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Full Length Research Paper

Fungal Lipolytic activity in *Jatropha curcas L.* fruit rot

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Isolation, identification and screening of lipid-degrading fungal organisms causing rot of unripe, ripe and dry fruits of *Jatropha curcas* L. were carried out, and determination of the fungus showing highest lipolytic activity on the substrate analysed from three different locations within Edo State, Nigeria was done using standard methods. Five fungal isolates (*Curvularia geniculata*, *Lasiodiplodia theobromae*, *Trichoderma harzianum, Mucor* sp. and *Penicillium* sp.) were recovered from the samples. *Penicillium* sp. had the highest frequency (37.5%) while *Mucor* sp. and *Trichoderma* sp. had the lowest (25%). Fungal isolates screened on solid agar showed low extracellular lipolytic activity detected by their non-production of a distinct clear zone of inhibition. In submerged fermentation, the growth of the lipolytic fungi ranged between 0.0492 (*Mucor* sp.) and 0.1539 g (*T. harzianum*). Spectrophotometric measurement (at 600 nm) of lipase production varied with *Mucor* sp. having the highest production of lipase. There was a significant difference (p≥0.05) between growth and lipolytic activities of the fungi. The study showed that *Mucor* sp. and *L. theobromae* are lipase producers and efficient lipid degraders. The implication of this is that these fungi could reduce the oil content of *J. curcas* seed and consequently its economic value.

Key words: Fungal isolate, lipase, *Jatropha curcas,* Nigeria.

INTRODUCTION

Jatropha curcas L., commonly known as Barbados nut, purging nut or physic nut, belongs to the family, Euphorbiaceae. It is believed to have originated from Central America, most probably Mexico (Makker and Becker, 2009). The plant is a poisonous, semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft) and growing in wastelands of almost any terrain or any kind of soil, even on gravel, sandy, acidic or alkaline soils with pH between 5.5 and 8.5 (Sharma et al., 2012). Plants from the same climatic zone show morphological

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differences with regard to the shape and size of the seeds and their protein and lipid content (Wolf et al., 1994). The plant is monoecious and flowers are unisexual, occasionally hermaphrodite. Ten stamens are arranged in two distinct whorls of five each in a single column in the androecium, and in close proximity to each other. In the gynoecium, the three slender styles are connate to about two-thirds of their length (Dehgan and Schutzman, 1994).

The plant is resistant to a high degree of aridity, allowing it to be grown in deserts. The seeds contain 27 to 40% oil (average: 34.4%) that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine (Makkar and Becker, 2009). The seeds are also a source of the highly poisonous toxalbumin and curcin (Abou-Arab and Abu-Salem, 2010). The seed cake can be used for fish or animal feed (if detoxified), biomass feedstock to power electricity plants, or as biogas or high-quality organic fertilizer. It can also be used as a bio-pesticide and for medicinal purpose (Henning, 2002).

Lipases are enzymes capable of catalysing the hydrolysis and synthesis of esters formed from glycerol and long-chain fatty acids (Svendsen, 2000; Sharma et al., 2001) and are produced by many microorganisms and higher eukaryotes (Elibol and Ozer, 2000; Kamimura et al., 2001). The ease with which enzymes could be isolated from microbes has made both bacteria and fungi predominant sources of lipase. However, fungi are the best lipase sources and are preferably used for industrial applications (Gupta and Soni, 2000; Mahadik et al., 2002).

Lipase producers have been isolated mainly from soil, or spoiled food materials that contain vegetable oil. Lipase production from a variety of bacteria, fungi and actinomycetes has been reported in several works (Kulkarni and Gadre, 2002; Maldonado et al., 2014). Lipase-producing fungi are present on a wide range of substrates in the ambient environment and these results could also provide basic data for further investigations on fungal extracellular enzymes (Griebeler et al., 2011). Among the available lipase-producing microorganisms, filamentous fungi belonging to various species of genera *Aspergillus, Rhizopus, Penicillium* and *Trichoderma* are described as prospective lipase producers (Lima et al., 2003; Kashmiri et al., 2006; Karanam and Medicherla, 2008). This study was undertaken to isolate, identify, screen and determine the most active lipase-producing fungus from *Jatropha curcas* fruit rot.

MATERIALS AND METHODS

Sample collection

Fruit samples of *J. curcas* L. (unripe, ripe and dry) used were obtained from three different locations, viz. Nigerian Institute for Oil Palm Research (NIFOR), Ugbowo and Upper Sakponba areas, in Edo State, Nigeria.

Preparation of medium and bacterial growth inhibition

Chloramphenicol at 0.02 g per 200 ml of medium was introduced into potato dextrose agar (PDA) to inhibit the growth of bacteria. Inoculation and transfer of culture were carried out on sterile inoculating bench CRC (Model HSB 60*180) after disinfection with methylated spirit.

Isolation of fungi associated with fruit rot

Small portions (including rotten and healthy) of 5 mm diameter were cut from the mesocarp with a flamed scalpel blade. These were sterilized in 0.1% mercuric chloride solution for 2 min and rinsed in three changes of sterile water, and thereafter dried with sterile tissue paper and crushed before plating in Petri dishes containing PDA medium. Inoculated Petri dishes were incubated at temperatures of 10 ± 2, 28 ± 2 and 35 ± 2°C for 3 to 7 days (Malik, 1996). After the period of incubation, different colonies of fungi associated with the fruit rot were each aseptically sub-cultured using a flamed inoculation loop, into a sterile plate containing PDA. The frequencies of the various colonies were observed; distinct colonies were sub-cultured to obtain pure isolates which were then maintained on PDA slants and stored at -4°C for further study. After ascertaining the purity of cultures, the fungi were identified on the basis of cultural, macroscopic and microscopic features with the help of suitable literature (Barnett and Hunter, 1998). Culture samples were also sent to the Commonwealth Mycological Institute, England for confirmation.

Identification of fungal isolates

Mycelia from the different fungal isolates were harvested. Slides were prepared. The prepared slides were examined under bright field (BF) or differential interference contrast (DIC) illumination microscope. Additional microscopic samples were made by gently pressing a piece of transparent tape onto a colony. A Leica DM 2500 microscope with bright field, phase contrast and DIC contrast optics was used to view the slides for spore shape, hyphae and colour. A spot camera (with spot imaging software) was mounted on the microscope and used for photomicrography. A Panasonic High Definition (HD) 1920×1080 camera with 14$_{MEGA}$PIXEL lens was used for colony photography.

Screening of the fungal isolates for lipase production on solid agar

A plate detection method containing a chromogenic substrate (Congo red) was used to screen the isolates for lipase-producing ability. The medium used for screening has the following composition in g/L: Peptone 10; $NaCl_2$ 5; Calcium chloride 0.1; Castor oil 1 ml; agar 50; Congo red 0.5; and distilled water 1,000 ml. The sterile medium was poured into plates and allowed to solidify. The agar plates were separately spot-inoculated with the fungal isolates and incubated at room temperature (28±2°C) for 14 days. Lipolysis was determined by the appearance of a clear zone of inhibition around the spot of inoculation. The diameters of the colonies and clear zones were measured from the 2nd (24 h after inoculation) to the 14th day (Gupta et al., 2004).

Screening of the selected isolates for lipase production using submerged fermentation

All the isolates were screened for lipase production in submerged fermentation medium. This was carried out using the modified

Table 1. Effect of temperature on growth of fungi isolated from *Jatropha curcas* fruits obtained from NIFOR.

Temperature (°C)	Sample	Growth (CFU)
35	Unripe	-
	Ripe	-
	Dry	20×10^{-5}
28	Unripe	
	Ripe	1×10^{-5}
	Dry	25×10^{-5}
10	Unripe	-
	Ripe	-
	Dry	-

- = absence of growth.

Table 2. Effect of temperature on growth of fungi isolated from fruits obtained from Ugbowo.

Temperature (°C)	Sample	Growth (CFU)
35	Unripe	-
	Ripe	2×10^{-5}
	Dry	4×10^{-5}
28	Unripe	30×10^{-5}
	Ripe	27×10^{-5}
	Dry	36×10^{-5}
10	Unripe	-
	Ripe	-
	Dry	-

- = Absence of growth.

Table 3. Effect of temperature on growth of fungi isolated from *J. curcas* fruits obtained from Upper Sakponba Road.

Temperature (°C)	Sample	Growth (CFU)
35	Unripe	-
	Ripe	-
	Dry	-
28	Unripe	33×10^{-5}
	Ripe	33×10^{-5}
	Dry	30×10^{-5}
10	Unripe	-
	Ripe	-
	Dry	-

- = Absence of growth.

method of Gupta et al. (2004). The sterile basal medium was inoculated with seed cultures of *Lasiodiplodia theobromae*, *Curvularia geniculata*, *Trichoderma harzianum*, *Penicillium* sp. and *Mucor* sp. Fermentation was carried out at room temperature (28±2°C) for 3 days for fungal isolates. Lipase production was determined by spectrophotometer at 600 nm (Gupta et al., 2004).

Data analysis

Experiments were performed in triplicate and the results were analysed statistically using SPSS version 16. The treatment effects were compared and significant differences were assessed with Duncan's multiple range test at $p \geq 0.05$.

RESULTS AND DISCUSSION

Five fungi were isolated from the fruit surface of *J. curcas* as the associated agents of fruit rot disease. The fungi are *Penicillium* sp., *Lasiodiplodia theobromae*, *Curvularia geniculata*, *Mucor* sp. and *Trichoderma harzianum.* At 35°C, there was no visible growth of fungi in the unripe fruit samples (Tables 1, 2 and 3). In addition, at 10°C, no growth was seen for both the unripe, ripe and dry samples. Fungal growth was noticed mainly at 28°C in the unripe, ripe and dry samples, with the highest colony-forming unit (CFU) count of 36×10^{-5} seen in dry sample from Ugbowo. The lowest CFU (1×10^{-5}) was recorded in the NIFOR ripe samples at 28°C. Lipases are able to catalyze hydrolysis, esterification, trans-esterification and lactonization or intermolecular esterification (Gupta et al., 2011). Lipase producers have been isolated mainly from soil, or spoiled food materials that contain vegetable oil. Lipase production from a variety of bacteria, fungi and actinomycetes has been reported in several works (Sztajer et al., 1988; Kulkarni and Gadre, 2002). The fungal isolates screened in this present investigation showed low extracellular lipolytic activity in solid substrate fermentation. Decrease in extracellular lipase production can be associated with either decrease in fungal growth or inactive nature of enzyme itself (Lui et al., 1995).

Table 4 shows the microscopic and morphological descriptions of the five fungi isolates from *J. curcas* fruit rot. Screening results of fungal isolates for lipase production and growth determination using submerged fermentation are presented in Table 5. The growth (dry weight) of fungal isolates in submerged fermentation ranged from 0.0492 (*T. harzianum*) to 0.1539 g (*Mucor* sp.). At 600 nm, *Mucor* sp. had the highest absorbance (0.284) as compared to *C. geniculata* which had the lowest absorbance of 0.022. However, in the liquid state (submerged fermentation), high lipolytic activities were observed. Lipase activity was highest in *Mucor* sp. followed by *L. theobromae* and *Penicillium* sp. Minimum activity was observed in *T. harzianum* and *C. geniculata*. Growth of the fungal isolates grown in PDA and lipolytic media is shown in Table 6. There were significant differences in fungal isolates grown in PDA medium

Table 4. Microscopic and morphological description of fungal isolates.

Isolate	Microscopic description	Morphological description
Penicillium sp.	Simple or branched conidiophores with round conidia.	Dark creamy mycelia growth
Lasiodiplodia theobromae	Conidia were unicellular, hyaline, ovoid to ellipsoid, with thick wall.	Whitish mycelia growth with granulated orange colour beneath
Curvularia geniculata	Septate brown hyphae were seen and had curved conidia and elongated conidiophores	Pinkish mycelia growth
Mucor sp.	Round and slightly elongated sporangiospores with sparsely or non-septate hyphae were noticed.	Whitish fluffy mycelia growth
Trichoderma harzianum	Hyaline septate hyphae with one-celled and round conidia.	Dark green mycelia growth

Table 5. Screening of fungal isolates for lipase production and growth determination using submerged fermentation.

Fungi isolate	Lipase production (absorbance at 600 nm)	Growth (dry weight in g)
Penicillium sp.	0.063	0.0889
Mucor sp.	0.284	0.1539
Lasiodiplodia sp.	0.142	0.1182
Curvularia sp.	0.022	0.0875
Trichoderma sp.	0.022	0.0492

Table 6. Growth of the fungal isolates in PDA and lipolytic media.

Fungi isolate	Growth (PDA medium) incubation time (Days)			Growth (lipolytic medium) incubation time (Days)		
	3	7	14	3	7	14
Penicillium sp.	0.70^{a}	1.77^{b}	5.07^{c}	1.53^{a}	3.43^{b}	4.10^{c}
Mucor sp.	4.70^{a}	8.00^{b}	8.07^{b}	4.10^{a}	8.00^{b}	8.00^{b}
L. theobromae	3.83^{a}	7.93^{b}	8.17^{c}	2.90^{a}	5.87^{b}	6.30^{c}
C. geniculata	7.50^{a}	7.83^{b}	8.00^{b}	5.50^{a}	7.90^{b}	8.00^{b}
T. harzianum	2.30^{a}	3.60^{b}	4.70^{c}	1.80^{a}	3.37^{b}	3.87^{c}

Values are means of triplicate determinations. Values in the same row with different superscripts are significantly different with Duncan's multiple range test at $p \geq 0.05$.

between 3rd day and 14th day. Similarly, the growth of fungal isolates grown in lipolytic medium varied significantly. Previous reports by Prakash and Sivakumar (2013) have also shown that lipase activity is high in Mucor sp. in an experiment on isolation and screening of degrading enzymes from mangrove-derived fungi. The result is similar to that of Lazer and Schroder (1992) who investigated fungal lipases which degrade lipids from palm oil. Savitha et al. (2007) reported that fungal strains of different genera were isolated from various sources of which three *Aspergillus* spp. and one *Mucor* sp. were found to be positive for lipase production. In the present study, maximum biomass production was attained at the late period of fermentation (14^{th} day) for *T. harzianum* and *Penicillium* sp., and 7th day for the other three fungi. This, however, is in contrast with the report of Kashimiri et al. (2006) who reported that maximum biomass production by *T. viride* was observed during the early hours of fermentation. This difference might be due to the species used. There was a significant difference in lipase production by the various fungi.

The present study showed that *Mucor* sp. and *Lasiodiplodia theobromae* are lipase producers and efficient lipid degraders of *J. curcas* seeds. If the activities of these fungi are not checked, they could lead to great losses in the *J. curcas* seed oil. This oil is currently being used for production of biodiesel- an environment friendly diesel, among other uses. It is suggested that further

research on production, characterization and purification of the fungi be carried out.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abou-Arabb AA, Abu-Salem MT (2010). Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatment on the anti-nutritional factors. Afr. J. Food Sci. 4(3):93-103.

Barnett HL, Hunter BB (1998). Illustrated Genera of Imperfect Fungi. (3rd edition). Burgess Publishing Company, Minneapolis. P.331.

Dehgan B, Schutzman B (1994). Contributions toward a monograph of neotropical *Jatropha* – phenetic and phylogenetic analyses. Ann. Bot. Gard. 81:349-367.

Elibol M, Ozer D (2000). Influence of oxygen transfer on lipase production by *Rhizopus arrhizus*. Process Biochem. 36(4):325-329.

Griebeler N, Polloni AE, Remonatto D, Arbter F, Vardanega R, Cechet JL, Di Luccio M, de Oliveira D, Treichel H, Cansian RL, Rigo E, Ninow JL (2011). Isolation and screening of lipase-producing fungi with hydrolytic activity. Food Bioprocess Technol. 4(4):578-586.

Gupta JK, Soni SK (2000). Industrial uses of enzymes. J. Punjab Acad. Sci. 2:75-80.

Gupta P, Upadhyay LSB, Shrivastava R (2011). Lipase catalyzed-transesterification of vegetable oils by lipolytic bacteria. Res. J. Microbiol. 6(3):281-288.

Gupta R, Gupta N, Rathi P (2004). Botanical lipases: an overview of properties. Appl. Microbiol. Biotechnol. 64(6):763-781.

Henning R (2002). Using the Indigenous Knowledge of *Jatropha:* The Use of *Jatropha curcas* L. Oil as Raw Material and Fuel. Indigenous Knowledge Notes 47 (August): 1–4. Accessed August 25, 2011. Available at https://openknowledge.worldbank.org/bitstream/handle/10986/10791/multi0page.pdf?sequence=1&isAllowed=y

Kamimura ES, Medieta O, Rodrigues MI, Maugeri F (2001). Studies on lipase-affinity adsorption using response-surface analysis. Biotechnol. Appl. Biochem. 33(3):153-159.

Karanam SK, Medicherla NR (2008). Enhanced lipase production by mutation induced *Aspergillus japonicus.* Afr. J. Biotechnol. 7(12):2064-2067.

Kashmiri MA, Ahmad A, Butt BW (2006). Production, purification and partial characterization of lipase from *Trichoderma viride*. Afr. J. Biotechnol. 5(10):878-882.

Kulkarni N, Gadre RV (2002). Production and properties of an alkaline, thermophilic lipase from *Pseudomonas fluorescens* NS2W. J. Ind. Microbiol. Biotechnol. 28(6):344-348.

Lazer G, Schroder FR (1992). Microbial Degradation of Natural Products. In. Winkelmann G (ed.) VCH, Weinheim. pp. 267-291.

Lima VMG, Krieger N, Sarquis MIM., Mitchell DA, Ramos LP, Fontana JD (2003). Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. Food Technol. Biotechnol. 41(2):105-110.

Lui R, Jiang X, Mou H, Guan H, Hwang HM, Li X (2009). A novel low-temperature resistant alkaline lipase from a soda lake fungus strain *Fusarium solani* N4-2 for detergent formulation. Biochem. Eng. J. 46(3):265-270.

Mahadik ND, Puntambekar US, Bastawde KB, Khire JM, Gokhale DV (2002). Production of acidic lipase by *Aspergillus niger* in solid state fermentation. Process Biochem. 38(5):715-721.

Makker HP, Becker KJ (2009). *Jatropha curcas* – a promising crop for the generation of biodiesel and value-added co-products. Eur. J. Lipid Sci. Technol. 111(8):773-787.

Maldonado, RR, Macedo, GA, Rodrigues, MI (2014). Lipase production using micro-organisms from different agro-industrial by-products. Int. J. Appl. Sci. Technol. 4(1):108-115.

Malik BS (1996). A Laboratory Manual of Veterinary Microbiology, Part III. Pathogenic Bacteriology and Mycology (4th ed.). pp. 137-146.

Prakash M, Sivakumar T (2013). Isolation and screening of degrading enzymes from mangrove-derived fungi. Int. J. Curr. Microbiol. Appl. Sci. 2(5):127-129.

Savitha J, Srividya S, Jagat R, Payal P, Priyanki S, Rashmi GW, Roshini KT, Shantala YM (2007). Identification of potential fungal strain(s) for the production of inducible, extracellular and alkalophilic lipase. Afr. J. Biotechnol. 6(5):564-568.

Sharma R, Chisti Y, Banerjee UC (2001). Production, purification, characterization, and applications of lipases. Biotechnol. Adv. 19(8):627-662.

Sharma S, Dhamija HK, Parashar B (2012). *Jatropha curcas*: A Review. Asian J. Res. Pharm. Sci. 2(3):107-111.

Svendsen A (2000). Lipase protein engineering (Review). Biochim. Biophys. Acta 1543(2):223-238.

Sztajer H, Maliszewska I, Wieczorek J (1988). Production of exogenous lipases by bacteria, fungi, and actinomycetes. Enzyme Microb. Technol. 10(8):492-497.

Wolf WJ, Schaer ML, Abbott TP (1994). Non-protein nitrogen content of defatting Jojoba meal. J. Sci. Food Agric. 65(3):277-288.