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Screening of Naturally Occurring Fungi for Lipase Production using Selected Agricultural Waste Products

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Abstract
The growing industrial demand for new sources of lipases with varying catalytic properties has prompted the isolation and selection of novel microbial strains. This study focused on isolating and screening fungi that produce lipase using selected agricultural waste products. The farm waste materials which include; wheat bran, rice husks, corn meal, and sugarcane bagasse, were collected aseptically from selected markets. They were sun-dried for 72 hours and pulverized. Naturally occurring fungi were obtained using the solid-state fermentation method. Isolation and identification of the fungi were performed using the dilution plate method and characterization respectively. Lipase production was screened using a medium containing a phenol red indicator. Five fungal isolates were obtained from wheat bran, two from rice husks, two from corn meal, and one from sugarcane bagasse. Out of these, four fungal isolates tested positive for lipase production, while one tested negative. From wheat bran the highest zone of colouration was observed in Aspergillus niger with 41.00±2.65, Aspergillus flavus with 34.00±12.29, Rhizopus stolonifer with 29.00±5.29, Geotrichum species with 24.33±7.09 and Fusarium species with 0.00±0.00. In rice husk, the highest zone of colouration was observed in Rhizopus stolonifera with 85.00±0.00 Aspergillus niger with 83.67±1.53, in corn meal the highest zone of colouration was observed in Aspergillus flavus with 85.00±0.00, Rhizopus stolonifer with 42.34±16.19. In sugar cane bagasse, the highest zone of colouration was observed in Rhizopus stolonifer with 85.00±0.00. It can be concluded that wheat bran, rice husk, corn meal, and sugarcane bagasse are potential substrates for lipase production using naturally occurring fungi.

Keywords: Agricultural waste products, Fermentation, Fungi, Lipase, Screening

INTRODUCTION
Agricultural industries generate significant wastes annually, and improper disposal of such waste can lead to environmental pollution and harm to human and animal health, agro-industrial waste is untreated primarily, leading to various issues related to climate change by increasing greenhouse gas emissions. Additionally, the reliance on fossil fuels further contributes to the production of greenhouse gases. Many industrial wastes remain untreated and under-utilized, resulting in disposal methods that include burning, dumping, or unplanned landfilling (Bos and Hamelinck, 2015). Promoting cleaner and renewable bioenergy resources is now a global priority (Okonko *et al*., 2019). Globally, it is estimated that around 147.2 million metric tons of fiber sources are available. Additionally, during the

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1990s, approximately 709.2 million metric tons of wheat straw residues and 673.3 million metric tons of rice straw were recorded (Belewu and Babalola, 2019). Agro-industrial residues are increasingly recognized for their high nutritional value, enhanced quality control, and classification as by-products of agro-industrial processes (Graminha *et al*., 2018). Lipases have become one of the leading types of biocatalysts, accounting for nearly 10 % of the enzyme market (Salihu *et al*., 2016). As fossil fuel reserves deplete due to high consumption in vehicular applications, there is an urgent need to develop cost-effective and sustainable energy alternatives, such as biodiesel. Lipases play a crucial role in this process as they hydrolyze acylglycerols into fatty acids and glycerol, facilitating esterification and transesterification reaction (Selvakumar and Sivashanmugam, 2017), due to its distinctive large catalytic potential, lipase is considered one of the most crucial industrial enzymes (Andualema and Gessesse, 2012), lipases are found in a variety of sources, including plants, animals, and microorganisms. Among these, microbial lipases are particularly advantageous for industrial applications. This is because they can be produced at a very high rate and do not require cofactors for their activity (Dey *et al*., 2017). Most lipases used for biotechnological applications are isolated from bacteria and fungi (Lin *et al*., 2016).

Fungi have become a significant source of lipases due to their capacity to secrete large amounts of extracellular enzymes, their ease of cultivation, and their ability to adapt to various substrates. Common lipase-producing fungi include Aspergillus, Rhizopus, Mucor, Penicillium, and Candida. Among these, *Aspergillus niger* and *Rhizopus oryzae* are extensively studied for their high lipase yield and stability (Sharma *et al*., 2021). Substrates like vegetable oils, agricultural residues (such as oil cakes, fruits, and fruit peels), and waste oils have been extensively studied as alternatives to refined media. These materials are abundant, cost-effective, and rich in lipids, making them ideal for promoting lipase production when cultivated on waste oils and oil seed cakes (Gupta *et al*., 2020). These substrates not only provide essential carbon sources but also induce lipase activity, as lipases are typically activated by fatty acids and triglycerides (Kumar *et al*., 2021).

Research has also examined the use of agricultural waste materials, such as rice bran, wheat bran, and coconut oil cake. These materials are rich in lignocellulosic compounds that support fungal growth and promote lipase production. Solid-state fermentation has proven to be especially effective with these substrates, as it simulates the natural habitat for many fungi (Akinmoladun *et al*., 2020). Furthermore, incorporating organic waste like fruit peels has been shown to enhance lipase production in various fungal strains, including *Mucor* spp (Patel *et al*., 2022). Enzymes of industrial interest have typically been sourced from submerged fermentation (SMF) due to their ease of handling. However, solid-state fermentation (SSF) has recently gained attention for producing higher yields and better product characteristics, especially when using low-cost agro-industrial residues as raw materials (Malilas *et al*., 2017).

The growing industrial demand for diverse lipases drives the isolation of new strains. Utilizing renewable resources, particularly agricultural wastes, for enzyme production is economically viable and helps mitigate pollution issues from waste disposal, promoting sustainable practices (De Azevedo *et al*., 2017), most research works focus on isolating naturally occurring fungi from contaminated soil samples Since soil serves as a reservoir for a large and diverse microbial community population, but the use of agricultural wastes as a substrate for isolating naturally occurring fungi is more economical than conventional substrates and also a means of converting wastes to wealth thereby controlling

environmental pollution. The study focused on isolating and screening fungi that produce lipase using selected agricultural waste products.

Materials and Methods

Collection and Processing of Samples

The samples were collected from selected markets within Ilorin, Kwara state, Nigeria. Samples include wheat bran, rice husk, corn meal, and sugarcane bagasse. The samples were sun-dried for 72 hours, after which the dried samples were pulverized and milled using Marlex Excellent Grinder (Mumbai, India) to obtain their powdery form.

Media Preparation

Potato dextrose agar (PDA) was prepared according to the manufacturer's instructions. It was sterilized at 121 °C for 15 minutes (Kusumo *et al.,* 2022).

Isolation of Natural Occurring Fungi from Selected Agricultural Wastes

Solid-state fermentation of the selected agricultural wastes to obtain naturally occurring fungi was carried out by measuring five (5) g of each substrate into sterile containers and ten (10) ml of distilled water was added to each of the substrates and thoroughly mixed. This was left for 7 days to allow natural fermentation for each of the substrates. The dilution plate method described by Piasai and Manoch (2009) was used to isolate fungi from the agricultural waste samples. One (1) g of each sample was mixed aseptically in 9mL of sterilized distilled water and shaken vigorously, appropriate serial dilutions were carried out and an aliquot of 0.1mL from the 10^3 test tube diluents was inoculated into sterilized potato dextrose agar (PDA) plate, incorporated with 0.1ml of streptomycin during preparation to inhibit bacterial growth, the plates were incubated at 25±2°C for seven (7) days.

Identification of Fungal Isolates

The fungal isolates were identified macroscopically and microscopically according to Ibrahim *et al*. (2019). The distinct colony plates were observed for their physical features and also viewed under the microscope at 40x immersion.

Screening of Fungal Isolates for Lipase Production

The fungal isolates were screened for their ability to produce lipase by inoculating into phenol red agar plates. This was prepared by using 1% (v/v) olive oil, 2% (w/v) agar, 0.1 % (w/v) $CaCl_2$, phenol red 0.01% (w/v), and a pH of 7.4. This was sterilized after which 20 ml of the medium was dispensed into petri dishes and organisms inoculated. The plates were incubated at 37 °C for 24 hours. Positive lipase production was observed by a change in the colour of the phenol red and the zone of colouration in diameter. (Makut and Bemgba, 2017; Abdulmumini *et al*., 2022).

RESULTS

Fungal Isolates Obtained from Selected Agriculture Waste Samples and Their Morphological Characteristics

A total of five fungal isolates were obtained from fermented wheat bran, two from risk husk, two from corn meal, and one isolate from sugarcane bagasse, and their characteristics are summarized in Table 1.

Table 1: Morphological Characteristics of Fungal Isolates

Fungal Isolate	Mycelia Colour	Spore Colour	Reverse Colour	Spore Shape	Hyphae	Probable Fungus
FW1 FR1 FC1 FS1	Whitish	Black	Whitish	Globose –ovoid	Non-septate	*Rhizopus stolonifer*
FW2 FR2	Yellowish black	Black	Creamy	Conidiospore	Septate	*Aspergillus niger*
FW3 FC2	Yellowish green	Whitish	Colourless	Large, ovoid	Septate	*Aspergillus flavus*
FW4	Whitish	Whitish	pale cream	Conidiospore	Septate	*Geotrichum* sp
FW5	Pinkish	Reddish	Colourless	Conidiospore	Septate	*Fusarium* sp

KEYS:
FW: Fungal isolates from wheat bran
FC: Fungal isolates from rice husk
FR: Fungal isolates from corn meal
FS: Fungal isolates from sugarcane bagasse

Screening Reaction of Fungal Isolate for Lipase Production

Four of the fungi isolates were positive while one of the isolates was negative shown in Table 2 below.

Table 2: Screening Reaction of Fungal Isolates for Lipase Production

Fungal Isolates	Screening reaction for lipase production
Rhizopus stolonifer	+
Aspergillus niger	+
Aspergillus flavus	+
Geotrichum sp	+
Fusarium sp	-

+ = positive - = negative

Zone of Clearance of Fungal Isolates

The zones of clearance shown by the fungal isolates obtained from the selected agricultural waste samples are measured in mm and presented in table 3.

Table 3: Zones of Clearance of Fungal Isolates Obtained from Selected Agricultural Waste Samples

Agricultural Waste Samples	Fungal Isolates	Zone of Clearance (mm)
Wheat Bran	*Rhizopus stolonifer*	29.00 ± 5.29^{ab}
	Aspergillus niger	41.00 ± 2.65^{bc}
	Aspergillus flavus	34.00 ± 12.29^{bcd}
	Geotrichum sp.	24.33 ± 7.09b
	Fusarium sp.	0.00 ± 0.00^{a}
Rice Husk	*Rhizopus stolonifer*	85.00 ± 0.00^{e}
	Aspergillus niger	83.67 ± 1.53^{e}
Corn Meal	*Aspergillus flavus*	85.00 ± 0.00^{e}
	Rhizopus stolonifer	42.34 ± 16.19^{d}
Sugarcane Bagasse	*Rhizopus stolonifer*	85.00 ± 0.00^{e}

Values are means of duplicate readings ± SD. Values with different subscripts on the column are significantly different at $p>0.05$

DISCUSSION

The study successfully isolated fungal strains from agricultural waste products, revealing varying levels of fungal diversity across the substrates. Wheat bran exhibited the highest diversity, yielding different types of fungal isolates. In contrast, only a type of fungal isolate

was obtained from fermented sugarcane bagasse, while corn meal and rice husk showed moderate diversity with fewer fungal isolates obtained. According to Ibrahim *et al*. (2019), wheat bran contains essential nutrients for fungal growth and has a lipid content of about 3-5%, making it a suitable substrate for fungal growth compared to other substrates. The study also noted that sugarcane bagasse contains 1-3% lipid content and lacks some basic nutrients, contributing to its minimal fungal isolates. The fungal isolates exhibited various morphological features as indicated in Table 1. Agricultural waste is a valuable substrate that supports the growth of diverse fungal isolates. Screening reactions of the fungal isolates was an evaluation of their lipase production potential. The naturally occurring fungal isolates; *Aspergillus niger, Aspergillus flavus*, *Rhizopus stolonifer,* and *Geotrichum* sp were positive for lipase production except for *Fusarium* sp. *Aspergillus* species have been extensively studied for their ability to produce various enzymes, including lipase (Bharathi and Rajalakshmi, 2019). According to Gholami-Shabani *et al*. (2022), *Aspergillus* species are being utilized as a reliable enzyme factory due to their notable ability to produce massive quantities of enzymes required for diverse industrial applications. Chijioke *et al*. (2018) opined that *Aspergillus* sp was used for lipase production and Abdulmumini *et al*. (2022) noted it had the highest zone of clearance indicating its suitability for lipase production.

Rhizopus sp has various applications in the industry including the manufacturing of different enzymes such as proteases, pectinases, phytases, cellulases lipases amongst others. Several researchers have documented the impact of diverse *Rhizopus* species in the production of lipase enzymes. Elhussieny *et al*. (2023) optimized culture conditions for the transesterification of waste frying oil using *Rhizopus stolonifer* biomass as a lipase inducer. Helal *et al*. (2021) affirmed the importance of *Rhizopus* spp and its industrial use as a lipase producer. They emphasized the utilization of lipase obtained from *Rhizopus oryzae* as an excellent product that has extraordinary roles in biodiesel and detergent production. Maldonado *et al*. (2016) also reviewed the importance of *Geotrichum* sp lipases and their applications in the industries. In this study, *Fusarium* sp isolated from fermented wheat bran which screened negative for lipase production in this study is contrary to the reviews of Bharathi and Rajalakshmi (2019) and Kumar *et al*. (2023). The varying zones of clearance of the naturally occurring fungi obtained from the different agricultural waste products is an indication that these fungi are potential lipase producers with the substrates as excellent sources. *Rhizopus stolonifer* had the highest zone of clearance of lipase activity with rice husk and sugarcane bagasse while *Aspergillus flavus* exhibited the highest zone of clearance with corn meal substrates. This variation in lipase production could be due to species differences and the ability to utilize the substrates (Hellen and Oliveira, 2009; Makut and Bengba, 2017). The isolation and screening of naturally occurring fungi for lipase production, utilizing agricultural wastes will solve problems on environmental pollution and enhance novel ways of production of lipase enzyme as a value-added product.

CONCLUSION

The study highlights the potential of agricultural waste products as substrates for lipase production. Naturally occurring fungi from the fermented substrates such as *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer,* and *Geotrichum sp*ecies screened positive for lipase production except for *Fusarium* sp. However, more research work can focus on scaling up lipase production utilizing the fungi and exploring its applications in various industries.

Conflict of Interest

Authors have no conflict of interest to declare**.**

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