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COMPARATIVE STUDY OF THE LIPOLYTIC ACTIVITIES OF SOME FUNGI AND LACTOBACILLUS SPECIES ISOLATED FROM SOME NIGERIA LOCAL FOODSTUFF

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ABSTRACT

This study is aimed at extracting lipase enzyme from three lactic acid bacteria and some fungi for a comparative study of their lipase activities. Lipase enzyme is useful in the degradation and detoxification of "bad"cholesterol in food and raw materials. In this study the submerged fermentation method was applied in the extraction of lipase from Lactobacillusplantarum, Lactobacilluscasei and Lactobacillus fermentumand the fungal isolates. Their corresponding lipase activity values were 1.2µmol/min/ml (Lactobacillusplantarum), 0.7µmol/min/ml(Lactobacilluscasei) and 1.9 µmol/min/ml (Lactobacillus fermentum). The fungi isolate namely G4 (Penicillium spp.), G5 (Rhizopus spp.), M3 (Aspergillus niger), M4 (Fusarium oxysporium) and M8 (Fusarium moniliforme), had the following corresponding lipase activity values: G4 (11.25 µmol/min/ml), G5 (2.92 µmol/min/ml), M3 (3.75 µmol/min/ml), M4 (2.92 µmol/min/ml) and M8 (6.25 µmol/min/ml). The result of the study indicates that microbial enzymes from fungi had better potentials as best sources of lipase degrading enzymes. The high level of lipase activity from fungi could be attributed to the fact that most fungi are spore formers and the multiplicity of spores could enhance increased lipase production. It is therefore recommended that researchers should source lipase enzymes both for environmental bioremediation and degradation of unhealthy fats in foodstuff from fungi species.

KEYWORDS: Lipase, fungi, Lactobacillus, degradation, fermentation.

INTRODUCTION

Fats or lipids which are broken down into glycerol and free fatty acids through a process called lipolysis are abundant in food and environment (Meynier and Genot, 2017). Lipolytic activities involve the application of a suitable lipase for the degradation of fats or lipids. These enzymes are secreted by several microorganisms using agro-industrial residues as potential substrates according to Hasan et al., (2009) and Sundar and Kumaresapillai, (2013). Lipase enzymes are active at the interface of aqueous and non-aqueous phases which distinguishes them from esterases (Pandey et al., 1999). Lipase is extensively distributed in plants, animals and microorganisms. The strains of Bacillus, Pseudomonas, Burkholderia, Acinetobacter Staphylococcus, and somefungi like Aspergillus terreusand Fusarium heterosporumare reported by some authors (Walavalkar and Bapat, 2001; Mrozik et al., 2006; Gayathri et al., 2013) to produce lipase. In terms of low production cost, greater stability and wider availability, microbial lipases are commercially significant than plant and animal lipases in the food industry. Lipases are commonly used in the production of a variety of products, ranging from fruit juices, baked foods, vegetable fermentation and in flavour development as reported by Hasan et al., (2005). Lipases are widely used in the processing of fatsand oils, detergents and degreasing formulations, foodprocessing, the synthesis of fine chemicals andpharmaceuticals, paper manufacture and production of cosmetics (Rubin and Dennis, 1997a, 1997b; Kazlauskas &Bornscheuer, 1998). Lipase can also be used to accelerate the degradation of fatty waste (Masse, et al., 2001 and Nunes et al., 2014).

Filamentous fungi are known to be good lipase producers and numerous fungal enzymes are utilized in various food industrial processes (Treichelet al., 2010 and Nunes et al., 2014). Since lipases produced by filamentous fungi are mainly extracellular, their extraction and purification are relatively easy. This reason may also contribute to the fact that fungal lipases belong to the most important groups of commercial enzymes. Several factors determine the lipase production of filamentous fungi in a culture medium; often the presence of an inducer (mostly oil) and the appropriate physiological parameters such as pH, temperature and oxygen levels are the most important factors according to Aravindan et al. (2007). However, to achieve the best yield for extracellular enzyme production, proper selection of the cultivation conditions is even more essential. More so, a single lipase showing

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various distinct biochemical properties can be obtained if different fermentation procedures are applied for the same fungus (Mateos et al., 2006).

It is worthy of note that several work has been done on the use of microorganisms in production or in determining the lipase production ability of microorganisms. This work is therefore aimed at comparing the lipolytic activity of fungi species and some Lactobacillusspecies isolated from some Nigeria local foodstuffs.

Materials and Methods

Sample collection

Maize and groundnut samplesinfested with fungiwere bought from a local market in Calabar, Southern, Nigeria and placed in a black polyethylene bag kept inside a cooler with ice packs and taken to Microbiology Laboratory, University of Calabar, Calabar. Fungal strains were isolated by spread plate technique using 1g of the infested food samples diluted in 99 ml of 0.1% peptone water solution and then milled in a stomacher for 2minutes. A 10-fold serial dilution with peptone solution was performed and 0.1ml of each diluted sample was transferred into duplicate Potato Dextrose Agar (PDA) plates supplemented with chloramphenicol (0.5mg/l) before being spread with a glass spreader and incubated at room temperature for at least 3-5days. The cells were counted and the colonies which showed different appearances were picked up for purification and then transferred toSabourad's Dextrose Agar (SDA) plates for the estimation of fungal load(Padmapriyaet al.,2011; Das and Pharm 2013; Khanomet al., 2013; Rana et al., 2014).Four yoghurt samples were bought from local shops in Calabar town, South Eastern Nigeria and screened for Lactobacillus species using theMRS agar. Lactobacillus isolates were subjected to some biochemical tests and furtheridentified using the API 50 CH test.

Isolation and Identification of Fungi and Lactobacilli species

The isolates from the samples were examined for morphological and biochemical characteristics according to Bergy`s Manual of determinative Bacteriology. The fungal isolates were coded as follows: G1, G2, G3…G8 and M1, M2, M3…M8 according to the substrates (groundnut (G) andmaize (M)) from where they were isolated whereas L1, L2 and L3 represents the Lactobacilli species isolated from yoghurt. All Gram positive and catalase negative isolates were identified using the API 50 CH test kit. Pure cultures of the lactic acid bacteria isolates were grown in MRS broth, their purity were checked before centrifugation to harvest all bacteria inoculum. These were then prepared in appropriate medium (API 50 CHI medium) and used immediately for identification. Broth cultures of the isolates were distributed into the 50 tubes of the API-test kit using a sterile micropipette. Care was taken to prevent the formation of bubbles. The test strips were incubated at 37^{o}C for 48hours and the results interpreted and recorded as a positive (+), negative (-) or doubtful (d).

Lipase production using Egg Yolk Emulsion and Nutrient Agar

Two eggs weighing about 50grams each were washed and soaked in 75% ethanol for 1hour to test for sterility. The eggs were cracked and the albumen carefully extracted to retain the yolk for the preparation of egg yolk emulsion. Equal volumes of yolk and sterile 0.85% saline were mixed and stared. Nutrient agar (NA) was then prepared at 14g NA in a 500ml distilled water and sterilized by autoclaving at 121^{o}C for 15mins. A 40mls volume of yolk emulsion was then added to the 500mls distilled water and NA mixture and shake gently, it was allowed to cool to about 45-48 ^{o}C. The preparation was aseptically poured into petri dishes and allowed to solidify. Thereafter, it was incubated for 12hours to confirm its sterility. After the 12-hour incubation, fungal isolates coded G1, G2 G3…G8 and M1, M2, M3…M8 were then inoculated and incubated at room temperature for 72hours andLactobacillus isolates coded L1, L2 and L3 were also incubated using anaerobic jar with strict adherence to microbiological methods. Opaque formation indicates lecithinase production whereas clear zone around the colonies indicates the production of lipase.

Determination of lipase activity using submerged fermentation

This was carried out in a culture rotary shaker incubator using an inoculation medium made from glucose (10.0g/L), peptone (20.0 g/L), sodium chloride (5.0 g/L), yeast extract (5.0 g/L), with pH 6.0. The production medium consists of the inoculation medium supplemented with salt solution. The salt solution was prepared from potassium hydrogen phosphate (2.0 g/L), sodium hydrogen phosphate (6.0 g/L), magnesium sulphate (3.0 g/L), ammonium sulphite (5.0 g/L), and calcium chloride (3.0 g/L).

The organisms that exhibited lipase production were subjected to submerged fermentation for the production of lipase enzyme. The spore suspension was prepared from the 72-hour culture by adding 5.0mL sterile water. A 5.0 mL spore suspension mixed with 45.0 mL of the inoculation medium was poured into a 250 Erlenmeyer flask. The total contents were incubated in a rotary shaker at 30 ºC for 48 hours. 10% inoculum was added with 45.0ml of production medium. It was incubated at 30 ºC for 7 days. At the end of 7 days fermentation, the biomass was treated with 50 ml of distilled water and stirred well for the extracellular Lipase to solubilize in aqueous media. After that it was filtered by muslin cloth. Residue was again treated with 50.0 mL of water and filtered. The filtrate was centrifuged at 4000 rpm for 60 minutes. The clear supernatant was taken as enzyme source (Sundar and Kumaresapillai, 2013). This method was used same for all the coded organisms (G4, G5, M3 M4 and M8) that showed lipolytic activity.

Lipase activity assay

Volumetric analysis was used to conduct the lipase assay activity, with olive oil as the substrates emulsion. 63.0 mL of emulsifying reagent was added to 27.0 mL olive oil and homogenized for 5minutes. In a conical flask 1ml of the substrate emulsion was added

with 0.8ml of 0.2M potassium phosphate buffer (pH 7.0). Thereafter 0.2ml of enzymatic extract was added. The whole contents were then incubated 28℃ for 10minutes. The reaction was brought to conclusion by the addition of 2ml of the mixture of acetone ethanol (1:1 v/v). Using phenolphthalein as indicator, the total contents were titrated against 0.05N Sodium Hydroxide (Awan et al., 2003; Sundar and Kumaresapillai, 2013). The end point was light pink in colour.

Calculation of lipase activity

The lipase activity for all the isolates that showed lipolytic activity were calculated using the formula shown below

$$\text{Lipase activity} = \frac{\Delta V \times N}{V_{(Sample)}} \times \frac{1000}{60}$$

Note that;

$\Delta V = V2 - V1$ (change in volume), V1= Volume of NaOH used against control flask, V2= Volume of NaOH used against experimental flask, N= Normality of NaOH, V (Sample) = Volume of enzyme extract

Extracellular lipase activity is measured in units per ml (U mL^{-1} or U/ml)

Results and Discussion

In the process of screening for both the fungi and Lactobacilli, a total of 19 isolates were obtained(Table 1).Among them,16 of which were fungi and 3 lactobacilli. The results showed that out of the sixteen (16) fungi isolates screened for lipolytic activity, five (G4, G5, M3, M4 and M8) were positive for zone of clearance, eight (G1, G2, G7, G8, M1, M5, M6 and M7) were positive for production of opaque precipitates and the remaining three (G3, G6 and M2) isolates showed normal growth without any activity. Whereas, for the Lactobacilli isolated, all the three species screened were positive for zone of clearance. The zones initial clearance observed is probably due to the immediate effect of free acid released onto the agar and on further incubation, the clearance zone turned a shade darker than the background and the completely change the whole plates which may be due to over release of acid. The zone of intensification could clearly be observed from the three lactobacilli plates and are uniquely the same as the result of Alder, (1952); Gillipiand Alder, (1952);Padmapriyaet al., (2011) and Sundar andKumaresapillai (2013)

With strict adherence to microbiological and biochemical analysis, the positive species were identified as G4 (Penicilliumspp),G5 (Rhizopusspp), M3 (Aspergillusniger), M4 (Fusariumoxysporium), M8 (Fusariummoniliforme), L1(Lactobacillusplantarum),L2 (Lactobacilluscasei), and L3 (Lactobacillusfermentum).

From the submerged fermentation procedure done, the results for all the isolates for zone of clearance were then used for enzyme activity with their various activities recorded in Table 2. Comparing the enzymatic activity of the fungi isolates, Penicilliumspp has the highest activity and lastly followed by Fusariumoxysporium. For the Lactobacilli isolates, Lactobacillusfermentum has the highest activity followed lastly by Lactobacilluscasei. These results are in accordance with the results of Padmapriya et al (2011); Sundar andKumaresapillai (2013).Comparing the activity of the Aspergillusniger, it is in conformity with the results of Sundar & Kumaresapillai (2013) which showed that Aspergillusnigerenzyme has a moderate enzyme activity and have more lipase activity than those of the bacterial group.Comparing the lipolytic activity of the fungi and Lactobacilli, it is shown clearly that fungal enzymes have more lipase activity than those of the bacterial group with the highest activity exhibited by Penicilliumspp.

Table 1: Results of Zones of clearance for some Lactobacillus and fungal isolates

S/N	Isolates	Zone of Clearance (cm)	Degree of opacity
1	G1	_	++
2	G2	_	++
3	G3	_	_
4	G4	3.5	_
5	G5	1.9	_
6	G6	_	_
7	G7	_	++
8	G8	_	+++
9	M1	_	++
10	M2	_	_
11	M3	2.1	_
12	M4	2.0	_
13	M5	_	+
14	M6	_	++
15	M7	_	++
16	M8	2.6	_
17	L1	1.5	_
18	L2	2.4	_
19	L3	2.0	_

+….positive, _ ….negative

Table 2: Enzyme production by microbial isolates using submerged fermentation method

S/N	Microbial Isolates	Lipase activity (µmol/min/ml)	Zone of Clearance (cm)	Degree of opacity	Probable organism
1	G4	11.25	3.5	_	Penicillium spp
2	G5	2.92	1.9	_	Rhizopus spp
3	M3	3.75	2.1	_	Aspergillus niger
4	M4	2.92	2.0	_	Fusarium oxysporium
5	M8	6.25	2.6	_	Fusarium moniliforme
6	L1	1.2	1.5	_	Lactobacillus plantarum
7	L2	0.7	2.4	_	Lactobacillus casei
8	L3	1.9	2.0	_	Lactobacillus fermentum

_….negative

The lipolytic activity demonstrated by the isolates coded G4, G5, M3 M4, M8, L1, L2 and L3 in egg yolk agar after incubation for 5-7days.

Key G1-G8 = Fungal isolates from groundnut;M1-M8 = Fungal isolates from maize;L1-L3 = Lactobacillus isolates from yoghurts

CONCLUSION

The high innate spore forming ability of the fungi may probably be the mainstay of its high lipase activity. Thus, microbial enzymes from fungi and likely spore forming organisms are recommended and considered as a good option to source for lipase degrading enzymes for the purposes of environmental bioremediation and degradation of noxious fats in foodstuff.

Competing interests: The authors declare that they have no competing interests.

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