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Screening of Lipase Producing Fungi from Groundnut and Engine Oil Contaminated Soil

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Abstract
Lipases are secreted by many fungi, yeast and bacteria. They are glycerol esters that catalyze the hydrolytic cleavage of ester bonds of water insoluble triglycerides to free fatty acids and glycerol. Soil is an excellent habitat of variety of enzyme producing microbes and the suitable habitat to isolate lipase producing microorganisms is the oil contaminated sites. The research was conducted to screen lipase Production from soil contaminated with groundnut oil and engine oil. Fungi were obtained from soil using serial dilution agar plate method from groundnut oil milling site and mechanic garage site. Fungi with different colony characteristics appeared on potato dextrose agar plates. These were explored for finding fungi with lipase enzyme producing capabilities. The lipolytic fungi were subjected to screening using tween 80 agar. The result showed that both soil have fungi capable of producing lipase but the soil from groundnut oil milling site had more lipase producing fungi because the precipitation zone observed was higher than that of soil from mechanic garage site. In conclusion, soil from groundnut oil milling site could be a good source for lipase producing fungi.

Keywords: Lipase, ground nut oil, engine oil, fungi, soil

INTRODUCTION
Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil-water interface and reverse the reaction in aqueous and non-aqueous media. The lipase enzyme has shown stability at extreme pH and temperature (Pooja*et al*., 2015).Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc many microorganisms such as bacteria, yeast and fungi are known to secret lipases. They are widely distributed in plants, animals and microbes, where their physiological role is to metabolize lipids. These are obtained either by extraction from animal or plants tissue or cultivation of microorganisms. Lipases are produced by many microorganisms, including bacteria, fungi, and yeasts (Akshita *et al*., 2017). Lipases occur widely in nature but only microbial lipases are significant. Microbial lipases are often more useful than enzymes derived from plants or animals because of the great variety of catalytic

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activities available. Since soil is a reservoir of a large and diverse microbial population, we investigated the ability of these microorganisms to produce lipases.

Among these microbial populations, the filamentous fungi are well known as perfect source of lipases since they produce extracellular enzyme. In addition, fungi enzymes are morestable and have more diverse properties compared to lipases from other sources. Some of the industrially significant lipolytic fungi are of the genera *Penicillium, Geotrichum, Aspergillus, Candida, Rhizomucor and Rhizopus* (Alima *et al.*, 2018). Lipolytic microorganisms have been establish in different environment such as effluent from pulp and paper industry, edible oil processing factories, dairy industries and soil contaminated with edible oil, petrol and diesel oil (Arun *et al.*, 2017) . The soil has a huge diversity of microbes that can be isolated and assessed for their capabilities as various enzyme production. Numerous techniques can be utilized for screening of lipolytic microorganism based on the detection of the occurrence of extracellular lipases. The utilization of a solid agar medium containing inducer substrates such as edible oils, triglycerides (triolein, tributyrin), Tween-20, Tween-80, and various dyes can be used (Baplu *et al*.,2014). Lipase has been widely used in biotechnological applications in diary industry such as food, cosmetic, detergent, leather and pharmaceutics, in bioremediation of environments polluted with spills containing hydrocarbons, waste from oil manufacturing or food processing (Alima *et al*., 2018).

MATERIALS AND METHODS

COLLECTION OF SOIL SAMPLE

Soil samples were collectedfrom ground nut milling site and engine oil from mechanic site for analysis. The soil sampleswere collected with unused plastic bag sealed with heavy-duty rubber bounds. All samples were labeled with a permanent waterproof maker.

ISOLATION OF SOIL FUNGI

The soil samples were collected from oil mills located at zai village in Dutse local government area of Jigawa State and engine oil from mechanic site in hakimi street in Dutse city, Jigawa State. Fungi were obtained by serial dilution agar plate method. One gram of each of the soil sample was separately added in test tube containing 4.5 ml of sterile distilled water followed by vortexing for 10 minutes to make soil suspension of 10^{-1} dilution. One ml of soil suspension from 10^{-1} dilution was transferred on to another test tube containing 4.5 ml of sterile distilled water to prepare 10^{-2} dilution. This step (withdrawal and transfer of soil suspension) was repeated until the preparation of 10^{-5} dilution. Each of the soil dilution was aseptically inoculated and uniformly spread in sterile petri plate containing the potato dextrose agar (PDA) medium. The inoculated PDA plates were kept at 28ºC for 7 days in an incubator. Different fungi appeared on PDA plates and were further subcultured on tween 80 agar plates.

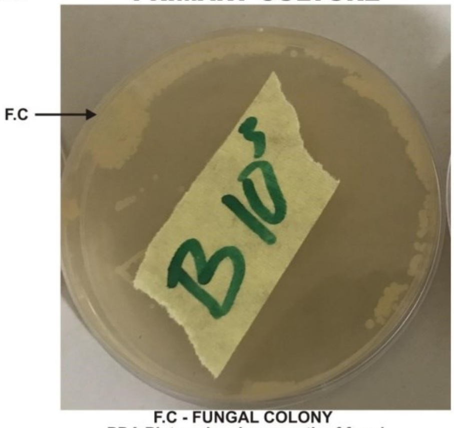
SCREENING FOR LIPASE ACTIVITY

The predominant fungion the PDA plate were isolated and screened for lipolytic activity. Lipolysis is observed directly by changes in the appearance of the substrate such as tributyrin which are emulsified mechanically in various growth media and poured into a petri dish. The fungal isolates were screened for lipolytic activity on agar plates containing tributyrin (1%, w/v), agar (2%, w/v), peptone (0.5g), beef extract (0.3g). Lipase production is indicated by the formation of clear halo zone around the colonies grown on tributyrin-containing agar plates (Kalpana *et al*, 2013).

RESULTS AND DISCUSSION

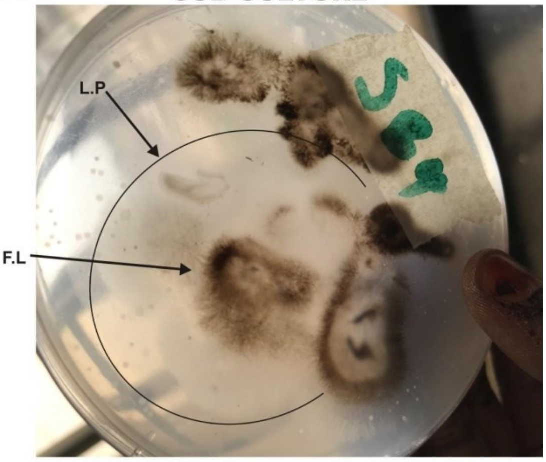
Result of the present study showed the presence of lipase producing fungi in *Aspergillus* spp from groundnut oil milling site and engine oil from mechanic garage site (Plate 1 and 3). From the result, the precipitation zone observed from the soil obtained from groundnut oil milling site was bigger than precipitation zone obtained from the soil of the mechanic garage shop. This indicated that soil from the groundnut oil milling site had more lipase producing fungi than soil from the mechanic garage site.

PLATE 3A **PRIMARY CULTURE**



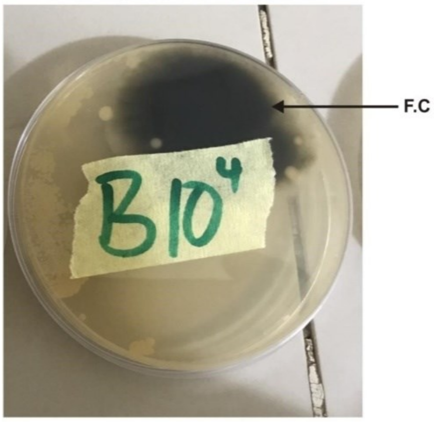
F.C - FUNGAL COLONY
PDA Plates showing growth of fungi

PLATE 1B **SUB CULTURE**



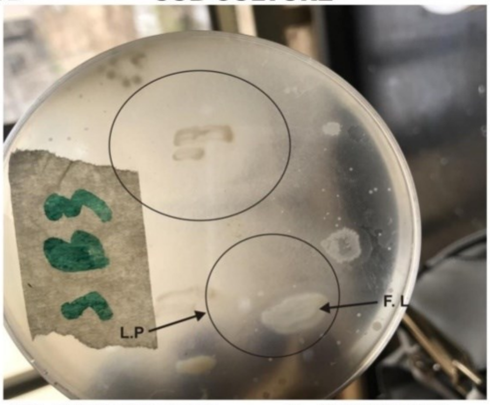
L.P - LIPASE PRECIPITATE F.L - FUNGAL LIPASE
Plates assay showing lipase production by the appearance of precipitate on tween 80 agar plate

PLATE 1A PRIMARY CULTURE



F.C - FUNGAL COLONY
PDA Plates showing growth of fungi

PLATE 3B SUB CULTURE



L.P - LIPASE PRECIPITATE F.L - FUNGAL LIPASE
Plates assay showing lipase production by the appearance of precipitate on tween 80 agar plate

The result of the present study indicated that both sands from groundnut milling site and mechanics garage site hadfungi capable of producing lipase. However, the activity of these fungi producing lipases varied between the sub cultures precipitation zone. That was because groundnut oil comes from plants and plants are also good sources of lipase. Soil has huge diversity of microbes that can be isolated and assessed for their capabilities as various enzyme productions. The soil being polluted with oil had very low water activity and low PH (4-5) and fungi are known to adapt best under such environmental condition. Groundnut contains 40-50 percent fat, these fat occurs in the form of different types of fatty acids such as oleic acids, linoleic acids, steric acids etc The soil being polluted with oil had very low water activity and fungi are known to adapt best under such environmental condition. Fungi make use of carbon as one of its macro element and groundnuts have high concentration of carbon and carbon is one of the main factors enhancing lipase production (Praveen *et al*., 2012).

CONCLUSION

The fungal strain *Aspergillus* spp screened from ground nut milling site has been shown to be capable of producing lipase. However, the present study requires greater research capacities (further purification of the crude enzyme).

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