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Isolation, Identification and Screening of Lipase Producing Fungi from the Soil Environment of Ilorin Metropolis

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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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Original Research Article

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ABSTRACT

This investigation was carried out to isolate, identify and screen for lipase producing fungal species present in the soil environment of Ilorin metropolis. Soil samples of approximately 200g each were collected randomly from eight different locations within the Ilorin metropolis for the investigation. Potato Dextrose Agar was used for the isolation of the fungal species by pour plate method. Six fungal species, *Penicillium* spp, *Acremonium*spp, *Mucor*s pp, *Rhizopus stolonifer, Aspergillus niger*and *Aspergillus flavus*were isolated and screened for their ability to produce lipases on tween-20 and phenol red agar. The results obtained for lipase production on tween-80 and phenol red after 5 days of incubation showed that four isolates were positive for lipase production which was indicated by diameter zone of clearance and visible precipitate of calcium monolaurate due to the deposition of calcium crystal. The result further revealed that *Aspergillus niger* had the highest lipase producing ability (having a diameter zone of clearance of 14 ± 0.05 mm), followed by *Rhizopus stolonifer* (having 10 ± 0.05 mm). *Aspergillus flavus and Mucor* sp had 6 ± 0.03 mm, 6± 0.01 mm respectively. *Acremonium* sp. and *Penicillium* sp. had no zone of clearance. These results demonstrate the presence of lipase producing fungi in the soil environment of Ilorin metropolis,Kwara State, and these can be harnessed locally for large scale production of the enzyme which is of value commercially in the production of leather, detergent,textiles and also as constituents of some special diets and pharmaceuticals.

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1. INTRODUCTION

The nineteenth-century industrial revolution accelerated activities that contributed significantly to environmental degradation (Narayanan, 2014). The current state of the environment, as well as the need to save it, has prompted manufacturers to devise solutions that incorporate sustainable production processes, one of which is enzyme catalysis (Sarma *et al*., 2018). Enzyme catalysis allows for functionality under milder temperature, pH, and pressure conditions, resulting in less energy consumption and the production of fewer unwanted by-products (Waltes*et al*., 2001).

Lipases (E.C.3.1.1.3. triacylglycerol ester hydrolases) are important industrial enzymes that catalyze the hydrolysis and synthesis of esters from glycerol and longchain fatty acids. These enzymes catalyze reversible reactions such as interesterification, aminolysis, and transesterification under specific conditions [1]. Molecular tools that can be used as parts of the microbialpool for lipase production in research at the laboratory and industry level [2] may provide a better understanding of previously discovered enzymes and their functional significance. Research aspects, reported lipase producing fungi were *Humicolal anuginosa*, *Fusarium* sp., *Mucor* sp., *Aspergillus* sp. *Rhizopus oryzae*, *Colletotrichum gloeosporioides*, *Alternaria dianthicola*, *Curvularia* sp., *Penicillium* sp., *Trichodermaviridae*, *Macrophomina phaseolina*, *Hypocrea pseudokoningii* etc [3]. Lipases are a ubiquitous enzyme found in a wide range of natural sources, including industrial wastes, vegetables, oil processing factories, and oil contamination of soil [4]. Lipid is a major component of the earth's biomass and is used in a variety of industries, including detergents, dairy, and textiles, surfactant production, oil processing, and microbial biodiesel [5].

Lipases have been isolated and identified in bacteria, fungi, plants, and animals [6]. Microbial lipases have been found to be more useful than plant and animal-derived lipases because they have a wider range of catalytic activities and microorganisms are easy to manipulate genetically and capable of rapid growth on low-cost media [7-10]. Furthermore, microorganisms are less affected by seasonal fluctuations, allowing for regular multiplication and the extraction of large amounts of lipases from microbial cells [11,12,13]. Microbial lipases, particularly those of fungi, are more potent and stable than their plant and animal counterparts (Singh and Mukhopadyay, 2012).

Microorganisms are ubiquitous in the sense that they can be found in almost any natural habitat (soil, water, air, leaves, and tree trunks), with soil serving as a reservoir for a variety of microorganisms (Metin and Bakir, 2019). Lipase-producing microorganisms have been isolated from soil (Colla*et al.*, 2016), the marine environment (Lodha, 2018), wastewater from the fish industry (Suharjono, 2015), waste volatile substances (Muthumari*et al.*, 2016), air (Abada, 2008), palm-oil mill effluent [14], silkworm intestine (Feng *et al.*, 2011), (Jagtap and Chobade, 2015).

The aim of this study is the screening for lipases producing fungi in the soil environment of Ilorin metropolis, Kwara State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This research focused on the soil environment of the Ilorin metropolis. Ilorin is the capital of Nigeria's Kwara State. Ilorin is situated at a strategic point between Nigeria's densely populated South-Western and sparsely populated middle belt, at latitude 8o24'N and longitude 4o10'E, 4o36'E.

2.2 Sample Collection

Random soil samples of 200 g were collected from eight (8) different locations, including Taiwo, Geri-alimi, Stadium road, ita-aman, Tanke, Challenge, G.R.A., and Apalara. The samples were taken at a depth of 4 inches below the soil surface with the help of a sterile hand trowel (pretreated with 70% alcohol). The samples were collected in polythene bags and transported to the Kwara State University Microbiology Laboratory in Malete for analysis.

2.3 Determination of Soil Physico-chemical Properties

Soil type, pH, and temperature were determined, as well as other physico-chemical properties.

2.3.1 Soil type

The soil types were determined by sieve analysis using the Unified Soil Classification System and a sieve apparatus [15].

2.4 Soil pH

A digital pH meter was used to determine the pH of the soil samples. Three grams of soil from each location were diluted in three milliliters of distilled water and stirred for five minutes [16]. After that, the pH meter's electrode was first inserted into phosphate buffer and then into the mixture. Thereafter, the readings were taken for each sample and an average of three consecutive readings were recorded.

2.5 Soil Temperature

A soil thermometer was used to determine the temperature of the various sites. Temperature readings were taken after the thermometer was inserted into the soil at a depth of 5cm and allowed to stand for 5 minutes. For each location, the average of triplicate readings was recorded [17].

2.6 Isolation and Identification of Fungal Isolates

Pour plate technique was used to isolate soil fungi [18]. A 0.1g soil sample from each location was evenly distributed on the bottom of a sterile Petri dish, into which was poured molten/cooled (40–45 ^{o}C) Potato Dextrose Agar (included with antibiotics Ampicillin to inhibit bacterial contaminants) and allowed to set. The plates were incubated for 5 days at 30°C. After 24 hours, colonies were counted, and distinct colonies were sub-cultured within 3–5 days to obtain pure cultures of the isolates. The macroscopic (cultural) and microscopic (morphological) characteristics of fungal isolates from pure cultures were used to identify them [19].

2.7 Screening for Lipase Production by the Isolates

Makut and Bemgba's method for preparing phenol red agar plates was used (2017).

The medium was made up of the following ingredients: phenol red 0.01 percent (w/v), 1 percent (v/v) olive oil, 0.1 percent (w/v) CaCl2, 2 percent (w/v) agar, and a pH of 7.4. After sterilization, aliquots of 20 ml of medium were poured into Petri dishes and organisms were inoculated. The plates were incubated at 37°C. The activity of lipase produced by the organisms was indicated by a change in the color of the phenol red. The amount of lipase produced by each isolate is indicated by the zone of clearance diameter.

Gonipath et al. [20] described a chemically defined medium (Tween-80 agar) that was used to screen the various fungal isolates for lipase production (2005). Peptone (15 g), sodium chloride (NaCl) (5 g), calcium chloride ($CaCl_2$) (1 g), Tween-80 (10 ml), and agar (15 g) were all dissolved in 1 litre of distilled water in the medium. The pH was adjusted to 6.0 using 1M NaOH. Approximately 20 ml of the medium was poured into Petri dishes and allowed to set. The fungal isolates were inoculated onto the plates and left to incubate for 48 hours at 28°C. The presence of a zone of clearance around the colonies and a visible precipitate of calcium monolaurate were used to determine whether the isolates produced lipase.

3. RESULTS

Table 1 shows the results of the physico-chemical properties of soil samples from various locations in the Ilorin Metropolis, while Table 2 shows the results of the cultural features of the fungi isolated, and Table 3 shows the results of Total Fungal Counts in soil samples from various locations. Table 4 shows the percentage frequencies of occurrence of fungal isolates, while Table 5 shows the results of lipase production by the fungal isolates.

4. DISCUSSION

The selected locations are among the well known in ilorin metropolis.Taiwo, Geri-alimi, Stadium Road, ita-aman, Tanke, and Challenge have sandy soils, whereas G.R.A and Apalara have sandy loamy soils. The pH values for all of the soil samples ranged from 6.5-8.2 on average. As a result, all of the soil samples examined were alkaline. The temperature range of soil samples from various locations ranged from 25 to 29^{0}C, which is most likely due to the fact that the study was conducted during the rainy season. These findings are similar to those of Makut and Bemgba [21].

Table 1. Physico-chemical properties of soil samples from different locations in Ilorin

S/N	Location	Soil Type	pH	Temperature (^oC)
1.	Taiwo	Sandy	6.5 ± 0.00	26 ± 1.63
2.	Geri-alimi	Sandy	7.1 ± 0.00	25 ± 1.21
3.	Stadium road	Sandy	6.9 ± 0.00	27 ± 1.22
4.	Ita-aman	Sandy-loamy	7.8 ± 0.00	28 ± 1.51
5.	Tanke	Sandy	7.3 ± 0.00	26 ± 0.74
6.	Challenge	Sandy	7.0 ± 0.00	29 ± 0.84
7.	G. R. A.	Sandy	6.9 ± 0.00	25 ± 1.13
8.	Apalara	Sandy-loamy	8.2 ± 0.00	28 ± 2.37

Table 2. Cultural characteristics of fungal isolates on Potato DextroseAgar

S/N	Isolates	Surface Color	Reverse Color
1.	*Aspergillusniger*	Black	Yellowish
2.	*Rhizopusstolonifer*	Grayish- white	Whitish pale
3.	*Aspergillusflavus*	Velvety- green	White Tan
4.	*Mucur*sp.	Fluffy- black	White
5.	*Acremonium*sp.	Whitish- cream	Yellow
6.	*Penicillium*sp	Greenish	Yellow

Table 3. Total Fungal Counts (TFC/g) in soil the different locations of Ilorin Sites

S/N	Location	Total Fungal Counts (TFC/g)
1.	Taiwo	$2.1 \times 10^2 \pm 0.06$
2.	Geri-alimi	$2.9 \times 10^2 \pm 0.05$
3.	Stadium road	$3.7 \times 10^{2.} \pm 0.07$
4.	Ita-aman	$1.0 \times 10^2 \pm 0.07$
5.	Tanke	$1.6 \times 10^2 \pm 0.04$
6.	Challenge	$2.3 \times 10^2 \pm 0.05$
7.	G. R. A.	$3.0 \times 10^2 \pm 0.03$
8.	Apalara	$2.8 \times 10^2 \pm 0.02$

Table 4. Percentage occurrence of fungal isolates in the soil of the different locations

Location									
S/N	**Fungal Isolates**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Occurrence** (%)
1.	*Aspergillus niger*	**+**	**-**	**+**	**+**	**+**	**-**	**+**	**+**	**80**
2.	*Rhizopus stolonifer*	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**100**
3.	*Aspergillus flavus*	**-**	**-**	**+**	**+**	**-**	**+**	**+**	**-**	**50**
4.	*Mucur* sp.	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**30**
5.	*Acremonium* sp.	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**20**
6.	*Penicillium* sp	**-**	**-**	**+**	**-**	**+**	**+**	**-**	**-**	**30**

KEY:(+) = Present; (-) = Absent; A = Taiwo; B = Geri-alimi; C = Stadium road; D =Ita-aman; E = Tanke; F = Challenge; G = GRA; H =Apalara

Table 5. Lipases production by isolates

S/N	Fungal Isolates	Zone of Clearance (mm)
1.	*Aspergillus niger*	14 ±0.05
2.	*Rhizopus stolonifer*	10 ±0.05
3.	*Aspergillus flavus*	6 ±0.03
4.	*Mucor* sp	6 ±0.01
5.	*Penicillium* sp.	0 ±0.00
6.	*Acremonium*sp.	0 ±0.00

Stadium road, G.R.A, Geri-alimi and Apalara had high fungal counts of 3.7 x 10^2, 3.0 x 10^2 2.9 x 10^2, and 2.8 x 10^2 respectively followed by those of the Taiwo and Challenge which had counts of 2.1 x 10^2 and 2.3 x 10^2 respectively.Tanke and Ita – aman had lowest counts of 1.6 x 10^2 and 1.0 x 10^2 respectively.

Among all the fungi isolated,*Rhizopus stolonifer* had the highest percentage of occurrence of 100%. This indicates that *Rhizopus stolonifer* was present in all the samples analysed. *Aspergillus niger* had 80% percentage of occurrence followed by *Aspergillus flavus* with 50% occurrence.*Mucor* sp and *Penicillium* sp 30% occurrence. Lastly, *Acremonium* sp had the lowest percentage occurrence of 20%.

Among all the six fungal species isolated, four organism which are *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor* sp were found to produce varying amount of lipase.The appearance of a zone of clearance and visible precipitate as a result of deposition of calcium crystal was used for the indication for lipase production. The diameter of the zone of clearance of the different isolates showed that *Aspergillus niger* had the highest,followed by *Rhizopus stolonifer* and then *Aspergillus flavus* and *Mucor* sp.

The importance of lipases in various industries cannot be overstated, and their use is growing in a number of areas. According to Davranov [22,23], extensive and ongoing screening for new microorganisms and their lipolytic enzyme will open new, simple synthetic routes and, as a result, new and faster ways to apply lipases to improving human life, including solving environmental problems.

5. CONCLUSION

The majority of the fungal species isolated in this study were found to produce lipases, which is not surprising. *Aspergillus niger*, on the other hand, had the highest lipase activity. Further research based on the findings of this study could lead to the development of high-yielding lipase-producing fungi for industrial production of these enzymes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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