Isolation and Identification of Lipase Producing Fungi From the Soil Environment of Keffi Metropolis

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Abstract: An investigation was carried out to isolate, identify and screen for lipase producing fungal species present in the soil environment of Keffi metropolis. Soil samples of approximately 200g each were randomly collected from ten different locations within the Keffi metropolis for the investigation. Sabouraud Dextrose Agar was used for the isolation of the fungal species by pour plate method using Direct Soil Inoculation technique. Five fungal species, *Acremonium* spp *Mucor* spp*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 of lipase production on tween-80 and phenol red after 5 days of incubation showed that all the 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 diameter of the zone of clearance of the various isolates revealed that *Rhizopus stolonifer* had the highest lipase producing ability (having a diameter zone of clearance of 12 ± 0.04 mm), followed by *Aspergillus niger* (having 10 ± 0.02 mm). *Acremonium* sp. and *Mucor*sp. had 8 ± 0.07 mm respectively, while *Aspergillus flavus* was able to produce just a minimal amount of lipases indicated by its zone of clearance (6 ± 0.04mm). These results demonstrate the presence of lipase producing fungi in the soil environment of Keffi metropolis, Nasarawa State, and these can be explored locally for the production of the enzyme which is of value commercially in the production of detergents, and also as constituents of some special diets and pharmaceuticals.

Keywords: Lipase production, soil, fungi, Keffi

Introduction

Enzymes are biocatalyst produced by living cells, and they catalyze specific reactions inside or outside the cells (Spiegel *et al.,* 1996). Lipases are categorized under lipolytic enzymes. Lipolytic enzymes are grouped into three main groups namely; esterases, phospholipases, and lipases (Arpigny and Jaeger, 1999). Lipases (triacylglycerolacyl hydrolases, EC3.1.1.3) are class of enzymes which catalyze the hydrolysis of long-chain triglycerides (Savitha *et al.,* 2007). Sharma *et al.* (2001) reported that lipases do catalyze hydrolysis of long chain acyl glycerols at an oil-water interface.

Lipases were first discovered in 1856 by Claude Bernard, when he studied the role of the pancreas in fat digestion (Peterson and Drablos, 1994). Since then different lipases have been identified in and isolated from bacteria, fungi, plants and animals. Microbial lipases are found to be more useful than those derived from plants and animals, since they have great variety of catalytic activities, and microorganisms are easy to manipulate genetically and capable of rapid growth on in-expensive media. Furthermore, microorganisms are less affected by seasonal fluctuations and as such, they can be multiplied regularly, and high amount of lipases may be obtained from the microbial cells (Iftikhar *et al.,* 2007; Hills andDegussa, 2003; Hellen and Oliveira, 2009). Microbial lipases, especially fungi lipases are more portent andstable than their plant and animal derivatives (Singh and Mukhopadyay, 2012) Most identified fungi produced their enzymes extracellularly and their production is easier and safer for industrial and research application (Schimid and Verger, 1998).

The production of lipases by microorganisms is apparently important from the economic and industrial stand point. Fungi are the most important enzyme producers, since their enzymes are produced extracellularly which facilitates their extraction from fermentation media (Arnold, *et al.,* 1975; Ferreira-Costa and Peralta, 1999). Filamentous fungi, especially those of the genera *Rhizopus, Mucor, Geotrichum, Aspergillus, Fusarium* and *Penicillium* are widely used as sources of lipases.

Lipases have enjoyed a wide range of application; their value cannot be underestimated in pharmacy, food industries, detergent, and leather industries among others. Lipases are important enzymes, which have wide range of application due to their advantage in enzymatic hydrolysis over chemical processes. Enzymatic hydrolysis involves less energy and a resultant higher quality of the derived product (Pratyoosh and Kshitiz, 2007). Lipases are used in different areas or industries, such as diary, food, leather industries, and production of cosmetics, pesticides, and pharmaceuticals (Shahani, 1975; Odera and Joh, 1986; Abd-alla, 1999). With the rapid development of enzyme technology application, lipases have become important in the Oleochemical industries, paper manufacturing, organic chemical processing, biosurfactant synthesis and agrochemical industries (Vulfson, 1994; Hiol *et al.,* 2000). Lipases have also found applications in waste management and also improvement of tanning techniques (Pandey *et al.,* 1999).Jaeger and Eggert (2002) reported that lipases constitute the most

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important group of biocatalysts for biotechnological applications.

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

Materials and methods

Study Area

This study was carried out on the soil environment of Keffi metropolis. Keffi metropolis is the headquarters of Keffi Local Government, Nasarawa State, Nigeria. Keffi is about 68 km away from Abuja, the Federal Capital of Nigeria, and 128 km from Lafia, the capital city of Nasarawa State. It is located on latitude 8º5'N and longitude 7º50'E, and is situated on an altitude 850 meters above sea level (Akwa *et al.,* 2007). Keffi metropolis is North-West of Lafia, the capital of Nasarawa State, Nigeria.

Sample Collection

Soil samples of about 200 g were collected by random sampling from ten (10) different locations namely; High-court, Township Stadium, Angwa Nepa, Kofan Goriya, Dadin Kowa, Kofar Hausa, G.R.A., Main Campus, Cross-3 and Angwa Lambu. The samples were collected with the aid of a sterile hand trowel (which was pretreated with 70% alcohol) at the depth of 4 inches below the surface of the soil. The samples were collected into polythene bags and conveyed to Microbiology Laboratory of Nasarawa State University, Keffi, for analyses.

Determination of Soil Properties

Certain physico-chemical properties of the soil, viz; soil type, soil pH and temperature were determined as discussed below.

Soil Type

The soil types were determined with the aid of a sieve apparatus by sieve analysis using the Unified Soil Classification System (Whitbread *et al.*, 1996).

Soil pH

The pH of the soil samples was determined by the use of a digital pH meter by standard method (AOAC, 1990). A sample of 3g of soil from each site was diluted into 3ml of distilled water respectively and stirred for 5 minutes. The electrode of the pH meter was then inserted into the mixture and readings were taken. An average of three consecutive readings was recorded for each sample.

Soil Temperature

The soil temperature of the various sites was determined using a soil thermometer. The thermometer was inserted into the soil at the depth of 5cm and allowed to stand for 5 minutes, after which temperature readings were taken. Like the pH, average of triplicate readings was recorded for each site or location (Dix and Webster , 1995).

Isolation and Identification of Fungal Isolates

The soil fungi were isolated by direct soil inoculation or soil plate method using pour plate technique as adopted by Makut and Ade-Ibijola (2012). Soil sample of 0.1g from each site was placed in even distribution onto the bottom of a sterile Petri dish to which molten/cooled (40 – 45ºC) agar was poured and then allowed to set. The plates were incubated at 30ºC for 5 days. Colonies were counted after 24 hours and thereafter distinct colonies were sub-cultured within 3 – 5 days in order to obtain pure cultures of the organisms. The medium used for the isolation is Sabroud Dextrose Agar (SDA) which was incorporated with antibiotics (Ampicillin and Tetracycline), 30mg/liter, to inhibit bacterial contaminants. Fungal isolates from pure cultures were identified based on their macroscopic (cultural) and microscopic (morphological) features with reference to David and Roland (2003).

Screening for Lipase Production by the Isolates

Phenol red agar plates were also prepared using the method described by Singh *et al.* (2006). The medium was made up of the following; phenol red 0.01% (w/v), along with 1% (v/v) olive oil, 0.1% (w/v) CaCl2, 2% (w/v) agar, and the pH adjusted to 7.4. Aliquot of 20ml of the medium were poured into Petri dishes (after sterilization) and organisms inoculated. The plates were incubated the temperature of 37ºC. A change in the color of the phenol red was an indication of the activity of lipase produced by the organisms. The zone of clearance diameter of each isolate indicates the amount of lipase produced by the isolate.

The various fungal isolates were screened for lipase production using a chemically defined medium (Tween-80 agar) as described by Gonipath *et al.* (2005). The medium contained peptone 15g, sodium chloride (NaCl) 5g, calcium chloride (CaCl2) 1g, Tween-80, 10ml and agar 15g, all dissolved into 1 litre of distilled water. The pH was adjusted to 6.0 using 1M NaOH. About 20ml of the medium was dispensed into Petri dishes and allowed to set. The fungal isolates were inoculated onto the plates and incubated at the temperature of 28ºC for 48 hours. The appearance of a zone of clearance around the colonies and visible precipitate of calcium monolaurate was used as an indication of lipase production by the isolates.

Results and Discussion

The results of the physico-chemical properties of soil samples of the different locations in Keffi Metropolis are presented in Table 1, while Table 2 shows the results of the cultural features of the fungi isolated, Table 3 shows the results of the Total Fungal Counts in the soil samples of the different locations. The percentage frequencies of occurrence of fungal isolates are presented in Table 4, while the results of

lipases production by the fungal isolates are presented in Table 5.

Table 1: Physico-chemical properties of soil samples from different locations in Keffi

Sites	Soil Type	pH	Temperature (°C)
High – Court	Sandy – loamy	7.6 ± 0.00	23 ± 2.43
Township Stadium	Clay	7.5 ± 0.00	28 ± 1.64
Angwa NEPA	Sandy – loamy	7.3 ± 0.00	24 ± 0.83
Kopan Goriya	Sandy	7.4 ± 0.00	30 ± 1.64
Dadin Kowa	Sandy	7.3 ± 0.00	30 ± 0.83
Kofar Hausa	Sandy	8.0 ± 0.00	31 ± 1.64
G. R. A.	Loamy	6.7 ± 0.00	24 ± 0.83
Main Campus	Sandy	6.7 ± 0.00	28 ± 2.43
Cross – 3	Clay	6.5 ± 0.00	28 ± 1.64
Angwa Lambu	Loamy	8.3 ± 0.00	27 ± 1.64

Table 2. Cultural characteristics of fungal isolates on Sabroud Dextrose Agar

Isolates	Top Color	Reverse Color
Acremonium sp.	White – Cream	Yellow
Mucor sp.	Fluffy – Black	White Tan
Rhizopus stolonifer	Grayis – white	White Tan
Aspergillus niger	Blakish – Brown	Yellow
Aspergillus flavus	Velvety – Green	White

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

Sites	Total fungal Counts (CFU/g)
High – Court	$1.5 \times 10^2 \pm 0.05$
Township Stadium	$3.0 \times 10^2 \pm 0.03$
Angwa NEPA	$2.1 \times 10^{20} \pm 0.05$
Kopan Goriya	$1.0 \times 10^2 \pm 0.04$
Dadin Kowa	$1.5 \times 10^2 \pm 0.05$
Kofar Hausa	$2.0 \times 10^2 \pm 0.04$
G.R.A	$2.0 \times 10^2 \pm 0.04$
Main Campus	$1.0 \times 10^2 \pm 0.04$
Cross – 3	$2.0 \times 10^{2.} \pm 0.04$
Angwa Lambu	$1.4 \times 10^2 \pm 0.05$

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

	Sites										
Fungal Isolates	A	B	C	D	E	F	G	H	I	J	**Occurrence (%)**
Acremonium sp.	+	+	-	-	-	-	+	-	+	+	50
Mucor sp.	-	-	+	-	-	+	-	-	-	-	20
Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+	100
Aspergillus niger	-	+	-	-	-	-	+	-	+	-	30
Aspergillus flavus	-	+	-	+	+	-	-	-	+	+	50

KEY:(-) = Absent**;** (+) = Present**;** A = High Court; B = Township Stadium; C = Angwan; NEPA; D = Kopan Goriya; E = Dadin Kowa; F = Kofar Hausa; G = GRA; H = Main; Campus; I = Cross -3; J = Angwa Lambu.

Table 5:Lipases production by isolates

Fungal Isolates	Zone of Clearance (mm)
Acremonium sp.	8 ± 0.05
Mucor sp.	8 ± 0.07
Rhizopus stolonifer	12 ± 0.04
Aspergillus niger	10 ± 0.02
Aspergillus flavus	6 ± 0.04

Discussion

The anlyses of the soil types of Keffi demonstrated that the soils in High Court and Angwa NEPA are sandy Loam. They both have partly sandy and Loamy particles mixed in an even proportion. The soils from Kopan Goriya, Dadin Kowa, Kofar Hausa and Main Campus were sandy, while those of Angwa lambu and GRA were loamy. However, the remaining locations (Township Stadium and Cross – 3) had clay soils. The Results of pH values indicated that Cross -3 had the most acidic pH among all the sites tested, while Angwa Lambu had the most alkaline soil, followed by Kofar Hausa. High court, Township Stadium and Kopan Goriya have a slightly alkaline pH, respectively. The temperature of the soil environment of Keffi as at the time of this investigation revealed that the soil environment of Keffi had temperature range from 23 – 31^{o}C. The soil type, pH, and temperature values obtained in this investigation are similar to those obtained in previous studies (Makut and Godiya, 2010; Makut and Owolewa, 2011).

Rhizopus stolonifer had the highest percentage of occurrence of 100%. This indicates that *Rhizopus stolonifer* was present in all the soil samples that were analyzed. The next to *Rhizopus* was *Acremonium* spwith a percentage of 50% (indicating that *Acremonium* spappeared at least in five locations),followed by *Aspergillus flavus* with a percentage occurrence of 40%, which also shows that *Aspergillus flavus* appeared in at least four locations. *Aspergillus niger and Mucor* sphad percentage occurrence of 30 and 20% respectively. The fungal organisms isolated were identical to those earlier reported by Makut and Godiya (2010); Makut and Owolewa (2011) and Makut and Ade-Ibijola (2012)

The six fungal species isolated were found to produce varying levels lipase The appearance of a zone of clearance and visible precipitate as a result of deposition of calcium crystal was used as an indicator for lipase production. The diameter of the zone of clearance of the various isolates revealed that *Rhizopus stolonifer* had the highest, followed by *Aspergillus niger*, and then *Acremonium* sp. and *Mucor* sp. which had, respectively. *Aspergillus flavus* had the least diameter zone of clearance. The variation in enzyme production could be attributable to species differences (Iftikhar *et al.,* 2007; Hellen and Oliveira, 2009).

The enormous uses of lipases in various industries cannot be over emphasized and it is on the increase several areas of applications. According to Davranov (1994), extensive and persistent screening for new microorganisms and their lipolytic enzyme will open new, simple routes for synthetic processes and consequently new and faster ways to the applications of lipases in adding value to human life including solving environmental problems.

Conclusion

It is evident that all the fungal species that were isolates in this investigation were found to produce lipases. However, *Rizopus stolonifer* had the highest lipases activity. Further studies based on the results of this investigation may lead to the use of high yielding lipases producing fungi that could be used for industrial production of these enzymes.

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