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Production of Microbial Enzymes from Ripe Plantain Fruits

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A B S T R A C T

Microbial enzymes have significant biotechnological application in industries. The purpose of this study was to isolate and identify microorganisms associated with ripe plantain fruits, determine the enzyme activity (lipase, protease, pectinase, cellulase and amylase) of the ripe plantain fruits samples and screen the isolated microorganisms for enzyme production. For isolation, standard isolation method was followed, while screening was done on different agar medium. Results revealed that. Day 5 of the fermentation period has the highest enzyme activity for all the enzymes in the fermented plantain fruit; lipase has the highest enzyme activity with a value of 1.3485 mg/mL/min while pectinase has the lowest with a value of 0.0014 mg/mL/min. *Bacillus* spp were screened positive for all the enzymes assayed for. Microorganisms were identified according to colony morphology and microscopic observation. This study contributes to catalogue of microorganisms that has been identified as enzyme producers and provides additional information to support future research about the industrial potential of these microorganisms that may produce enzymes and other metabolites of industrial importance.

Introduction

Plantain (*Musa paradiasiaca*) is an important staple starchy food in Nigeria, which belong to the family of Musaceae. It is a monocotyledonous, perennial fruit cultivated in the tropical and sub – tropical regions of the world (Stresses *et al.*, 2006). It is highly perishable fruit, which can be available all year round. The large quantity of plantains provides the potential for industrial use. Plantain fruits are a great source of lignocellulosic biomass which is renewable, chiefly unexploited and inexpensive. It has a lot of nutritional benefits accounting to its high demand in the market. They are used as food, beverages, fermentable sugars, medicines and flavouring (Nelson *et al.*, 2006). Plantains contribute to a healthy and balanced diet, providing a rich source of

vitamins and mineral to fuel the body. Plantain is composed mainly of water (around 75 percent) and carbohydrates (32percent). Carbohydrate in plantain is made up of the sugars and starches. The sugars and starches that make up this fraction are present in varying concentrations according to the state of the fruit's ripeness (FAO, 2014). The major change during the ripening process of plantain is the conversion of starch to sugar.

Many microorganisms such as bacteria, mould, and yeast produce a collection of multipurpose enzymes with extensive diversity of structures and industrial applications. Many microbial enzymes, such as amylases, cellulases, lipases, pectinases and proteases extracellularly produced. Amylases, starch-degrading enzymes, have numerous biotechnological applications. These enzymes are used in textile and garments, paper industries, starch liquefaction, food, adhesive and sugar production and pharmaceuticals (Bajpai *et al.*, 1989). Cellulases, sugar degrading enzymes are used in textile industry for bio-polishing of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness (Hill *et al.,* 2006). Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juice and in baking, while de-inking of paper is yet another emerging application (Sangrila *et al.,* 2013).

Lipases, lipid degrading enzyme are versatile tool for biotechnology. It is applicable in multiple industries such as agrochemical, pharmaceutical, cosmetic and perfume, taste and flavor industries, textile, food and dairy, detergent and surfactant industries, fat and oil, leather and paper production, chemical and waste water treatment *et al.,* . Especially lipases are applied for biodiesel production (Hasan *et al.,* 2006). Pectinases a group of enzymes that contribute to the degradation of pectin by various mechanisms. Acidic pectic enzymes are widely used in the production and clarification of fruit juices and wines (Rachana, 2017).

They are also very important in maceration and solubilization of fruit pulps. Alkaline pectic enzymes have been used in several areas, including retting and degumming of fiber crops, textile processing, coffee and tea fermentations, paper and pulp industry, and oil extraction (Kiro, 2010). Proteases, enzyme which catabolizes protein by hydrolysis of peptide bonds are generally used in detergents, food industries meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds. They also have medical pharmaceutical applications (Hamid *et al.,* 2008). The increase in world enzyme demand has led to sourcing for alternative substrate for the production of microbial enzyme; hence, agricultural wastes are readily accessible around the world as residual wastes for the production of these enzymes.

Materials and Methods

Collection of sample

Unripe mature plantain fruits (*Musa paradiasca*, Linn) were obtained from a farmland in Arigidi Akoko, Ondo State, Nigeria. The plantain fruits were kept in a sterile air tight polythene bags and transported to the Microbiology Postgraduate Laboratory, Federal University of Technology, Akure for further analysis.

Ripening of plantain

Unripe plantain fruits were made ripened naturally for 9 days.

Preparation of plantain fruits

The ripened plantain fruits were washed under running water to remove dirt, peeled and grinded using an electric blender (Model QBL-18L40). The grinded plantain fruits were divided into two portions; A and B. Portion A was left unfermented while Portion B was fermented for 5 days.

Isolation of bacteria and fungi from samples

Serial dilution was done to reduce the microbial population. One millilitre (1 ml) aliquot of the fermented sample and 1 g of the unfermented sample were separately transferred into 9 ml of sterile distilled water in a test tubes. At every 24 hours, samples were aseptically withdrawn from the fermentors, serially diluted. A stepwise serial dilution was carried out until the required dilution was obtained. A 1 ml aliquot (10^{-6} and 10^{-4} dilution factors for bacteria and fungi isolation respectively). Nutrient agar (NA), nutrient broth (NB), Potato dextrose agar (PDA), Potato dextrose broth (PDB) were prepared according to manufacturer's specification for the isolation of bacteria and fungi. Nutrient agar and potatoes dextrose agar was prepared for bacteria and fungi respectively. The media after cooling down were aseptically poured in Petri dishes under laminar flow hood using pour plate technique and allowed to solidify. Samples were aseptically introduced into the Petri dishes using sterile pipette. The plates were later incubated at 37^0C for 24 hours for bacteria and 25^0C for 72 hours for fungi. After 24 hours of incubation, the plates were examined for bacteria. Colonies and spore forming units formed on the media were counted and sub-cultured on freshly prepared nutrient agar media to obtain pure culture of bacteria. Thereafter, the plates were incubated at 37^0C for 24 hours. The pure isolates were stored temporarily on slants and kept at 4^0C for further use (Fawole and Oso, 2007), while after 72 hours of incubation, the plates were examined for fungi on potato dextrose agar. Isolates were sub-cultured on freshly prepared potato dextrose agar media to obtain pure culture. Thereafter, the plates were incubated at 25^0C for 72 hours (Cheesbrough, 2010). The Bacteria isolate were observed using microscopy, Gram staining, sugar fermentation test, biochemical tests such as urease test, catalase test, citrate utilization test and indole test while the morphological, cultural and microscopy identification of fungi isolates was examined based on the colour, types and shapes of spores, conodia and hyphae; further stained with two drops of lactophenol-cotton blue dye and viewed under light microscope (Fawole and Oso, 2007).

Enzyme activity

The enzyme activity of both the unfermented and fermented ripe plantain fruits sample was determined using the techniques for cellulase, protease, lipase, pectinase and amylase respectively according to the method of (Bernfeld, 1951; Miller, 1959; Ladd and Butler, 1972; Mandels *et al.*, 1976; Maia *et al.*, 1999)

Microbial screening for enzyme production

Culture media specific to each enzyme were used for primary screening of enzymes production by following the methods for cellulase, protease, lipase, pectinase and amylase respectively according to the method of (Hankin, *et al.*, 1971; Akpomie *et al.*, 2012; Lisdiyanti *et al.*, 2012; Saowapar *et al.*, 2014; Hnin *et al.*, 2015; Toshi and Sudhir, 2017).

Statistical analysis

The experimental design was done in triplicate. The data obtained were subjected to one way analysis of variance (ANOVA) SPSS

version 2.0. Differences were considered significant at P<0.05.

Results and Discussion

Microbial isolation and identification

The total bacterial counts (10^6 cfu/ml), fungal mean counts (10^4 sfu/ml) is shown in Figure 1 and 2. The total bacterial count in the unfermented sample was 10.21, while that of fungi was 16.00. The total bacterial counts for the fermented sample ranged from 13.11 to 38.01, while the range for fungi was 21.22-38.00. The identified bacteria isolate from ripe plantain fruits during fermentation include; *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Lactobacillus plantarum, Leuconostoc mesenteriodes, Streptococcus feacalisand Lactobacillus fermentum.* The Moulds identified during fermentation include; *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum. Saccharomyces cerevisae*and *Candida utilis* were the yeasts isolated during fermentation as presented in tables 1, 2 and 3 respectively.

Enzyme activity and microbial screening for enzyme Production

The enzyme activity (mg/ml/min) of the unfermented sample and the fermented plantain fruit sample is represented in figure 3. The highest cellulase activity was on day 5 (0.6940) of the fermentation period. The enzyme activities for all the enzymes assayed for were low in the unfermented sample. Among all the assayed enzymes, lipase showed the highest enzyme activity. All the isolated microorganisms exhibited one or more enzymatic activity. *Bacillus* spp exhibited enzyme activity to all the enzymes. *Bacillus* spp were the only bacteria that exhibited pectinase activity, while all the fungi except *C. albicans* and *Penicillumi notatum* exhibited pectinase activity. All the isolate exhibited amylase activity (Table 5).

The lignocellulosic structure of plantain fruits may be a factor responsible for the low microbial population in the unfermented sample (Agbor *et al.,* 2011; Akhtar *et al.,* 2012). The presence of bacteria such as *B. cereus,* and *B. subtilis* in the unfermented sample agrees with the findings of Oriola *et al.,* (2017) when plantain fruits were fermented for 3 days during production of "Agadagidi." The presence moulds such as *A. niger, A. flavus* and *P. notatum* in the unfermented sample could be a result of them being plantain fruits microflora which agrees with the finding of Oriola *et al.*, (2017) when *A. flavusA. fumigatus* and *P. notatum* were isolated from uncrushed plantain fruits. The dominant population of *A. flavus* particularly in the unfermented sample may be due to the presence of nutrients available within the plantain fruits for utilization. Nasrin *et al.*, (2017) attested to this when combination of molasses and jackfruit were used as a substrate for mutant strain of *A. niger* for citric acid production. Slightly acidic environment of the plantain fruts may be responsible fortheir adaptation (Murali *et al.,* 2017).

Lactobacillus spp are vital organisms for fermentation process which may be responsible for their presence from during fermentation. Kalui and co-researchers (2010) documented in a review that these bacteria are essential in spontaneously fermenting food and that these microorganisms produce lactic acid as an important product from the energy yielding fermentation of sugars. These conditions created by *Lactobacillus* spp could favour the growth of fungi particularly on day 4 and beyond, thus, Fungi, *Lactobacillus* metabolise sugars within the plantain fruits which is converted to organic acids.

Table.1 Morphological and biochemical characteristics of bacteria isolated during fermentation of ripe plantain fruits

	A	B	C	D	E	F	G	H
Colour	Cream	Pale yellow	White	White	Yellow	Cream	White	White
Shape	Rod	Cocci	Rod	Rod	Cocci	Rod	Cocci	Rod
Edge	Entire	Entire	Irregular	Irregular	Entire	Irregular	Entire	Irregular
Elevation	Flat	Raised	Flat	Flat	Raised	Circular	Circular	Circular
Surface	Rough	Smooth	Smooth	Rough	Smooth	Smooth	Smooth	Smooth
Gram	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Coagulase	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Spore	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve
Motility	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Oxidase	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Indole	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
MethylRed	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Urease	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Starch	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve
Glucose	AG	AG	-	A	-	AG	AG	AG
Sucrose	-	A	A	-	-	-	AG	-
Maltose	A	A	AG	A	-	A	AG	AG
Lactose	AG	A	-	-	A	AG	AG	AG
Fructose	-	A	AG	A	A	A	AG	AG
Galactose	AG	AG	AG	-	-	AG	A	A

Key: A – *Escherichia coli* **B -** *Staphylococcus aureus* **C –** *Bacilus subtilis* **D –***Bacilus cereus* ***E*** *-* *Streptococcus faecalis* **F–***Lactobacilus plantarum* ***G–****Leuconostoc mesenteriodes* *H—Lactobacillus fermentum*

A = Acid present and gas absent, AG = Acid and gas present, -ve = Negative, +ve = Positive, - = Absent

Table.2 Morphological and biochemical characteristics of yeast isolated during fermentation of ripe plantain

	A	B
Cultural Characteristics		
Colour	Cream	Brown-black
Size	Medium	Medium
Surface	Smooth	Smooth
Shape	Oval	Cylindrical
Spore	+	+
Mycelium	-	-
Sugar Fermentation		
Glucose	FA	FA
Maltose	FA	FA
Lactose	A	-
Fructose	FA	FA
Sucrose	FA	FA
Galactose	FA	-

Key: A – *Saccharomyces cerevisiae* B - *Candida utilis*

A = Acid, FA = Fermentation and Assimilation, - = Absent, + = Present

Table.3 Morphology and microscopy characteristics of mold isolates during fermentation of unripe plantain fruits

Isolates	Cultural Characteristics	Spores/Conidia arrangement under the Microscope	Identity of Isolates
A1	The surface is rough. Spores are granular flat, often with radial grooves yellow at first but quickly become bright yellow green with age. Sizes of colonies are medium.	Conidia are globose to sub globose with pale green colour. Septate hyphae with long condiophore bearing the conidia	*Aspergillus flavus*
A2	The surface is smooth with brown mycelia growth. With whitish colonies large in size.	An upright conidiopore that terminates in a swelling, bearing phialides at the apex radiating from the entire surface. Conidial are one celled and densely packed. Spores are black.	*Aspergillus niger*
A3	The surface is smooth. Dark greenish colour with white boundaries on plates.	Broom like structure with condioshpere bearing the conodia	*Penicillium notatum*

Table.4 The occurrence of bacteria and fungi during fermentation

	Fermentation Period (day)					
	Unfermented Sample	**1**	**2**	**3**	**4**	**5**
Escherichia coli	+	+	-	-	-	-
Staphylococcus aureus	+	+	-	-	-	-
Bacillus subtilis	+	+	+	-	-	-
Bacillus cereus	+	+	-	-	-	-
Streptococcus faecalis	-	+	+	+	-	-
Lactobacillus plantarum	-	-	+	+	+	+
Lactobacillus fermentum	-	-	-	+	+	+
Leuconostoc mesenteriodes	-	-	-	+	+	+
Saccharomyces cerevisiae	+	+	+	+	+	+
Candida utilis	+	+	+	+	+	+
Aspergilus flavus	+	-	-	-	-	-
Aspergilus niger	+	+	-	-	+	-
Penicillum notatum	+	-	-	-	-	-

Table.5 Microbial screening for enzyme production

Isolates	**Amylase**	**Protease**	**Lipase**	**Pectinase**	**Cellulase**
Lactobacillus fermentum	+	+	-	-	-
Bacillus subtilis	+	+	+	+	+
Bacillus cereus	+	+	+	+	+
Staphylococcus aureus	+	-	+	+	-
Escherichia coli	+	+	+	-	-
Lactobacillus plantarum	+	+	+	-	-
Leuconostoc mesenteriodes	+	+	+	-	-
Streptococcus feacalis	+	+	+	-	-
Saccharomyces cerevisiae	+	+	+	+	+
Candida albicans	+	+	+	-	-
Aspergillus flavus	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Penicillium notatum	+	+	-	-	+

Legend: + Positive, - Absent

Figure.1 Bacterial counts of sample during fermentation

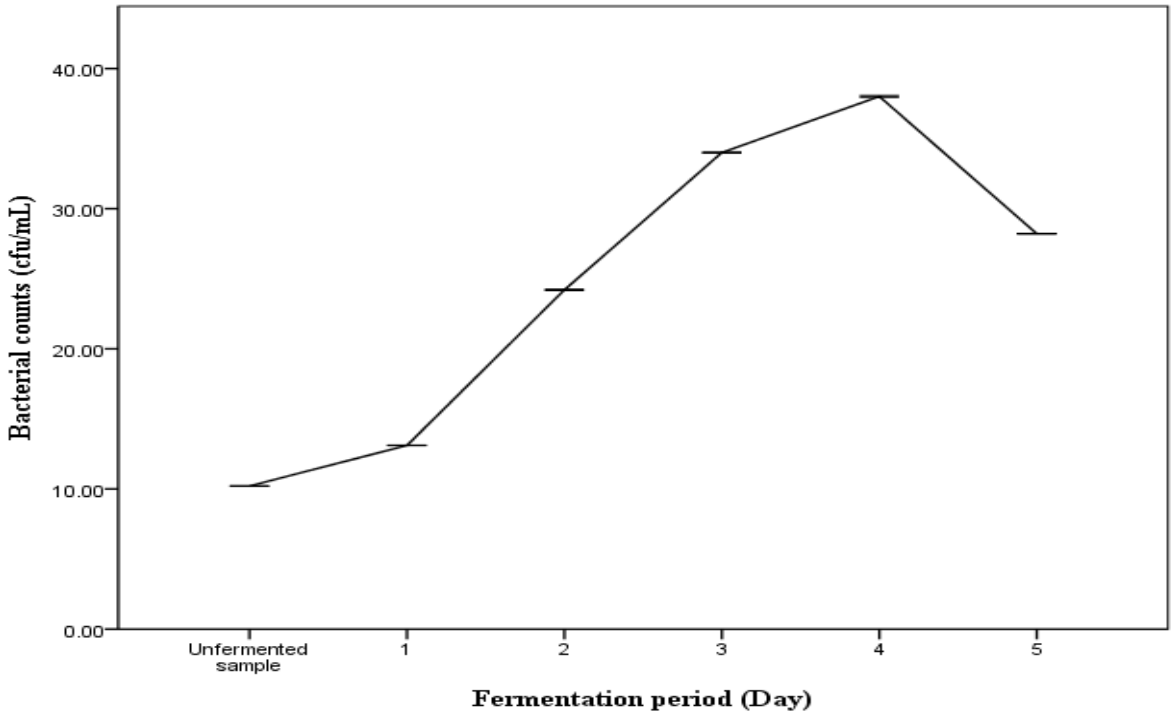


Figure.2 Fungal counts of sample during fermentation

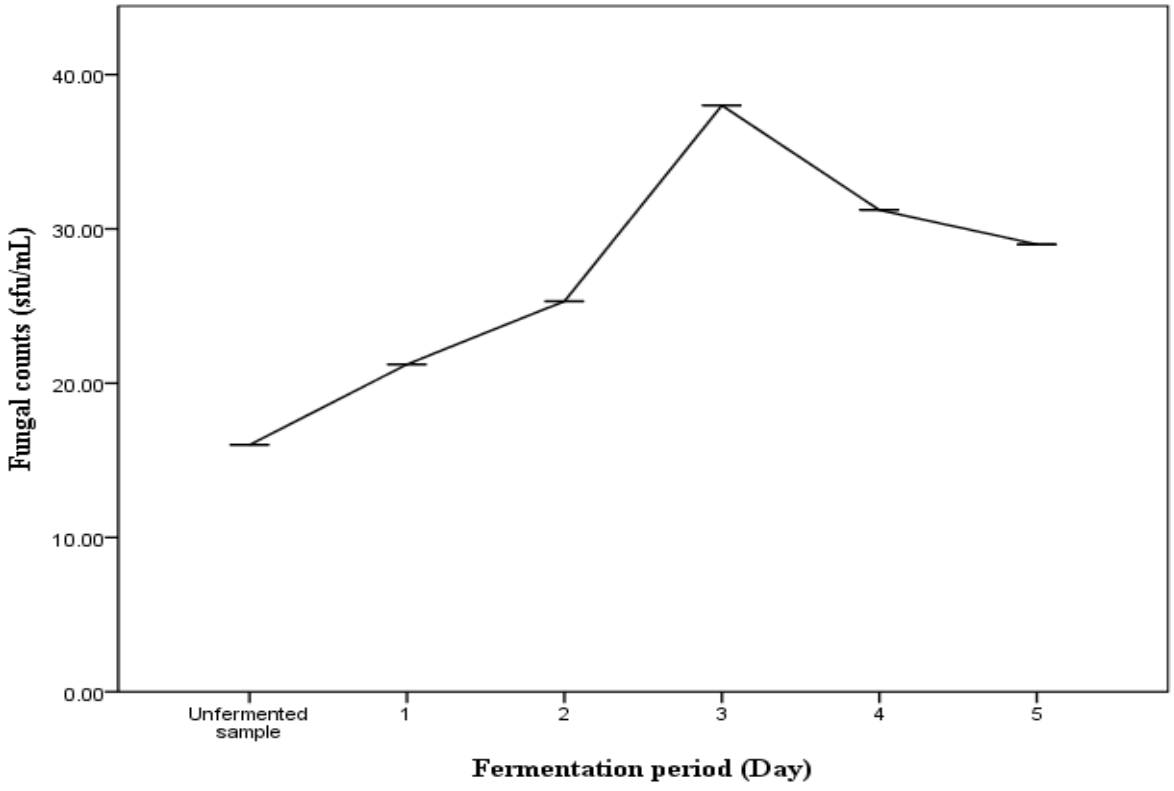
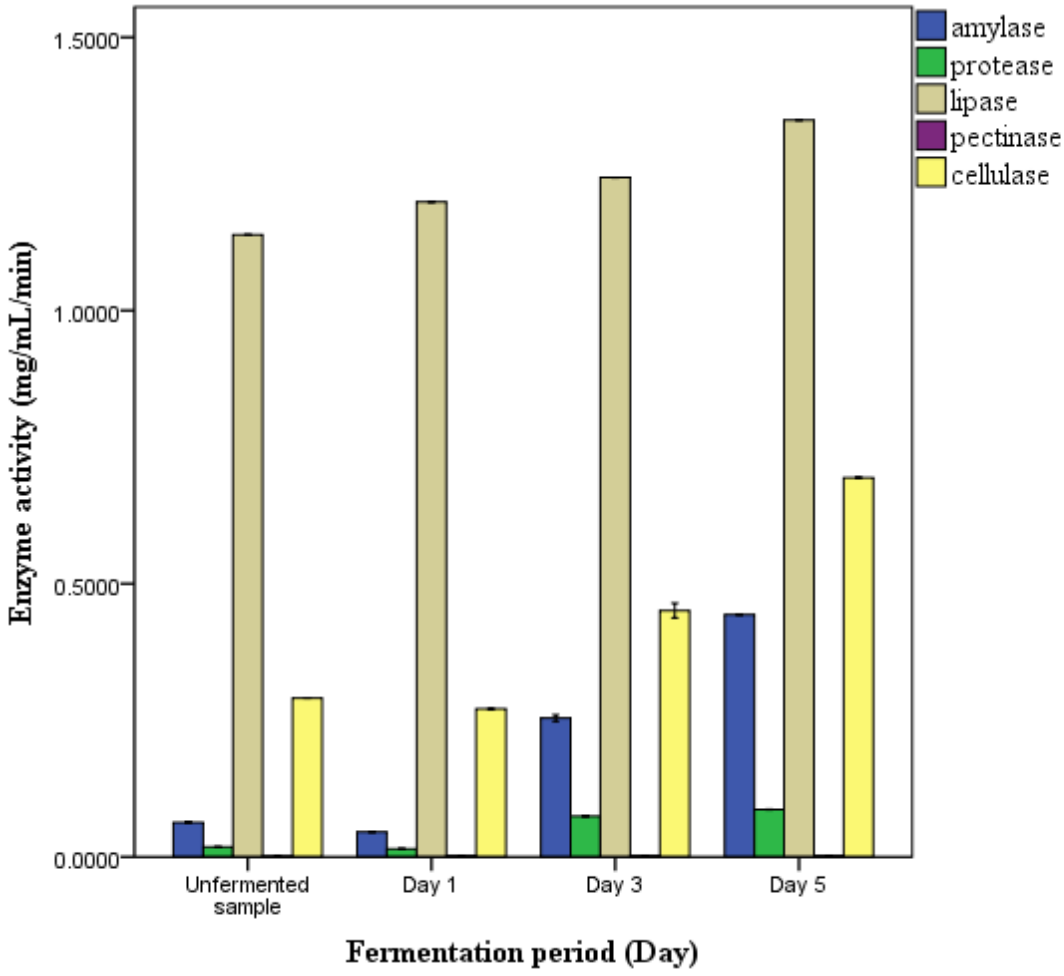


Figure.3 Enzyme activity of sample during fermentation



The growth and population of other microorganisms maybe due to the creation of enabling environment; which favour their survival and development of these set of microorganisms. These conform to the report of Ogbonnaya and Chukwu (2012).

Saccharomyces spp, could utilize the sugars present within the plantain fruits for the facilitation of the fermentation process in order to accumulate ethanol in the presence of oxygen particularly during early days of degradation period (Walker *et al.,* 2016; Alonso-del-Real *et al.,* 2017). However, *S. cerevisiae* becoming dominant on day 4 and 5 may be due to the environmental conditions that facilitate their survival, growth, development and proliferation. *Candida* spp, yeast catabolizes sugars into carbon dioxide in the presence of oxygen may create an enabling environmental condition which may have facilitated the occurrence of *S. cerevisae* during fermentation (Azhar *et al.,* 2017).

The availability of light may be responsible for the growth of *A. niger* and *A. flavus* particularly in the unfermented sample, fermented sample of day 4 which also agrees with the findings of Shehu and Bello while studying the effect of environmental factors on the growth of *Aspergillus* spp. associated with stored grains (Shehu *et al.,* 2012). Also,

as the fermentation period increases, relative humidity and light intensity decreases. Thus, these factors could be applicable to bacteria and yeast that grew on the unfermented ripe plantain fruits.

Ripe plantain fruits could serve as a fermentation media for the production of enzymes. The production of enzymes by microorganisms in the fermentation media depend on the availability of suitable and utilizable substrate (Adeleke *et al.,* 2017). More so, Rashid *et al.,* (2016) concurred that major and minor elements contained within a substrate can be fermented or synthesized by microorganisms using various enzymes. Also, the capacity of microorganisms to produce extracellular enzymes is influenced by environmental conditions such as temperature, pH, aeration, inoculums age and the presence of inducer or repressor substrates (Nigam, 2013). Solid state fermentation for the production of enzymes offers advantages over the conventional method of submerged fermentation (Cruz *et al.,* 2013). Submerged method of fermentation used may also be attributed to the low enzyme activities (Colla *et al.,* 2015).

The low amount of enzyme activity of all the enzymes assayed for particularly in the unfermented sample may be due to complex structure such as cellulose, hemicellulose and lignin which is important for its utilization and digestibility (Tejado *et al.,* 2007; Cao *et al.,* 2012; Li *et al.,* 2016; Murali *et al.,* 2017). The progressive increase in enzyme activities may be due to the hydrolytic (water) effect on the plantain fruits which may increase the surface area and remove hemicellulose. This is in agreement with the findings of Rodolfo (2014) that water treatments at elevated temperatures (200-230°C) and pressures can increase the biomass surface area and remove hemicellulose. The enzyme activity of the plantain fruits may have increased if it temperature and pressure were raised. The presence of cellulase in plantain fruits agrees with the separate reports of Sethi *et al.,* (2013) and Philip *et al.,* (2016) which states that enormous amounts of agricultural, industrial and municipal cellulose wastes contains cellulose. The increased cellulase activity at the early period of fermentation could be due to the reduced amount of disaccharide cellobiose which could be present within fermented plantain fruits which seems to be a more potent inhibitor of cellulase (Gao *et al.,* 2016). Payne *et al.,* (2015) documented that a large number of compounds such as glucose, mannose, galactose, xylose, ethanol and various ions can act as possible inhibitor for cellulase.

The unfermented sample offered reduced accessibility to cellulose and hemicellulose and degradability for enzymatic or chemical action which agrees with the findings of Barakat *et al.,* (2014) and Gao *et al.,* (2016). The presence of *Bacillus* spp. may contribute to the production of cellulase in the plantain fruits during fermentation. Separate findings of Akhtar *et al.,* (2012) and Saowapar *et al.,* (2014) agrees with this finding when different species of *Bacillus* produced cellulase.

Aspergillus niger,and *A. flavus* that were positively screened for cellulase production agrees with the separate findings of Jabasingh (2011), Liu *et al.,* (2011) and Amorea and Faracoa (2012) that *A. acculeatus, A. fumigatus, A. niger* were producers of cellulase. Yeasts such as *S. cerevisae* and *Candida* spp have shown capacity to produce cellulase (Maki *et al.,* 2009). *Lactobacillus* spp being protease producers agrees with the finding of Hnin *et al.,* (2015) that proteolytic activity is an important characteristic of lactic acid bacteria. *Bacillus* spp production of protease agrees with the findings of Hamza and Woldesenbet (2017) when *Bacillus* sp. Cab44 was observed to hydrolyse casein.

Oyeleke *et al.,* (2010) documented that *A. flavus* and *A. fumigatus* were able to produce extracellular protease. Oyeleke *et al.,* (2010) observed that when *A. flavus* and *A. fumigatus* were subjected to the same temperature of 30^0C, *A. flavus* was able to produce the highest amount of protease. Fungi are better producers of pectinase when compared to bacteria based on the findings of this research. This agrees with different documentations of (Raju and Divakar 2013; Roosdiana *et al.,* 2013; Kavuthodi *et al.,* 2015 and Reddy *et al.,* 2016) that most of the Bacterial isolates (mostly *Bacillus* spp and *Pseudomonas* spp) such as; *Pseudomonas fluorescence* and *B. subtilis*, *Bacillus* sp. MFW7, *B. cereus* and *Staphylococcus aureus* were reported as good pectinase producers. *Bacillus subtilis* being a producers of pectinase agrees with the report of Mariam and Aruna (2017) that *B. subtilis* strain arium 1115 produced the highest quantity of extracellular pectinase out of the arrays of *Bacillus* spp assayed for.

All bacterial isolates were able to break down soluble starch. *Bacillu*s spp. were screened positive for all enzymes evaluated for. *Bacills subtilis* had the highest zone of clearance. The presence of these amylolytic bacterial in the soil agreed with an earlier report by Omemu *et al.,* (2005) as cited by Oyeleke *et al*.,(2010), that soil is known to be a repository of amylase. The bacterial isolates of *Bacillus* spp. also showed a high ability to secrete pectinase with low ability to secrete protease. Although, Rodarte *et al*.., (2011) reported that *Bacillus cereus* did not show positive result in protease qualitative test. *Staphylococcus aureus*in this study did not show the ability to secrete protease and cellulose, this did not agree with the findings of Appak (2006) who stated that *Staphylococcus* spp. produced lipases and proteases more than any other enzyme group.

The various *Bacillus* spp isolated from the ripe plantain fruit sample was the best producer of lipase based on the zone of hydrolysis. Lactic acid bacteria isolated from the plantain fruits during fermentation exhibiting lipase activity concurred with the documentation of Padmapriya *et al.,* (2011) that *Lactobacillus* spp is a producer of lipase. *Candida albicans* and *A. flavus,* screened positive for lipase production agrees with the documentation of Singh *et al.,* (2016) that *C. Antarctica, C. lipolytica* and *A. flavus* are producers of lipase.

Fungi are better producers of pectinase when compared to bacteria based on the findings of this research. *Bacillus* spp. isolated were indicated to be a producer of pectinase. This agrees with different documentations of Raju and Divakar (2013); Roosdiana *et al.,* (2013); Kavuthodi *et al.,* (2015) and Reddy *et al.,* (2016) that most of the Bacterial isolates (mostly *Bacillus* spp. and *Pseudomonas* spp.) such as; *Pseudomonas fluorescence* and *B. subtilis*, *Bacillus* sp. MFW7, *B. cereus* and *Staphylococcus aureus* were reported as good pectinase producers. *Bacillus subtilis* being a producers of pectinase agrees with the report of Mariam and Aruna (2017) that *B. subtilis* strain arium 1115 produced the highest quantity of extracellular pectinase out of the arrays of *Bacillus* spp assayed for. pH could also be a factor for low pectinase production in which acidic pH values were observed during the fermentation of the plantain fruits. According to the finding of Mariam and Aruna (2017), *B. subtilis* strain arium 1115 produced the highest amount of pectinase at pH 9 (21.44 U/mL). Temperature is another factor to consider for pectinase production. *Bacillus* species have demonstrated slightly higher temperature for pectinase production such as marine *B. subtilis* and *B. circulans* at 40°C (Joshi *et al.,* 2013; Raju and Divakar, 2013). However, findings show *B. cereus, B. firmus* I-10104, *B. cereus* and *B. endophyticus* and *B. coagulans* exhibited maximum pectinase production at 37°C (Namasivayam *et al.,* 2011; Aaisha and Barate, 2016).

Amylase activity in the plantain fruits during fermentation may be as a result of the presence of concentration of hemicellulose carbohydrate particular starch within the plantain fruits. This agrees with the findings of Singh *et al.,* (2012) when amylase and xylanase content of rice bran, corn cob, wheat bran, wheat straw, and sugarcane bagasse were described in relation with the composition of starch and hemicellulose. The progressive increase in amylase activity in the fermented plantain fruits could be due to the availability of microorganisms such as *B. cereus, B. subtilis, Lactobacillus* spp., *A. flavus, C. albicans,* and *S. cerevisiae* in the fermenting substrate. These consortiums of microorganisms may contribute to the high amylase production. Akpomie *et al.,* (2012) reported that *Bacillus* spp and *Lactobacillus* sp have ability to produce amylase. Hence, *Bacillus* spp. are known to be commercial producer of amylase. Progressive increase in temperature could also be another factor that led to the progressive increase in amylase production which may be a satisfactory condition that made the microorganisms to produce more amylase. Akpomie *et al.,* (2012) attested to this fact that gradual increase in amylase activity was observed from 26 ^{o}C to 45 ^{o}C and beyond this range it declined. Thus, temperature contributes to the factor responsible for the secretion of amylase.

It is concluded that microorganisms utilized ripe plantain fruits as substrate for production of some metabolites of industrial applications. Farmers needs to be oriented about the usefulness of plantain fruits for production of substances such as enzymes that are valuable in industries thus, can be applied for its biotechnological application in food, pharmaceutical and medical industries.

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