspergillus niger* and 2.92 µmol/min/ml for *Rhizopus* sp. [22] reported a lipase activity range of 36.32 – 43.56 µmol/min/ml for *Fusarium* spp. isolated from dairy effluent and diesel contaminated soil and a lipase activity range of 33.37-49.81 µmol/min/ml for *Aspergillus* spp. isolated from the same sources. Lipase production by fungi varies according to strain, growth medium, culture condition, pH, temperature, oxygen levels, and carbon and nitrogen sources [19, 22]. The low lipase activities of the fungi used in the present study could mean that the fungal strains had inherently low lipase expression capacities. Variation in lipase activity could also be related to differences in the lipase activity determination methods used by the different researchers. It is important to note that lipase activity values reported in this study are values from a non optimized lipase production process. Proper selection of cultivation conditions is very essential for maximal enzyme production. In line with this, [23] rightly stated that various distinct biochemical properties can be expressed by a single fungus if subjected to different incubation conditions. It is therefore necessary to optimise the lipase production process so as to determine the best lipase production capacities of the fungi used in this study.

Table 3. Occurrence of fungal isolates in soil samples from different locations in Jos North Local Government Area

Fungi Isolates	Katako	Angwan Rukuba	Terminus	Gada-Biyu	Farin-Gada	Total	Occurrence (%)
Aspergilus niger	+	+	+	+	+	5	100
Aspergilus ochraceus	+	-	+	+	-	3	60
Rhizopus stolonifera	+	+	-	+	-	3	60
Penicillium sp.	-	+	+	-	-	2	40
Rhizopus sp.	+	-	+	+	-	3	60
Fusarium sp.	-	-	-	-	+	1	20

Key: (+) =Present, (-) =Absent.

Table 4. Lipolytic activities of fungal isolates on phenol red agar

Fungal Isolates	Hydrolysis Zone Diameter (cm)
Rhizopus stolonifera	3.69 ± 0.06^{a}
Aspergillus ochraceus	0.97 ± 0.05^{b}
Aspergillus niger	1.73 ± 0.05^{c}
Penicillium sp.	0.87 ± 0.01^{d}
Rhizopus sp.	3.54 ± 0.01^{e}
Fusarium sp.	1.14 ± 0.01^{f}

Means on same row with different superscripts are significantly different at P = .05, One way Anova. Data are means ± SD (n = 3)

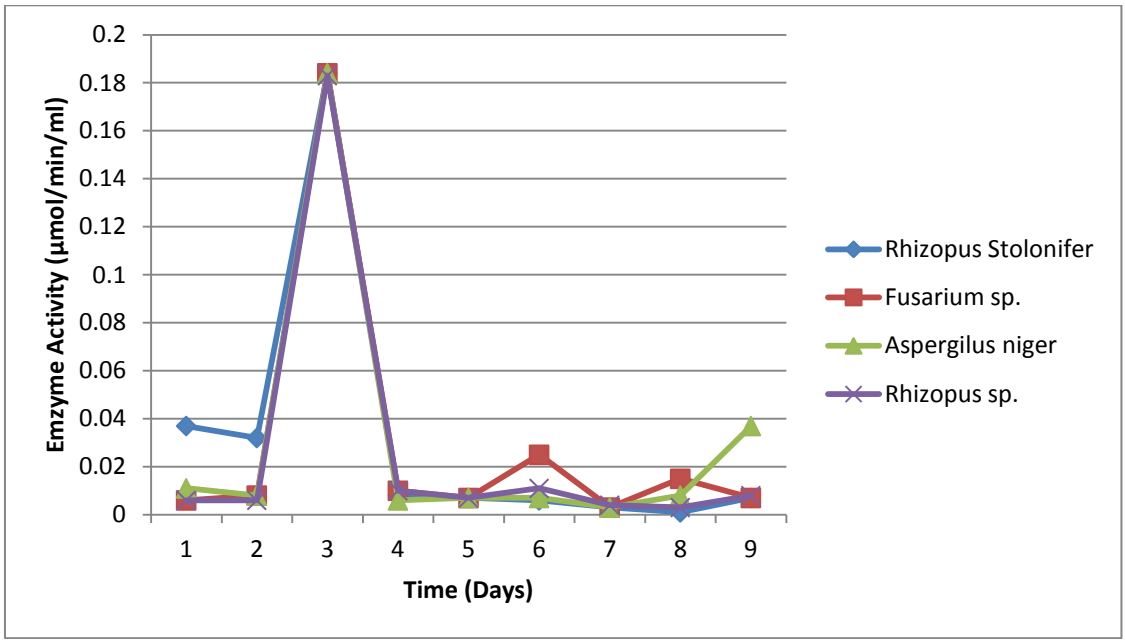

Fig. 1. Lipase activity of fungal isolates

4. CONCLUSION

The findings from this study show that the soil environments of Jos North Local Government Area of Plateau State, Nigeria harbour lipase-producing fungi including *Rhizopus stolonifer, Fusarium* sp., *Aspergilus niger,* and *Rhizopus* sp. The required time for best lipase production by these fungi is three days. Lipase production capacities of the fungi were relatively low making it necessary for optimization studies to be carried out as a means of maximizing the lipase-producing potentials of the fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mehta A, Bodh U, Gupta R. Fungal lipases: A review. Journal of Biotech Research. 2017;8:58-77.
2. Stoytcheva MS, Gisela M, Roumen Z, José ÁL, Velizar G. Analytical methods for lipases activity determination: A review. Current Analytical Chemistry. 2012;8:400-407.
3. Pereira MG, Vici AC, Fachini FDA, Tristao AP, Cursino-Santos JR, Sanches PR, Jorge JA, Polizeli MT. Screening of filamentous fungi for lipase production: *Hypocrea pseudokoningi* a new producer with a high biotechnological potential. Biocatalysis and Biotransformation. 2014;32(1):74-83.
4. Sharma R, Christi Y, Banerjee UC. Production, purification, characterization and application of lipases. Biotechnology Advances. 2001;19:627-662.
5. Makut MD, Bembga US. Isolation and identification of lipase-producing fungi from the soil environment of Keffi metropolis. Nigerian Journal of Microbiology. 2017;31(1):3630-3635.
6. Optimisation of process parameters for production of lipase in solid-state fermentation by newly isolated *Aspergillus* species. Indian Journal of Biotechnology. 2004;3:65-69.
7. Rajesh EM, Arthe R, Rajendran R, Balakumar C, Pradeepa N, Anitha S. Investigation of lipase production by *Trichoderma reesei* and optimization of production parameters. EJEAFChe 2010;9:1177-1189.
8. Pratyoosh S, Kshitiz G. Ecological screening for lipolytic molds and process optimization for lipase production from *Rhizopus oryzae* KG-5. Journal of Applied Sciences in Environmental Sanitation. 2007;2(2):35-42.
9. Hiol A, Jonzo MD, Rugagi N, Drueti D, Sandra L, Commeau LC. Purification and characterization of an extracellular lipase

from thermophilic *Rhizopus oryzae* strain isolated from pal fruit. Enzyme Microbial Technology. 2000;26:421-430.
10. Hassan F, Sha AA, Hameed A. Industrial applications of microbial lipases. Enzyme and Microbial Technology. 2006;39(2):235-251.
11. Elemuo NG, Ikiensikimama SS, Ubani CE, Egwim EC, Osuoha JO. Production and characterization of lipase enzyme expressed by crude oil-contaminated soil isolates. Universal Journal of Microbiology Research. 2019;7(1):1-6.
12. Jeffers A. Soil texture analysis "The Jar test. Assessed 12 September 2018. Available:https://hgic.clemson.edu/factsheet/soil-texture-analysis-the-jar-test/
13. Watson ME, Brown. pH and lime requirements. Northern Central Regional Research Publication. 1998;221:13-17.
14. Domsch KH, Graxas W, Anderson TH.Compendium of soil fungi. UK, London: Academic Press; 1980.
15. Samson RA, Hoekstra ES, Van Oorschot CAN. Introduction to Food –Borne Fungi. 2nd ed. Netherlands: Institute of the Royal Netherlands Academy of Arts and Sciences.
16. Cray JA, Bell AN, Bhaganna P, Mswaka AY, Hallsworth JE. The biology dominance; can microbes behave as weeds? Biotechnology. 2013;(6):453-492.
17. Dajanta K, Wongkham S, Thirach P, Baophoeng P, Apichartsrangkoon A, Santithum P, Chukeatirote E. Comparative study of proteolytic activity of protease-producing bacteria isolated from Thua nao. Maejo International Journal of Science and Technology. 2009;3:269–276.
18. Roy S, Das I, Munjal M, Karthik L, Kumar G, Kumar S, Rao KVB. Isolation and characterization of tyrosinase produced by marine actinobacteria and its application in the removal of phenol from aqueous environment. Frontiers in Biology. 2014;9(4):306–316.
19. Okoro CU, Ajaba M. Comparative study of the lipolytic activities of some fungi and Lactobacillus species isolated from some Nigerian local foodstuff. Global Journal of Pure and Applied Sciences. 2018;24:141-145.
20. Rihani A, Tichati L, Soumati B. 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.
21. Colla LM, Rezzadori K, Camara SK, Debon J, Tibolla M, Bertolin TE, Costa J A V. A solid-sate bioprocess for selecting lipase-producing filamentous fungi. Naturforsch. 2009;64c:131-137.
22. Alabdalall AH, Alanzai NA, Aldakeel SA, Abdulazeez S, Borgio JF. Molecular, physiological, and biochemical characterization of extracellular lipase production by *Aspergillus niger* using submerged fermentation. Peer J. 2020;8:e9425.
23. Diaz JC, Rodriguez JA, Roussos S, Cordova J, Abousalham A, Carriere F, Baratti J. Lipase from the thermotolerant fungus *Rhizopus homothallicus* is more thermostable when produced using solid state fermentation than liquid fermentation procedures. Enzyme and Microbial Technology. 2006;39:1042-1050.

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