ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FOUND IN MAIZE SLURRY FROM SELECTED MARKETS WITHIN ABUJA

BY
SURAYYA ABDULLAHI
191211022

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DECLARATION

I, SURAYYA ABDULLAHI with matriculation number **191211022,** hereby declare that this project report titled **"Isolation and Identification of Lactic Acid Bacteria found in Maize Slurry from Selected Markets within Abuja"** was carried out by me under the supervision of **MORENIKE FADAYOMI**

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SURAYYA ABDULLAHI Date

CERTIFICATION

This is to certify that **SURUYYA ABDULLAHI** with matriculation number **191211022,** carried out this research work titled **"Isolation and Identification of Lactic Acid Bacteria found in Maize Slurry from Selected Markets within Abuja"** in partial fulfilment for the award of the degree of Bachelor of Science in **Microbiology,** under my supervision and the work has not been previously submitted either in part or full for the award of any other degree in this or any other University.

_____ _____

Morenike Fadayomi Date

Supervisor

_____ _____

Assoc. Prof. Sani S.D. Mohammed Date

Head of Department

_____ _____

External Examiner Date

DEDICATION

ACKNOWLEDGEMENT

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Abstract

The presence of Lactic acid bacteria in maize slurry sold in Abuja markets were investigated. This was carried out by using standard microbiological techniques. The aim of the study was to isolate and identify lactic acid bacteria from maize slurry sold in Abuja. Out of the four (4) samples collected two (2) were positive for lactic acid bacteria and also Escherichia coli was also isolate from the maize slurry sample collected. Proximate analysis reveals the presence of all the required contents such as protein, fibre, fat, carbohydrate, and moisture content. With carbohydrate content been the highest content in the maize slurry. The presence of all the content shows how nutritious the maize slurry is. Antibiotic sensitivity test was carried out using antibiotic impregnated multi disc containing ten different antibiotics. The antimicrobial susceptibility profile indicated that Lactic acid bacteria were found to be resistant to streptomycin and augmentin while susceptibility was seen among ciprofloxacin and Rifampin and amoxil. It is highly recommended that maize slurry should be prepared and handled under good sanitary and hygienic practices to avoid contamination.

Chapter One

Introduction

1.1 Background of the Study

Maize and it's uses

Maize (*Zea mays*) also known as corn is a cereal grain that is one of the staples widely grown and consumed in Nigeria. Maize is the third most widely cultivated food crop in Nigeria Maize as a crop is highly yielding, easy to process and readily digested. It is a versatile crop that grows on a cross range of agroecological zones (Faria *et al*., 2017).

There are principally two types of maize (white and yellow variety) that are produced in Nigeria. Maize is planted for its grains that are used for making flour or eaten as vegetable Maize can be eaten as whole grain when boiled or roasted. It can also be used in its prepared form as pap (maize porridge) and Eko (Agidi) which is an extracted starch meal obtained after the cooking (boiling) of a prolonged soaking and fermentation of maize slurry.

Fermentation of Maize

Maize slurry (*Akamu*) is a product of fermentation of maize (corn), millet or sorghum. Maize slurry *(Akamu*) can be processed into a whitish or yellow-like custard depending on the variety of maize used for the fermentation. *Akamu* is classified among the breakfast foods (Abiola *et al.,* 2017). It is the first native food given to babies at weaning. Preparation of *Akamu* involves the steeping of the maize grains for two days and wet milling thereafter. Water is added to the mash and is sieved through a clean cloth. The filtrate is allowed to sediment for a day and dewatered with a clean cloth sac. These are the typical stages of *Akamu* and do not include some of the stages of (Eka *et al*., 1999). Fermented foods are largely consumed in Africa where they constitute a bulk of diet. Majority of Nigerian fermented foods are products obtained though

lactic acid fermentation such as Eko (locust bean), fufu (mashed cassava or plantain), Iru (corn starch meal), Fermented cabbage, cucumber, pumpkin as well as Yoghurt, Palm Wine, Burukutu (fermented guinea corn) Kununzaki (fermented millet) etc (Osungbaro, 2009). Lactic acid bacteria are involved in the processing of Maize slurry from fermentation and equally during storage. The *Lactobacillus* species have been to be the major lactic acid bacteria that are present in maize slurry. These species include *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus amylovorus* while other lactic acid bacteria such as *Pedicoccus acidlactici* and *Pedicoccus pentasaceus* are also found in maize slurry (Onuorah et al., 2019).

Importance of Lactic acid bacteria

Lactic acid bacteria are usually found in decomposition of plants and lactic products and produces lactic acid as the major metabolic end product of carbohydrate fermentation. In other words, lactic acid bacteria are found in carbohydrate rich materials especially fermented foods. Lactic acid bacteria and other metabolic process contribute to the organoleptic and textural profile of a food item. The industrial importance of lactic acid bacteria is further evinced by their generally recognized as safe (GRAS) status, due to their ubiquitous appearance in food and their contribution to the healthy microflora of human mucosal surfaces (Farinde *et al*., 2015). The lactic acid bacteria produce a proteinaceous toxin known as bacteriocins. In food fermentation industry, bacteriocins is a global interest because they inhibit the growth of many spoilage and pathogenic bacteria and thus extend the shelf life of foods. Bacteriocins are typically considered to be narrow spectrum antibiotics, though this has been debated (Gadaga *et al*., 2008).

Fermented foods are more easily digestible over unfermented food. This is because there is a partial or complete hydrolysis of some substances present in the substrate which will enhance

their digestion (Falegan *et al.*, 2014). Fermented foods generally enrich the human dietary through development of a wide diversity of aromas, flavours, and textures in foods (Farinde *et al*., 2015).

1.2 Statement of the Research Problem

Maize slurry is a popular Nigeria fermented food that contains several fermentative organisms such as Lactic acid bacteria. These lactic acid bacteria sometimes exhibit antibacterial activity against bacteria and food borne pathogens while the also aid in the fermentation of the maize slurry. During the processing of the maize slurry, contamination may occur as a result of poor handling or hygiene which will result to an increased in the number of bacteria present in the maize slurry, which are easily noted by off taste, color change and off odor. Hence therefore, this research is aimed at isolating and characterizing of Lactic acid bacteria found in maize slurry from selected markets within Abuja.

1.3 Justification of the Study

Microorganisms found in maize slurry production occur through several namely; exposure, handling, and use of contaminated materials for preparation. The following genera of microorganisms are known to participate in the fermentation of maize slurry production. Includes, Staphylococcus, Escherichia, Pseudomonas, Enterococcus, Klebsiella, Streptococcus, Corynebacterium, Aspergillus, Saccharomyces and several species of the genu Lactobacillus are found in the fermentation of maize slurry production. Lactobacillus species have been the major lactic acid bacteria that are present in maize slurry. (Onuorah et al., 2019).

This research will explore the activity of the different lactic acid bacteria that are involves in the fermentation process of maize slurry purchased from different Markets located in Abuja.

1.4 Research Aim and Objectives

The aim of the research is to Isolate and characterize lactic acid bacteria found in maize slurry sold in markets within Abuja.

Objectives of this study are to:

i. Culture and isolate Lactic acid bacteria from different maize slurry samples sold within Abuja
ii. To characterize the isolated lactic acid bacteria from maize slurry.
iii. To carryout Proximate analysis on the Maize slurry samples collected from the market
iv. To carry out antibiotic sensitivity testing on isolates

1.5 Significance of the Study

Maize slurry has been one of the popular staple foods consumed within Abuja and its environs. It constitutes a major source of food for man especially the infants. Due to contamination by different bacterial during the processing and/or storage it has become a public health concern. The result of this research will reveal presence of lactic acid bacteria in maize slurry collected within Abuja.

Chapter Two

Literature Review

2.1 Overview of Maize

Maize (*Zea Mays*) is the most important cereal in the world after wheat and rice, with regard to cultivation areas and total production. The name maize is derived from the South American Indian Arawak-Carib word "Mahiz". It is also known as Indian corn or corn in America. It was introduced into Nigeria in the 16th Century by the Portuguese. The global production of maize is estimated to about 300million tonnes per year. 145million (or about 50 percent) of which are produced in USA alone. In Nigeria its production is quite common in all parts of the country, from the North to the South with an annual production of about 5.6million tonnes. The country's maize crop covers about 1million hectares out of nine million hectares it occupies in Africa (Jitngarmkusol *et al*., 2018).

Maize is prepared and consumed in a multitude of ways which varies from region to region is from one ethnic group to another ethnic group. For instance, maize grains are prepared by boiling or roasting as paste ('eko'), abado', and 'elekute' in Nigeria and 'kenke' in Ghana, or as popcorn which is eaten all over West Africa. Traditional methods of preparations and uses of maize are restricted to definite localities or ethnic groups. This trend was also noted in the traditional preparation and uses of cassava (*Manihot esculenta crantz, Euphorbiaceae*) (Onyerekua *et al*., 2019).

2.2 Uses of Maize

2.2.1 Human Food

Maize & cornmeal (ground dried maize) constitute a staple food in many regions of the world. Maize is central to Mexican food. Virtually every dish in Mexican cuisine uses maize.

One form of grain or cornmeal, maize is the main ingredient of tortillas, tamales, pozole, atole and all the dishes based on them, like tacos, quesadillas, chilaquiles, enchiladas, tostadas and many more. In Mexico even a fungus of maize known as *Huitlacoche* is considered a delicacy. Maize which is a major source of starch is a major ingredient in home cooking and in many industrialized food products. Maize is also a major source of cooking oil (corn oil), and of maize gluten. Maize starch can also be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, and sweetener; and also fermented and distilled to produce grain alcohol. Sometimes maize is used as the starch source of beer (Banigo *et al*., 2017).

2.2.2 Chemicals

Starch from maize can also be made into plastics, fabrics, adhesives, and many other chemical products. The corn steep liquor, a plentiful watery by product of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms (Plaami, 2017).

2.2.3 Medicinal Uses

A crop which is highly edible and nutritious as maize, also has some medicinal uses among the local people. It is used to cure many diseases, which it had over the years proved to be very effective.

These include:

The Water filtered through charcoal obtained from maize stalk can be used as a treatment to cure gonorrhea (Abdulrahman, 2016). While an infusion obtained from stigma of maize inflorescence can be used for treatment of diseases of the urinary tract or passage (Abdulrahman *et al*., 2016).

Maize is consumed in many forms in different parts of the world, from maize grits, polenta and corn bread to popcorn and products such as maize flakes. The grain is fermented to give ogi in

Nigeria and other countries in Africa and is decorticated, degermed and precooked to be made into arepas in Colombia and Venezuela (Herrero *et al*., 2016).

Maize is also widely used to make beers in Benin for example; malt is obtained by germinating the grain for about five days. The malt is then exposed to the sun to stop germination. The grains are lightly crushed in a mortar or on a grinding stone. The malt is cooked and the extract is strained off, cooled and allowed to stand. After three days of fermentation, it is ready to be drunk as beer. The lime cooking process for maize is particular to Mexico and Central America, although today technology has been exported to other countries such as the United States (Herrero *et al*., 2016). A dough prepared from lime-cooked maize is the main ingredient for many popular dishes such as atole, a beverage with a great variety of flavors, and tamalitos, made by wrapping the dough in maize husks and steam-cooking it for 20 to 30minutes to gelatinize the starch. This form is usually prepared with young chipilin leaves (*Crotalaria Longirostrata*) the flowers of loroco (*Fernaldia Pandurata*) or cooked beans mixed with the dough thus improving the nutritional quality of the product and its flavour (Faria *et al*., 2017). There are many other ways to convert maize into interesting and acceptable forms for human use (Abdulrahman, 2016).

2.3 Maize Slurry Production

Traditional process of making "ogi" has a number of slight variations described by several authors. "Ogi" is traditionally prepared in batches on a small scale of two or three times a week, depending on demand. The clean grain is steeped in water for one to four days to soften. Once soft, it is grounded with a grinding stone, pounded in a mortar or grounded with a power mill. The bran is sieved and washed away from the endosperm with plenty of water. Part of the germ is also separated in this operation. The filtrate is allowed to ferment for 24 to 72 hours to produce

slurry which when boiled gives the ogi porridge. “Ogi” is usually marketed as a wet cake wrapped in leaves, or it may be diluted to 8 to 12percent solids in water and boiled into a pap or cooked to a stiff gel (Osagie *et al*., 2018).

Akinrele (2014) reported that the souring of the maize took place spontaneously without the addition of inoculants or enzymes. He identified the organism involved in this unaided fermentation and investigated their effects on the nutritive value of the food. The moulds he identified are *Epholosporium, Fusarium, Aspergillus* and *Penicillium* species and the aerobic bacteria as *Corynebacterium* and *Aerobactor species,* while the main lactic acid bacterium he found was *Lactobacillus plantarum*. There was also yeast identified as *Candida mycoderma, Saccharomyce cerevisiae* and *Rhodotorula sp.* Although “ogi” is supposed to have an improved vitamin-B contents the result observed are quite variable, at least for thiamine, riboflavin and niacin. Adeyemi *et al*., (2014) identified the carboxylic acids of maize fermentation. They found 11 acids, with lactic, acetic and butyric acids being the most important. The ogi making process is quite complex, and the porridge can also be prepared from sorghum, rice, millet and maize. Therefore, laboratory procedures have been developed to learn more about the process and introduce changes to convert the grains to food more efficiently. These studies have been useful in evaluating varieties of cereal grains for their efficiency in making “ogi” from whole male are kernels (79.1 percent) and dried milled flour (79.8percent) (Ukpabi *et al*., 2015).

The commercial manufacture of ‘ogi’ does not differ substantially from the traditional method. Modifications have been introduced or added, such as the dry milling of maize into a fine meal or flour and subsequent inoculation of the flour-water mixture with a culture of *lactobacilli* and yeast. In view of the importance of ‘ogi’ in the Nigerian diet, large scale production is indicated. The material could be dried and packaged in polythene bags for a good

shelf life. Some of the modification which include spray drying the slurry or drum drying (Osagie *et al*., 2018).

2.4 Nutritional and some Chemical changes of Maize Slurry

2.4.1 Chemical changes

The process of fermenting maize, sorghum, or millet to produce maize slurry not only removes parts of the maize kernels such as seed-coat and the germ, but also involves washing, sieving and decanting all of which induce changes in the chemical composition and nutritive value of the final product. Adeyemi *et al*., (2014) reported on specific nutrients of a number of 'ogi' samples produced in different ways unfermented and fermented with *Aerobactor cloacae, Lactobacillus plantarum* and a mixture of the two bacteria. He also compared the values found with those from the traditionally fermented products. Judging from the ratio of amino nitrogen to total nitrogen, the author reported that protein was degraded to a very small amount by any bacterial species. When compared with the unfermented 'ogi', *Aerobacter cloacae* appeared to synthesis more riboflavin and niacin, which did not take place with *L. plantarum*. Traditionally produce 'ogi' had more thiamine and slightly lower the values of riboflavin and niacin than that made with maize and *A. cloacae.*

Osagie *et al*., (2018) reported on the proximate composition of Ogi made from common whole maize which were uncooked, and freeze- dried or cooked and freeze-dried after fermentation. Changes were relatively small in all major nutrients, with a slight increase in fiber and a decrease in ash content when compared with whole maize. These authors also reported on amino acid content, they found no differences maize flours and 'ogi' for all amino acids including the essential ones. The maize slurry samples, however had about twice the amount of serine and somewhat higher values for glutamic acid. *Osagie et al*., (2018) reported that ogi

processing did not decrease the protein content of maize, but total and available lysine were significantly reduced. On the other hand, tryptophan levels were more stable and in two samples increased, probably because of fermentation. These authors also found an increase in neutral detergent fibre and ash but no change in ligin. Adeyemi *et al*., (2014) found a decrease in protein, either extract, ash and crude fiber in ogi as compared with maize that was processed as a whole grain or dry milled.

2.4.2 Nutritional Changes

Abdulraham *et al*., (2006) found a substantial decrease in protein quality of drum dried common maize ogi, which they ascribed to the drying process. These same authors reported significant losses on lysine. Several authors have more recently tested maize and sorghum and reported that fermentation improved the nutritional value of the product. It has been indicated that some of the microorganisms responsible for ‘ogi; fermentation, such as *Enterobacter cloacae* and *Lactobacillus plantarum* use some of the amino acids for growth. This together with the elimination of the germ from Kernels explains that the very low protein quality of ogi and similarly produced maize products Abdulraham *et al*., (2016).

2.4.3 Microbial Properties of Maize Slurry

Three distinctive fermentative phases are characterized with Maize slurry production. At steepings, gram negative organisms predominate especially *Achromobacter* and *Klebsiella spp*. Following the milling and sieving, gram negative organisms and lactic acid bacteria especially *streptoccus spp* dominates. The final stage of souring is dominated by non-homofermentative lactic bacteria especially *Lactobacillus plantarum* and *Pediococcus gunther* which leads to the involvement of yeast as a minority component. *Saccharomyces cerevisiae* dominates the steeping stage while *Candida* survives in the finished product. Fermentation temperature is the major

factor affecting the type of organisms involved in the production process. The lactic acid bacteria *Lactobacillio sp, Corynebacterium sp* and *Enterobacter sp* were among the major organisms responsible for the fermentation and nutritional improvement of maize slurry (Jitngarmkusol *et al*., 2018).

At 15^0C, gram negative organisms survive in the products at the end of seven days; whereas at 33^0C and 37^0C, the flora is more heterogenous and the end product is accompanied by an odd odour and taste. Large fermentation rods suggestive of *Bacillus spp*. are also targeted to be the causal agent of proteolysis and the putrial colour of 'ogi'. Also, the gas evolution that is found during steeping is attributed to the presence of *Klebsiella aerogenes* (Jitngarmkusol *et al*., 2018).

2.4.4 Biochemistry of Maize slurry

When the grain is fermented, there is an increase in pH. The raw 'ogi' contains much less protein than the parent cereal because some soluble proteins are lost in steeping, washing with water and during mashing. Acid reacting substances are present during mashing. Acid reacting substances are also present during ogi fermentation and increases as the fermentation progresses (Oyerekua *et al.,* 2019).

Lactic acid is the primary volatile acid of ogi fermentation, acetic acid is the main volatile acid followed by butyric acids other volatile acids of fermentation are formic acid, propionic acid, isobutyric acid, isohexonic acid etc. During steeping in water it contains a high number of volatile acids unlike finished products, because the bulk of the acid produced in the later stages of the fermentation is leached out into the water. These acids appear in form of filing on the surface of the water Ukpabi (2015).

2.5 Properties of Lactic Acid Bacteria

In recent years, more and more attention has been paid to the metabolism of lactic acid bacteria. Lactic acid bacteria (LAB) are a type of gram-positive bacteria that use carbohydrates as the only or main carbon source (George *et al.,* 2018). Lactic acid bacteria are generally cocci or rods, and have strong tolerance to low pH. Although lactic acid bacteria include more than 60 genera, the frequently genera occur in food fermentation generally include *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Enterococcus, Weissella,* etc. (Mokoena, 2017). But it has recently been proposed to merge *Lactobacillaceae* and *Leuconostocaceae* in one family *Lactobacillaceae*. The genus Lactobacillus was also reclassified into 25 genera (Zheng *et al.,* 2020). As a fermentation strain, lactic acid bacteria should have several important metabolism characteristics, such as the ability to produce acid and aroma, the ability to hydrolyze protein, the ability to produce viscous exopolysaccharides and the ability to inhibit bacteria. LAB are widespread in nature and predominate of microflora in milk and milk products; many species are involved in the daily manufacturing of dairy products (Ayad *et al.,* 2004).

The lactic acid bacteria used in the dairy fermentation can roughly be divided into two groups of the basis of their growth optimum. Mesophilic lactic acid bacteria have an optimum growth temperature between 20^0C and 30^0C and the thermophilic have their optimum between 30^0C and 45^0C. Traditional fermented products from sub-tropical countries harbor mainly thermophilic lactic acid bacteria, whereas the products with mesophilic bacteria originated from western and northern European countries.

The lactic acid bacteria can be mainly divided into two groups based on the end products formed during the fermentation of glucose. Homofermentative lactic acid bacteria such as *Pediococcus, Streptococcus*, and *Lactococcus* produce lactic acid as the sole product of glucose

fermentation. Heterofermentative lactic acid bacteria such as *Weissella* and *Leuconostoc* produce equimolar amounts of lactate, CO_2 and ethanol from glucose (Kuipers *et al.,* 2000).

LAB have been extensively used in food fermentation, including the production of milk products, and its proteolitic activity is very important in producing flavor compounds of end product (Moulay *et al.,* 2013). Proteolytic system of LAB is important for the growth of microorganisms and it is involved in casein utilization within LAB cells and give contribution to the development of organoleptic properties of fermented milk products (Moulay *et al.,* 2013). Milk fermentation process has relied on the activity of LAB, which play a crucial role in converting milk as raw material to fermented milk products. In milk fermentation industry, various industrial strains of LAB are used as starter cultures. Starter cultures of LAB were obtained from a sequence activity and passed a process of isolation, selection and confirmation. Several behaviors as the characteristics of each individual selected strains of LAB has been established and used in the production of fermented milk products industrially. The most important properties of LAB are their ability to acidify milk and to generate flavour and texture, by converting milk protein due to their proteolytic activities. The mild acid taste and pleasant fresh are characteristics of fermented milk products such as yoghurt and cheese (Griffiths and Tellez, 2013).

The lactic acid bacteria grows favorably under low concentration of NaCl (0%, 2.5%) yielding high counts. At higher concentration of NaCl, the growth of lactic acid bacteria are been impeded or yield low counts (Naveen et al., 2013).

2.6 Taxonomy of Lactic Acid Bacteria

The taxonomy of lactic acid bacteria have been used based on Gram reaction and the production of Lactic acid from various fermentable carbohydrates. According to the current taxonomy, they

belong to the phylum, Firmicutes, class Bacilli, order Lactobacillales and families include *Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae,* and *Streptococcaceae*. The classification of LAB into different genera is highly based on morphology, mode of glucose fermentation, growth at different temperature, configuration of Lactic and growth at high salt concentration

2.6.1 Lactobacillus

Lactobacillus is the largest genus within the group of lactic acid bacteria. To date, it contains 168 species, some of which are used in the manufacture of fermented dairy, sourdough, meat, vegetable foods, or used as probiotics. The genus *Lactobacillus* belongs to the phylum Firmicutes, class Bacilli, order II *Lactobacillales*, and family *Lactobacillaceae* (Salam, 2016). *Lactobacillus* are Gram-positive, catalase-negative, non-spore-forming, rod-shaped bacteria that produce lactic acid as the major end product of fermentation. Lactobacillus species can be divided into three groups:

Obligately homofermentative (Group I) including*: L. acidophilus, L. delbrueckii, L. helveticus, L. salivarius.*

Facultatively heterofermentative (Group II) including: *L. casei, L. curvatus, L. plantarum, L. sakei* and

Obligately heterofermentative (Group III) including*: L. brevis, L. buchneri, L. fermentum, L. reuteri*.

Since the phylogenetic relatedness of lactobacilli was investigated by sequencing the 16S rRNA of all species successfully described up to now and this permitted the allotment of the lactobacilli to the following groups: *Lactobacillus buchneri* group (bu), *Lactobacillus casei* group (ca), *Lactobacillus delbrueckii* group (de), *Lactobacillus plantarum* group (pl), *Lactobacillus reuteri*

group (re), *Lactobacillus sakei* group (sa), and *Lactobacillus salivarius* group (sl). In contrast, *Lactobacillus brevis* and *Lactobacillus perolens* as well as the related species *Lactobacillus bifermentans* and *Lactobacillus coryneform* is are uniquely positioned among the *lactobacilli. L. bifermentans* is lactose negative strain and has the ability to convert lactate to acetate, the organism is also able to form H2, which is untypical of *lactobacilli*, *Lactobacillus coryneform* is usually produce lactic acid in a D form whereas most of *Lactobacillus* produce mainly L form of lactic acid (Salam *et al*., 2016).

2.6.2 Leuconostoc

Leuconostoc spp. are Gram-positive, nonmotile, catalase-negative, asporogenous bacteria. Their cells are spherical, oval, or c*occobacillar*y and usually occur singly or in pairs. The species possessing rod-shaped cells had been classified in the genus *Leuconostoc* but were later reclassified into the genus *Fructobacillu*s (Hassan *et al.*, 2011).

They are mesophilic and psychrotolerant/psychrotrophic bacteria. Their optimum growth temperature is generally around 25–30^{0}C. Very weak growth occurs at temperatures higher than 40^{0}C. Certain species, in particular *Leuconostoc gelidum* and *Leuconostoc inhae*, can grow at temperatures lower than 4^{0}C and are sometimes recovered as spoilage microbes from cold-stored meat and fish products (Hassan *et al.,* 2011). They are acid-sensitive microorganisms and typically grow well with an initial pH between 6 and 7. Their growth is markedly inhibited by an acidic pH less than 5 *Leuconostoc* spp. are obligately heterofermentative LAB and metabolize glucose via the phosphoketolase pathway. They produce 1 mol each of lactate, ethanol, and CO_2 as the end products from the metabolism of 1 mol of glucose (Hassan *et al.,* 2011). The major lactic acid produced is of the D-isomer. In contrast to obligately homofermentative LAB, *Leuconostoc spp*. have the ability to metabolize pentoses, i.e., arabinose, ribose, and xylose, and

produce lactate and acetate. Acetate instead of ethanol is produced from acetyl-phosphate using acetate kinase, and this process generates an extra 1 mol of ATP. This means that these microorganisms are able to obtain 2 mol of ATP from the metabolism of pentoses. Acetate production and generation of an extra 1 mol of ATP can be also seen during glucose metabolism when an external electron acceptor (e.g., pyruvate or oxygen) is supplied (Hassan *et al.*, 2011). They are generally good fermenters of simple carbohydrates and may metabolize a wide variety of carbohydrates, including monosaccharides, disaccharides, oligosaccharides, sugar alcohols, and gluconate. This may be due to their diverse habitats, as described above. Different carbohydrates are generally available under different environments. Dairy-origin *Leuconostoc spp., e.g., Ln. mesenteroides subsp. cremoris and Ln. lactis,* are relatively poor fermenters when compared to other *Leuconostoc* spp. Lactose metabolism is not common in the genus Leuconostoc (Hassan *et al.,* 2011).

2.6.3 Lactococcus

Lactococcus constitutes one of the several genera forming the LAB family. This genus is a homo-fermenter that ferments sugars, releasing only one by-product, lactic acid. The subspecies L. *lactis subsp. lactis and L. lactis subsp. cremoris* are predominant in unpasteurized raw milk and cheese. Although these microorganisms are abundant in raw milk, they are reintroduced in pasteurized milk because the milk sterilization process significantly reduces or eradicates the *lactococc*i population in raw milk. The *Lactococcus* genus *(L. lactis)* is vital in cheese making, particularly acidification through fermentation of lactose. As a result of this acidification, adaptation and replication of pathogenic microorganisms are mitigated. Additionally, the *Lactococcus* genus is capable of proteolysis, a process which converts amino acids into flavor complexes (Aseel 2019).

2.8 Importance of Lactic Acid Bacteria in Maize Slurry Fermentation

Fermentation is one of the oldest methods in food preservation. It is well known that the production of beer, wine, and other alcoholic drinks is impossible without certain yeast, so also Lactic acid bacteria are very important in product whose production is based on lactic fermentation (Slavica *et., al* 2014). They contribute to the taste and texture of fermented products and inhibits food pathogens and spoilage bacteria by producing growth-inhibiting substances and large amounts of Lactic acid. As agents of fermentation, LAB are involved in making yoghurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats. Lactic acid as one of the metabolites produced by LAB has various industrial applications such as a preservative, acidulant, and flavor in food, textile and pharmaceutical industries. It can also be used for the production of lactate-esters, propylene glycol, propylene oxide, acrylic acid, 2,3-pentanedione, propanoic acid and acetaldehyde (Rachel and Bukola, 2012). They are responsible for maintaining a balance of the micro-biota of healthy host and they have the ability to colonize the gastrointestinal tract and ferment carbohydrate which produces lactic acid as the major metabolic end product that aid digestion (Philip *et al.*, 2017).

Chapter Three

Materials and Methods

3.1 Study Area

The study area for this research is the Federal Capital Territory Abuja. The largest city and the capital of Nigeria, Abuja can be found roughly in the central part of the country, situated in the west central region of the African continent located on Latitude and longitude coordinates are: **9.072264, 7.491302**. Administratively, Abuja is the center of the Federal Capital Territory and is a city with the area close to 275 square miles. It has an annual temperature range from 26°C – 32°C, relative humidity of 40 – 60% and annual rainfall of 1469mm. it is mainly a planned community and a modern residential center which can be considered one of the most up-to-date cities of the region. The downtown area is situated in the eastern part of the municipal area of the city, and the central districts of Abuja include Central Business District, Wuse, Garki, Asokoro, and others.

3.2 Collection of Samples

The research will be done at the Abuja. Maize slurry will be purchased from Wuse market located inside Abuja. The experiment will be carried out in microbiology laboratory of Nile University of Nigeria. The maize slurry will be collected from Five different samples from Wuse market, Galadimawa market, Utako market and Garki new market will be collected aseptically in other to avoid further contamination and transported in a cold chain to the school laboratory inside Nile University of Nigeria, Abuja for microbiological investigation.

3.3 Preparation of Media

3.3.1 Preparation of Man Rogosa de-Sharpe (MRS) Agar

The MRS agar was prepare according to the manufacturer's instruction. About 62.2 g of MRS agar was dissolved in distilled water (1 L) and heated to dissolve. The media was sterilized by autoclaving at 121 °C for 15 min then allow to cool at 45 °C before dispensing into petri-dish. The MRS Agar is a special media for isolation of Lactic Acid Bacteria.

3.3.2 Preparation of Nutrient Agar

According to manufacturer instructions, 28g of Nutrient agar was weighed into conical flask and was dissolved with 1 liter of distilled water, mixed gently and boil for some minutes to ensure the media was dissolved properly by boiling for 1 minutes. The conical flask was tightly closed with a sterile cotton wool and wrapped with a foil paper to avoid contamination. Then autoclaved at 121 °C for 15 minutes; the media was allowed to cool at 45 °C then dispensed into petri dish allowed to solidify before use.

3.4 Isolation of Lactic Acid Bacteria from Maize slurry

3.4.1 Serial dilution

About 1 ml of pap was dispensed aseptically into a sterile test tube to which 9 ml of sterile distilled water had been previously added. The mixture was shaken to homogenize and a dilution factor of 10^{-1} was obtained. Then 1 ml of this dilution (10^{-1}) was pipetted and dispensed aseptically into another sterile test tube containing 9 ml of sterile distilled water this makes a mixture of one in hundred dilutions i.e., 10^{-2}. The process was repeated until a dilution of seven-fold was obtained.

3.4.2 Inoculation

The working area was cleaned and sterilized with 70% alcohol after which the solidify agars (Nutrient agar and MRS agar) were labelled properly prior to inoculation. The wire loop was

sterilized using a bunsen flame then allow to cool. With the aid of the sterilized wire loop about 0.001 ml of the dilution 10^{-5} of the Maize slurry was picked and aseptically streak into a solidify Nutrient agar after which the petri dish plate was cover upside down. After streaking the wire loop was sterilized again and the same process was repeated for MRS agar. The standard streaking method was employed for this study so as to obtain single colonies on the petri dish. After inoculation both plates (Nutrient agar and MRS agar) were incubated inside the incubator at a temperature of 35 ± 2°C for 24 – 48 hours (Ochei *et al.*, 2000).

3.5 Morphological Characteristics of Isolates

Microscopic and cultural methods will be proper identification of the isolated lactic acid bacteria. They include carrying out gram staining reaction to reveal the color, shape and size of the lactic acid bacteria while the cultural method will reveal the texture, color and shape of the lactic acid bacteria.

3.5.1 Gram staining techniques

Gram staining was carried out on the isolates. A smear of the culture was made on a clean grease free slide labeled with each isolate code and heat fixed to dry. The smear was then stained with crystal violet for 60 seconds after which it was rinsed in water. Few drops of Lugol's iodine solution (Gram's iodine) were added and allowed for 60 seconds. The smear was decolourized with 95% ethanol for 30 s and immediately rinsed with tap water. The slide was counter stained with Carbon Fuchsin for 1 min and rinsed with water and then dried with Whatman filter paper. Gram-positive cells are purple while Gram-negative cells are red (Ochei *et al.*, 2000).

3.6 Biochemical Characteristic of the Isolates

This will be carried out using the following biochemical reagents and media; these are sugar fermentation test (glucose, lactose and sucrose), catalase, motility test, growth test at different

temperature (4°C, 37°C and 80°C), growth test at different salinity (5%, 10% NaCl) and growth at different acidity (pH level 2, 3, 4). Confirmatory identities of the bacteria will be made using the Bergey's Manual of Determinative Bacteriology Cheesebrough (2014).

3.6.1 Catalase test

A microscopic slide was placed inside a petri dish. Using a sterile inoculating loop, a small amount of microorganism from 24-hour pure culture was placed onto the microscopic slide. 3% H_2O_2 solution was added to each of the slides and a portion of the bacterial colony was mixed with it. Production of bubble indicated the presence of catalase enzyme in the bacteria (Reiner, 2010).

3.6.2 Motility indole urea (MIU) test

Following incubation for 18-24 h at 37°C, the colony in tube was observed for the presence of motile organisms. For indole production, 0.1 ml of chloroform was first added to each test tube and allowed for 15 min. Then 1ml of Kovac's reagent was added and then examined for red color development within 10 minutes as an indication of indole production (Ferdous *et al*., 2013).

3.6.3 Methyl red test

Methyl red test was performed to determine the ability of the bacteria to oxidize glucose with the production of high concentration of acidic end products. Glucose phosphate broth was inoculated with the isolates and incubated for 2-5 days at 37°C. Then 5 drops of methyl red indicator (0.2%) was added and carefully observed for immediate development of a red color which indicates a positive test for acidic products (Ferdous *et al*., 2013).

3.6.4 Growth at Different Temperature

This was carried out by inoculating a single isolate into 5ml of MRS broth. Three different test tubes containing the MRS broth were used. Each test tubes were incubated at different

temperature of 4°C and 37°C for 24 hours. Growth was observed based on the presence of turbidity.

3.6.5 Growth at Different Salinity

This was carried out by inoculating a single isolate into 5ml of Phosphate Buffer Saline. Two concentrations (5% and 10%) of the Phosphate buffer saline were prepared. The isolate was inoculated into each of the test tubes containing different concentration of the phosphate buffer saline and incubated at 37 °C. Turbidity indicates the presence of growth was checked at 3 hour and 6 hours.

3.6.6 Growth at Different Acidity

Before inoculation 0.1N of Hydrochloric acid was added by drops into three different test tubes containing 5ml of MRS broth and the pH value was checked using pH meter until the target pH value (2 and 4) is achieved. A single colony of lactic acid bacteria was inoculated into each of the test tubes and the incubated at 37 °C for 90 minutes. Results was read based on the presence of turbidity which indicates growth.

3.7 Proximate analysis of Maize Slurry

The proximate compositions of the Maize slurry were determined using standard analytical methods. All measurements were done in duplicates and values presented in percentage.

3.7.1 Ash content determination

The ash content was determined using the method described in AOAC (AOAC, 2006).

5g of the sample was weighed into a crucible in a muffle furnace and heated at 550 °C for six hours until it became gray ash. The dish was removed from the muffle furnace using crucible

tong and placed in a desiccator to cool. When cooled it was re-weighed and the weight of ash was obtained by the difference.

3.7.2 Moisture content determination

The moisture content of the samples was determined using AOAC method (AOAC, 2006).

The Petri-dish was washed thoroughly and placed in oven to dry. 5g of the sample was then placed in a preweighed. Petri dish, and then placed in an oven to dry at 105 ℃ for two hours. The dish and dry sample were transferred to a desiccator to cool at room temperature before being weighed again. The experiments were repeated until constant weight was obtained.

The Moisture content was calculated as Initial weight – Final weight.

3.7.3 Fat content determination

Fat was determined using soxhlet fat extraction method (Onwuka et al., 2016).

250 ml boiling flask was washed thoroughly and dried in oven at 105 °C for 30 minutes and then placed in a desiccator to cool. 2g of the dried maize slurry sample was then weighed accurately into labeled thimbles. Cooled boiling flask was filled with 200 ml of petroleum ether and boiled at 40 – 60 °C. The extraction thimble was plugged lightly with a cotton wool and the boiling flask containing the petroleum ether was placed in the extraction thimble to boil and the soxhlet apparatus was allowed to reflux for six hours. The thimble was removed carefully, and the petroleum ether on top of the container was collected and drained into another container for reuse. When the flask was free of petroleum ether, it was removed and boiled for an hour at 105°C. It was finally transferred from the oven into a desiccator to cool before weighing.

3.7.4 Fibre content determination

Crude Fibre content was determined by Weende's method (AOAC, 2006).

2g of the Maize slurry sample was weighed into a 250ml conical flask and 200 ml of 1.25 % H_2S0_4 was added and the mixture was boiled under reflux for 30 minutes. The solution was filtered with Whatman filter paper; the residue was rinsed thoroughly with hot water until it was no more acidic when tested using pH paper. The residue was transferred into a 250 ml beaker and 200 ml of 1.25 % NaOH was added and boiled for 30minutes in a digestion apparatus after which it was filtered and rinsed with distilled water until the filtrate was neutral when tested with pH paper. The residue was transferred into a crucible and placed in electric oven at 100 °C for eight hours to dry. It was then removed and placed in a desiccator to cool before weighing. After weighing, the sample was incinerated, cooled in a desiccator and reweighed.

3.7.5 Protein determination

Protein content of the sample was determined using the Kjeldahl method (AOAC, 2006). The total nitrogen was determined and multiplied by a conversion factor of 6.25 to obtain the protein content. 0.5g of the sample was weighed into a Kjeldahl digestion flask. A tablet of selenium catalyst was added to it. 20ml of H_2SO_4, 10g of $NaSO_4$, 1g of $CuSO_4$ were also added to the flask and digested by heating under a fume cupboard till the solution digested completely and changed to blue color. The solution was carefully removed and allowed to solidify for 24 hours until a white colour was obtained. The solution was dissolved with 100 ml of distilled water in a 200 ml volumetric flask. Then 60 ml of 40 % of NaOH and two pieces of zinc metal were added to the solution in the Kjeldahl distillation apparatus. The mixture was distilled until a total of 50 ml distillate was collected into 250 ml conical flask containing boric acid and was titrated with 0.1N H_2SO_4. The end point of the titration was observed when the color of the distillate changed to the initial color of the mixture of boric acid and screen methyl red indicator which was light pink.

3.7.6 Carbohydrate determination

The carbohydrate content of the test sample was determined by estimation using the arithmetic difference method (AOAC, 2006)

%CHO = 100 – (% fat. + % ash + % fiber + % protein)

3.8 Molecular Identification:

This will confirm and identify the lactic acid bacteria to specie level using DNA extraction, PCR and DNA sequencing.

3.9 Antimicrobial Susceptibility

A disc diffusion technique, which is a reliable and accurate technique, will be employed for this study. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial agent. This is placed on a plate of sensitivity testing agar uniformly inoculated with the test organism (*Lactic acid bacteria*). The antimicrobial diffuses from the disc into the medium, and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the sensitivity of the organism, Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc (NCCLS, 2017).

3.9.1 Kirby-Bauer Disc Diffusion Technique.

All the confirmed *lactic acid bacteria* strains were be subsequently tested for resistance based on the Kirby-Bauer disk diffusion method, using multi-disk format including: streptomycin 10µg, septrim 1.25µg, chloramphenicol 30µg, sparfloxacin10µg, ciprofloxacin 5µg, amoxil 10mcg, augmentin 30mcg, gentamycin 10mcg, pefloxacin 5mcg, ofloxacin 5mcg. will be used for the susceptibility testing. Inhibition zone diameter was measure, recorded and interpreted based on the CLSI criteria standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials (CLSI, 2021).

3.10 Growth Test for Lactic Acid Bacteria

3.10.1 Growth test at different temperature

- A small amount of the bacterial colony was inoculated into MRS Broth.
- Incubate at 4^{o}C, 37^{o}C for 4 days.

3.10.2 Growth test at different salinity

- 25g of NaCl was dissolved into 500 mL of water to make a 5% solution.
- 10g of NaCl was dissolved into 100 mL of water to make a 10% solution.
- 3.77g of MRS Broth was dissolved into 80 mL of the 5% and 10% NaCl solution in a well labelled conical flask.
- Stirred well using a magnetic stirrer to dissolve completely in water.
- Autoclave at 121°C and 15 lbs pressure for 15 minutes and let them cool.
- 5 mL of the broth was dispensed into a well labelled test tubes.
- Inoculate one or two colonies into 5% & 10% NaCl broth.
- Incubate the tube at 37°C for 48 hours.
- Examine tubes for turbidity after 48 hours and if negative again at 72 and 96 hours.

3.10.3 Growth test at different acidity

- The test tubes were numbered (1,2,3&4) one test tube for each sample.
- The test tubes were half-filled with hydrochloric acid solution.
- 1cm^3 of the solution was dispensed into a measuring cylinder.
- Distilled water was added into the cylinder upto 10cm^3 mark.
- The test tubes will have the solution in them with pH2 and pH4.

- A drop of the universal indicator was added to each sample and rock till the color change.
- Then, the color of the solution was compared with the pH indicator chart.
- 3.77g of MRS Broth was dissolved into 80 mL of the pH2 and pH4.
- Autoclave at 121°C and 15 lbs pressure for 15 minutes and let them cool.
- 5 mL of the broth was dispensed into a well labelled test tubes.
- Inoculate one or two colonies into pH2 and pH4 broth.
- Incubate the tube at 37°C for 48 hours.
- Examine tubes for color change after 48 hours and if negative again at 72 and 96 hours.

Chapter Four

Result

Out of the four (4) maize slurry sample collected from four market within Abuja. Two (2) were positive for lactic acid bacteria. The other bacteria isolated was *Escherichia coli*. The mean and

standard deviation colony of the bacteria count obtained was 46.5 ± 6.35. They lactic acid bacteria were further characterizes for identification using gram staining reaction and biochemical test and growth test in pH and NaCl medium at different temperature. All results obtained were analyzed and displayed in tables.

4.1 Bacteria Colony count from Maize slurry

From Table 4.1 shows the total bacteria count. Serial dilution was carried out on the isolates from the maize slurry. Maize slurry collected from Wuse market had high count of bacteria 105 x 10^5 followed by Garki new market 39 x 10^5, Galadimawa market 27 x 10^5 and the least been

Utako market 15 x 10^5. The mean value and standard deviation of the total lactic acid count is 46.5 x 10^5 ± 6.35

Table 4.1: Bacteria Colony count from Maize slurry

Name and sources of sample	Number of Factor	Number of Colonies	Total LAB Count cfu/ml
WUM	10^5	105	105 x 10^5
GDM	10^5	27	27 x 10^5
UTM	10^5	15	15 x 10^5
GNM	10^5	39	39 x 10^5
Mean ± Standard deviation		**46.5 ± 6.35**	

Key: **WUM**: Wuse Market, **GDM**: Galadimawa, **UTM**: Utako Market, **GNM**: Garki New Market

4.2 Proximate Analysis of Maize slurry

The result of proximate composition from Table 2 revealed that the moisture content of the maize slurry as 10.3 ± 0.7, protein content 8.3 ± 0.4, Fat content as 2.1 ± 0.1, Total ash 2.8 ± 0.2, fibre content 2.0 ± 0.1 while the total carbohydrate was recorded as 74.5 ± 3.9.

Table 4.2: Proximate Analysis of Maize Slurry

Composition	Maize Slurry

Moisture content	10.3 ± 0.7
protein content	8.3 ± 0.4
Fat (ether extract)	2.1 ± 0.1
Total ash	2.8 ± 0.2
Crude fibre	2.0 ± 0.1
Total carbohydrate	74.5 ± 3.9

4.3: Gram Staining Reaction of Bacteria isolates

From Table 4.3, it shows the gram staining reaction of the lactic bacteria isolated from the fermented cow milk. Gram staining reaction was carried out as the first step for identification. All the isolates were gram positive, purple in color and had rod shape.

Table 4.3: Gram Staining Reaction of Bacteria Isolates

S/N	Isolates	Color	Shapes
WUM	10^4	Purple	Coccobacilli
	10^5	Purple	Coccobacilli
GDM	10^4	Pink	Rod
	10^5	Pink	Rod
UTM	10^4	Pink	Rod
	10^5	Pink	Rod
GNM	10^4	Purple	Cocci
	10^5	Purple	Cocci

Key: **WUM**: Wuse Market, **GDM**: Galadimawa, **UTM**: Utako Market, **GNM**: Garki New Market

Table 4.4: Biochemical Test Results of Lactic Acid Bacteria

From Table 4.4, it shows the biochemical test reaction of the bacteria isolated from the maize slurry. Biochemical reaction was carried out in other to identify and characterize the lactic acid

bacteria. From the table above based on the biochemical reaction two (2) of the isolates are Lactic acid Bacteria; they are *Lactobacillus spp* and *Streptococci spp.*

Table 4.4: Biochemical Test Reaction of the Lactic Acid Bacteria

Sample code	Mot	Ind	Cata	Lact	Suc	MR	Glu	Probable Organism
WUM	-	-	-	+	+	-	+	*Lactobacillus spp.*
GDM	+	+	+	+	**v**	+	+	*Escherichia coli*
UTM	+	+	+	+	**v**	+	+	*Escherichia coli*
GNM	-	-	-	+	+	-	+	*Streptococci spp.*

***Key**: (+): Positive, (-): Negative, **Cata**: Catalase, **MR**: Methyl red, **Ind**: Indole, **Mot**: Motility, **Suc**: Sucrose, **Glu**: Glucose, **Lact**: Lactose.*

4.5 Growth Characteristics of Lactic acid Bacteria at different Acidity and Salinity Concentration.

The bacteria isolated from this study shows high resistance to low pH and high salinity concentration at 4 °C and 37°C. Only isolates from Wuse market and Garki new market shows negative results at 5% NaCl at a temperature of 4 °C.

Table 4.5: Growth Characteristics of LAB at different Acidity and Salinity Concentration

Sample code	pH		Salinity		Temperature	
	pH2	pH4	5% NaCl	10% NaCl	4 °C	37 °C
WUM	-	++	++	+++	++	+++
GDM	++	++	++	+	++	++
UTM	+++	++	+	++	++	+
GNM	++	++	++	++	-	++

Key: (-): Negative, (+): Mild, (++): Moderate, (+++): Heavy

WUM: Wuse Market, **GDM**: Galadimawa, **UTM**: Utako Market, **GNM**: Garki New Market

4.6 Antibiotic resistance and susceptibility pattern of Bacteria isolated from Maize slurry

From table 4.6, it reveals that there is high susceptibility rate of bacteria to the antibiotics used in this study. High susceptibility was seen among rifampin, ciprofloxacin, erythromycin and ampiclox while resistance was seen among streptomycin and augmentin and intermediate resistance was only seen with pefloxacin.

Table 4.6: Antibiotic resistance and susceptibility pattern of Bacteria isolated from Maize slurry

Antimicrobial	Number of Isolates n= 4		
	Resistant	Intermediate	Susceptible
S	4 (100%)	0 (0.0%)	0 (0.0%)
PEF	2 (50%)	1 (25%)	1 (25%)
ERY	1 (25%)	0 (0.0%)	3 (75%)
R	0 (0.0%)	0 (0.0%)	4 (100%)
CPX	0 (0.0%)	0 (0.0%)	4 (100%)
AMX	2 (50%)	0 (0.0%)	2 (50%)
APX	1 (25%)	0 (0.0%)	3 (75%)
CN	2 (50%)	0 (0.0%)	2 (50%)
AU	4 (100%)	0 (0.0%)	0 (0.0%)
OFX	2 (50%)	0 (0.0%)	2 (50%)

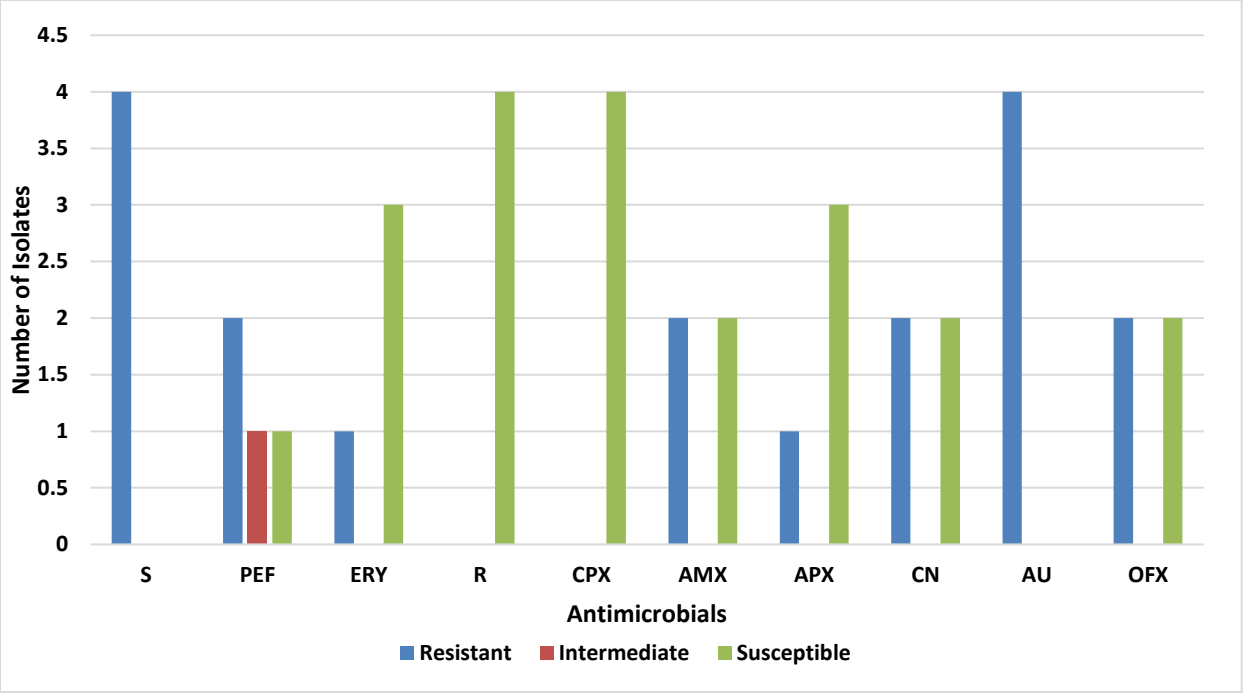

Figure 2: **Antibiotic resistance and susceptibility pattern of Bacteria isolated from Maize slurry**

From the histogram chart above, the resistant antimicrobials are as follows; streptomycin (4) and augmentin (4), while highly susceptible antimicrobials are rifampin (4), ciprofloxacin (4), erythromycin (4) and ampiclox (4).

Chapter Five

Discussion, Conclusion and Recommendation

5.1 Discussion

This study has revealed the presence of lactic acid bacteria from the samples of maize slurry collected from the four different market in Abuja, one sample was collected each from each market. Two (2) isolates of Lactic acid bacteria were isolated from the four (4) samples collected. The detection of lactic acid bacteria from maize slurry samples is in agreement with the findings of Bukola et al., (2019) who reported *Lactobacillus spp* which is a member of the lactic acid bacteria in maize slurry in her research work titled Evaluation of Ogi (Corn Caramel) from Maize and Sorghum for Isolation and Characterisation of Lactic Acid Bacteria (LAB).

Table 4.1 shows the total bacteria count. Serial dilution was carried out on the isolates from the maize slurry. Maize slurry collected from Wuse market had high count of bacteria 105 x 10^5 followed by Garki new market 39 x 10^5, Galadimawa market 27 x 10^5 and the least been Utako market 15 x 10^5. The mean value and standard deviation of the total lactic acid count is 46.5 x 10^5 ± 6.35. High count seen in Wuse market may be as a contamination during processing or storage.

Table 4.2 shows the proximate analysis result of maize slurry collected from the market in Abuja. High contents of carbohydrate were noted from the maize slurry collected from Abuja and therefore is in agreement to the findings of Akin et al. (2019) who also reported high carbohydrate content. Also, protein, fat and fibre content were also revealed by the proximate analysis. This therefore proves the fact of how nutritious the maize slurry is man.

Table 4.3 shows the gram staining reaction of the isolates from maize slurry. Gram staining reaction is a requirement for identification of bacteria. The isolates showed gram positive, purple in color and appear cocobacilli and cocci in shape. This method of identification

was employed by Evurani *et al*., (2019) in his study. The positive cocci and cocobacilli isolates were then further identified using the available biochemical test reactions.

Table 4.4 shows the results of biochemical reactions carry out on the gram positive cocci and cocobacilli isolates from maize slurry. Biochemical tests done on each of the isolates include catalase, methyl red, indole and oxidase including the *Escherichia coli*. The results for the biochemical test done on the isolates reveal the results of the biochemical test reaction as seen in Table 4.4. Biochemical test reaction was then merge with the gram staining result and read using the Bergey manual of bacteriology for identification, the isolates were presumptive *Lactobacillus spp* and *Streptococci spp*.

Table 4.5 shows the results of the growth at acidic and salinity medium. The results obtained in this research showed that during the spontaneous fermentation of maize slurry, the pH of maize slurry shows activity even at acidic medium. This tells the reason why the gastric content is acidic and how lactic acid bacteria are able to survive the acidic medium even at low and high temperature. Also, the lactic acid bacteria were also able to survive at salinity concentration of 5% and 10% at 4 °C and 37 °C. These results are in agreement with the result of Ola Oluwa *et al.* (2013).

Table 4.6 shows the antibiotic resistant and susceptibility pattern of Lactic acid bacteria to the antibiotic used. Results reveals that there is high susceptibility rate of the bacteria to the antibiotics used in this study. High susceptibility was seen among rifampin 4 (100%) and ciprofloxacin 4 (100%), erythromycin 3 (75%) and ampiclox 3 (75%) while high resistance was seen among streptomycin 4 (100%) and augmentin 4 (100%) and intermediate resistance was only seen with pefloxacin 1 (25%). The reported resistant antibiotics in this study is similar to the findings of Obire *et al*., (2015) who reported high resistance of amoxil and gentamycin to

Lactic acid bacteria isolated from maize slurry collected from Benue state. And also, the finding of Herrero et al. (2016) who also reported high resistant of streptomycin to lactic acid bacteria.

5.2 Conclusion

The maize slurry contained a considerable amount of LAB. Some of these LAB had the ability to act as probiotics and at the same time produce antibacterial substances to inhibit the growth of pathogens and food-borne spoilage bacteria. However, some of these LAB produces bacteriocins, are considered sometimes to be opportunistic pathogens. Thus, further studies are required to select LAB able to be used as starters to improve the hygiene and safety of maize slurry in general, to improve human's health and also as probiotic in infant formulas.

5.3 Recommendation

It is advocated that individuals that prepare maize slurry should practice good health hygiene to reduce the microbial load of organisms found in maize slurry. It should be prepared in a clean environment and wash their hands properly before and after preparing the pap in other to avoid contaminating the maize slurry. Maize slurry should be stored under appropriate temperature after production and the materials used during the preparation should be washed and kept properly to avoid contamination and excessive fermentation by the fermenters present in the pap so as to avoid outbreaks of diseases. Also, further studies for identification of LABs into specie level is require so as to know the type of LAB involve in fermentation.

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