EVALUATING THE PROBIOTIC POTENTIALS OF BACTERIA ISOLATED FROM SOME LOCALLY FERMENTED NIGERIAN FOODS

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BENIN CITY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

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CERTIFICATION

This is to certify that the project work was carried out by Toyin-Joyce Anifowose ISHOLA (MRS) with matriculation number PG/LSC2015473 in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under the supervision of;

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DEDICATION

I dedicate this work to God Almighty for His unfailing love, help, strength and for being my source of wisdom and knowledge.

ANTI-PLAGIARISM TEST

We, the undersigned, attest and declare that the thesis of Toyin-Joyce Anifowose ISHOLA titled Evaluating the probiotic potetials of bacteria isolated from some locally fermented Nigeran foods has successfully passed the anti-plagiarism test and does not violet any copyright regulations.

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ABSTRACT

Fermented foods are known to contain active components such as probiotics and antioxidants. Probiotics are living microbes which when taken in sufficient amounts confer health benefits. Due to lactose intolerance in some individuals and the high fat content of dairy foods, other means of obtaining probiotics have been explored. The aim of this study was to evaluate the probiotic potential of some bacteria isolated from Nigerian fermented foods. Samples of fufu, ogi, tuwo, palm-wine, ogogoro, iru, ogiri and ugba were purchased from New Benin Market in Benin City, Edo State, Nigeria. Enumeration and isolation of bacterial and lactic acid bacteria were carried out using nutrient agar and De Man, Rogosa and Sharpe agar, using pour plate technique. Cultural, morphological and biochemical tests were employed to identify the bacteria isolated. The bacterial isolates were subjected to acidic pH of 3 and bile salt concentration of 0.3%. The antibiotic susceptibility of the isolates were determined using the disc diffusion method. The antibacterial activity of the isolates were tested against three test pathogens, *Escherichia coli*, *Salmonella* sp. and *Klebsiella* sp. using the agar well diffusion technique Kirby Bauer disk diffusion method. The mean heterotrophic bacterial counts of Nigerian fermented foods ranged from 5.00±0.28 (ogiri) - 8.70±0.42×10^7 cfu/g (iru), while the lactic acid bacterial counts ranged from 0.80±0.28 (iru) – 5.00±0.42×10^4 cfu/g. *Bacillus* sp,, *Citrobacter* sp., *Lactobacillus* sp.[1], *Lactobacillus fermentum*[2] and *Streptococcus* sp.[2] were isolated from ogi, *Bacillus subtilis*[3] and *Streptococcus* sp.[1] were isolated from the ugba, *Klebsiella pneumonia* were isolated from fufu, *Lactobacillus fermentum*[1] were isolated from tuwo, *Bacillus subtilis*[1] were isolated from iru, *Lactobacillus* sp.[2] were isolated from palm-wine while *Bacillus subtilis*[2] were isolated from ogiri-egusi and *Escherichia coli* isolated from ogogoro. The result of the acid tolerance tests revealed that percentage survivability ranged from 55.60% (*Streptococcus* sp.[1]) – 200.00% (*Citrobacter* sp.[1]). Percentage survival of bacterial isolates to bile salt concentration of 0.3% ranged from 147.80% (*Streptococcus* sp.[2]) – 462.50% (*Escherichia coli*). The antibiotic resistance index ranged from 2 (*Streptococcus* sp. and *Bacillus subtilis*[3]) –8 (*Escherichia coli*). The antibacterial activity of the isolates ranged from 2mm-10mm with *Escherichia coli* and *Citrobacter* species having no activity against any of the test pathogens. *Bacillus subtilis* E2 passed all the test criteria, so it can be recommended as a potential probiotics, while *Escherichia coli* was suspected to be a contaminant due to observed antibiotics resistance. To avoid contaminants in fermented foods, proper hygienic measures, production procedures, storage and cooking should be ensured.

CHAPTER ONE

1.0 INTRODUCTION

Background of study

The increasing awareness of consumers towards a healthy life style has resulted in an ever-growing demand for food products with versatile health benefits including, for example, food items containing probiotic bacteria. The term probiotics refers to live microbial cultures which, when consumed by people or animals (in the form of dehydrated cells or fermented products), can positively affect their health by improving the properties of the original microbiota (Sathyabama *et al*., 2014). Due to the prolonged use of antibiotics as infection treatments, more pathogenic bacteria have developed resistance to these molecules (Galán *et al*. 2013). There is, therefore a need for microorganisms which are not hazardous to human health and which at the same time are effective against such pathogens. One possible mechanism is when healthy bacteria produce substances which are harmful to the pathogens or when they can compete with them for space and nutrients (i.e. colonizing the intestinal cells of the colon). The isolation and screening of microorganisms from natural sources has always been a very powerful strategy to obtain useful and genetically stable bacterial strains (Adnan and Tan, 2006). For example, it is well known that spontaneously fermented foods with mixed cultures are potential substrates to grow bacterial strains. Indeed, in many cases, such microorganisms are particularly able to withstand stress factors due to the complex environment they were isolated from. Lactic acid bacteria (LAB) are a very important microorganism group comprising several probiotic bacteria, among which *Lactobacillus* spp. has been reported to be the most active and safe (i.e. non-pathogenic) microorganism (Salminen and VonWright, 1998). Fermented dairy foods have been conventionally associated with probiotics, some of which include cereal-based products (cassava), condiments and beverages.

According to the World Health organization (WHO) and Food and Drug Organization (FAO), probiotics are live microorganisms that, when administered in the right proportion, confer health benefits on the host (Nwagu *et al*., 2020). Probiotics for example are non-antibiotic growth-promoting additives directly consumed by humans to favourably influence host intestinal microbiota and immune

system functions (Yadav and Jha, 2019). The commonly reported probiotics include the lactic acid bacteria *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Bacillus*, and the yeasts, *Saccharomyces* and *Candida* (Bron *et al*., 2012). Parker RB was the first to use the concept of probiotics as foods with health effects in 1974 before the FAO and WHO adopted it (Bottalico *et al*., 2020). More recently, WHO recognized probiotics as a healthy food choice, rendering them highly relevant regarding nutrition and health (Santacroce *et al*., 2019). *Lactobacillus* and *Bifidobacterium* are bacteria genera commonly used as probiotics (Fijan, 2014). Some probiotic strains have successfully managed bacterial and viral diseases, including respiratory infections (Lopez *et al*., 2021).

Extensive studies showing the efficacy of probiotics against various diseases emphasize their immunomodulatory and stimulatory prop probiotics are associated with beneficial effects for humans, they contribute to intestinal microbial balance and play a role in maintaining health (Soccol *et al*., 2010). Benefits of consumption of probiotics include the prevention and treatment of infantile diarhoea, colon cancer, constipation, antibiotic induced diarrhoea, hypercholesterolaemia, lactose intolerance, vaginitis and intestinal infections (Marchand and Vandenplas, 2000) Probiotics play a vital role in increasing host resistance to colonization by exogenous, potentially pathogenic organisms. This is achieved through different mechanisms such as production of lactic acid, hydrogen peroxide or acetic acid which increases the acidity of the intestine and inhibits the reproduction of numerous pathogenic bacteria (Reid *et al*., 2003). For probiotic bacteria to be effective, they must possess a number of specific properties. One such property is the ability to survive in acidic and bile-containing media as they have to undergo these conditions during their passage through the gastrointestinal tract (Klaenhamer and Kullen, 1999). Moreover, to have beneficial effect on human health, among other eventual functional properties they must show antibacterial behavior towards pathogenic strains, either by producing antimicrobial agents (bacteriocins, organic acids) or by reducing the adhesion of pathogenic bacteria (Gareau *et al*., 2010). In addition, to qualify for a probiotic, a microbial strain must have the ability to exert beneficial effect on the host animal, e.g., increased growth or resistance to disease, be nonpathogenic and non-toxic, be found in the finished product as viable cells in large numbers, be able

to survive and metabolize in the gut environment, and show stability in storage and field conditions. These properties are attributed to the ability of the microorganism to produce acids and/or bacteriocins, and other metabolites which not only have the capacity to boost the host immune systems but also favorably impacts its competitiveness against other microbes. Some probiotics are known to have the ability to stimulate, modulate, and regulate immune response in the host, modulate the release of hormones in the gastrointestinal tract (Kristensen *et al*. 2016), and regulate acute and chronic inflammation in intestinal mucosal tissue caused by inflammatory bowel disease (IBD) progression (Bakirtzi *et al*., 2016).

However, there is paucity of studies on the possible probiotic quality of foods or the probiotic nature of microbial strains actively involved in fermentation especially Bacillus. According to (Burgain *et al*., 2011), probiotics represent about 65% of the global functional food market and have been incorporated into numerous foods amongst which are dairy and non-dairy products (chocolates, cereals and juices). Though probiotics are living organisms, dead bacteria and bioactive compounds produced by live cells can also exhibit probiotic qualities (Chugh and Kamal-Eldin, 2020). Fermentation is a chemical change in foods which is brought about by enzymes from living microorganisms. It is a food processing technique practiced by man for centuries in various parts of the word, especially Africa. The Nigeria indigenous fermented foods constitute a group of foods that are produced in homes, villages and small-scale cottage industries at prices within the means of majority of consumers. They are often referred to as "traditional" fermented foods because they originated hundreds of years ago before written records (Salminen and VonWright, 1998). Fermented foods are important in the Africa diet where they may constitute major staples, adjunct to staples, beverages and condiments. Most African foods produced by fermentation can be used as a main course food, as a side dish or as a flavouring agent to make food more enjoyable or to add aroma to food (Hesseltine, 1983). The fermented foods are derived from various substrates including roots and tubers, legumes, pulses, oilseeds, cereals, saps of palm trees, etc. the tropical climate provides temperatures suitable for such processes in Nigeria for whom such foods are commonplace develop a liking for their strong odors and flavors. Fermentation

leads to a general improvement in the nutritional quality of foods, it is a process that serves as a mean of providing a source of nourishment for large rural populations, it enhances the nutrient content of foods through the synthesis of proteins, vitamin, and essential amino acids (Zhang *et al*., 2010).

1.1 AIM AND OBJECTIVES

The aim of this study was to evaluate the probiotic potential of bacteria isolated from some Nigeria fermented foods.

The specific objectives were to;

i. enumerate, isolate and identify bacteria from some Nigeria fermented foods;
ii. determine the tolerance for acidic pH (3) and bile salt (0.3%) of the bacteria isolated from fermented foods;
iii. evaluate antibacterial activity and antibiotic susceptibility potential of the bacteria isolated from fermented foods.
iv. determine the sodium chloride (Na(cl) tolerance of the bacteria isolated from fermented foods; and
v. determine the haemolytic activity of the bacteria isolated from fermented food

CHAPTER TWO

LITERATURE REVIEW

2.1 FERMENTED FOODS

Food may be processed by fermentation, which is accomplished with the help of microbes, particularly yeast and lactic acid bacteria (LAB). Despite being an antiquated method of food preservation, it remains a customary practice among indigenous people in Africa and the majority of the developing world, typically carried out on a local or family basis (Pundir et al., 2013). Because of their distinct flavor, texture, and color, customers prefer fermented foods over their unfermented equivalents. In developing nations, one of the primary nutritional components is fermented traditional foods and drinks, some of which are consumed as light meals, sauces, or refreshments (Chugh and Kamal-Eldin, 2020).

The preservation of fermented foods' quality and palatability is due to the production of organic acids and a wide variety of antimicrobial agents by lactic acid bacteria (LAB), which have been identified from a variety of fermented foods (Nout et al., 1997). Locally, African cultures employ lactic acid bacteria (LAB) to make a range of fermented foods, including as drinks, fermented fruits and vegetables (such roots or tubers), fermented milks, and fermented meats. Lactic acid and alcoholic fermentations are the most widely utilized forms of fermentations that are used to produce fermented foods and drinks. Alkaline fermentation and fermentation of amino acids are two further forms (Steinkraus et al., 1997). Rural communities are known to be consuming fermented foods laden with probiotic microflora, from which they derive health benefits (Moyane and Jideani, 2013). However, despite an increasing interest in probiotic LAB, there is a paucity of literature regarding novel and emerging uses of LAB as probiotics, especially from the African continent. Additionally, strict regulation by international bodies has resulted in limited probiotic products passing the clinical trial stage. It is established that LAB controls diarrhea and has antimicrobial properties.

In developing nations, particularly Nigeria and other West African nations, plant sources—most notably legumes, grains, and tubers—make up a sizable share of the protein consumed. These plant sources are typically used as an inexpensive way to get energy and protein. Though a wealth of knowledge on the fermented foods high in protein found in Southeast Asia and other countries (Campbell-Platt, 1987). There is a dearth of knowledge on the fermented foods high in protein that are popular in Nigeria and throughout Africa. Depending on their ethnicity or regional preferences, about 80% of Nigerians consume a variety of fermented foods and drinks. The techniques for fermentation of these staple foods are well known by particular ethnic groups and are passed on from generation to generation in certain communities. Common fermented foods, food condiments and beverages in Nigeria are derived from the local staples or other foods prevalent in various Nigerian localities. Such staples include fufu (akpu), ogi, tuwo; fermented beverages include palm wine, ogogoro (distilled palm wine), condiment include, ogiri-isi (Ricinus communis), locust beans, ugba, etc. Fufu or Akpu (cassava meal), (Sathyabama et al., 2014).

Soaked and fermented cassava is eaten as akpu. In Eastern Nigeria, this food is frequently consumed, particularly by elder Igbo tribes who consider other cassava products, such as gari, to be inappropriate. However, a lot of young individuals find the smell or perfume of fufu to be quite irritating. Lactic acid bacteria during the fermentation of cassava provide this scent (Okafor, 1981). The tubers are essentially sliced into big pieces (20 cm long), skinned, cleaned, and then immersed in water for four to five days in earthenware pots or in a gently running stream. During this time, the cassava tuber ferments and softens, releasing HCN in the soak water and giving rise to the distinct flavor of retted cassava meal. It is important to keep the tuber properly immersed to guarantee softening. After being broken down in clean water and sieved, the starchy particles that pass through the sieve are let to settle for three to four hours. While the sediments are packed into a fabric bag, knotted, compressed, and put under intense pressure to release extra water, the water is being decanted. The final dish is formed into balls and cooked for thirty to forty minutes in boiling water (100oC). The cooked material is ground into a paste called akpu in a mortal using a pestle and mortar. This paste can be consumed

with stew, soup, or sauceDry maize (Zea mays) that has been fermented is used to make ogi or akamu. It is a well-liked baby weaning meal and breakfast cereal among Nigeria's Igbo-speaking population. In essence, the procedure involves soaking the corn in water for one to three days, during which time the microorganisms (yeast and lactic acid bacteria) ferment the grain, create acids, and give it a distinct smell (Umo and Fields, 1981). Using a mortar and pestle or a blender, the softened maize is crushed into a meal after being cleaned. To get rid of additional fibrous elements and pieces of the hull, the ground material is combined with water and sieved. The almost pure starch suspension, or filtrate, is put in a saucepan to settle the starchy particles. After decanting the supernatant, the wet starch is transferred into cloth bags, pressed, and dried until a semi-solid starch is achieved. This is kept in a cold location so that samples of the stock may be extracted and cooked to create ogi, also known as akamu, a corn gruel. To improve the flavor, kambu is typically served hot with table suhar. It is eaten by itself as a meal for the disabled, with milk added to increase its nutritional content. Additionally, it may be used to prepare foods for weaning. It is occasionally eaten with akara, or bean balls, which is a common breakfast dish across Nigeria, particularly in the southern regions (Okoli and Adeyemi, 1989).

Rice, millet, guinea corn, maize, or any other cereal can be used to make tuwo. To ferment, the grain is submerged in water in a calabash for the whole night. Spread out on a mat, the wet seed is allowed to dry in the sun. It is pounded into a fine powder and then mixed with hot, boiling water to create a smooth paste. After fermentation, this is kept at ambient temperature for one to four days to allow for optimum taste development. As a preservative, potash is occasionally added. African Yam beans (Owoh) (Sphenostylis stenocardia), cotton plant seeds (Gossypium hirsutum), melon seeds (Ogiri) (Citrullus vulgaris), and fluted pumpkin seeds (Ogiri) (Telfairia occidentalis) were among the Leguminosae used to manufacture fermented sauces (Chukwu et al., 2019). Prior to ingestion by humans and animals, these seeds undergo a natural fermentation process that enhances their nutritional value, improves digestibility, and detoxifies them. However, using starter cultures in the production process would result in better nutritional value, safety, and shelf-life quality. According to reports, the use of Lactobacillus species as starter cultures during fermentation has improved the nutritional value,

digestibility, accessibility, safety, and quality of fermented foods, suggesting that these species may have probiotic properties (Garcia et al., 2021). Due to inadequate cleanliness during production, shipping, and storage, fermented condiments have only currently gained local commercial acceptance (Obafemi et al., 2021). The condiments' distinct taste, sticky texture, and pungent smell are caused by the release of λ-polyglutamic acid metabolite, which is produced by the prolonged fermentation of protein components by fermenting microorganisms during spontaneous fermentation (Ugwuanyi and Okpara, 2019).

The African locust bean, or Parkia biglobosa, is a nutrient-dense vegetable that is high in protein and a few other healthy ingredients. For the majority of individuals whose protein consumption is poor because animal protein sources are expensive, it provides a cheap supply of protein. According to earlier research, locust beans are highly digestible (74%–97%) when pepsin and trypsin are added (Elemo et al., 2011). This is in addition to its great culinary and medicinal agent commercial values (Liman et al., 2010). African locust bean-based condiments fermented by Bacillus species depend heavily on the processes of lipolysis and proteolysis (Allagheny et al., 1996). According to reports, the primary metabolic activity that occurs during African locust bean fermentation is proteolysis (Odunfa, 1985). In Nigeria, a delectable culinary spice known as "iru" is made by fermenting the seeds of the African locust bean tree, or Parkia clappertoniana keay. There are two kinds of "iru": "iru woro," which is a firmer fermented product, and "iru pete," which is a softer, pastier substance that has fermented to include mashed cotyledons. Additionally, the spice gives soup taste and thickness. Parkia biglobosa are traditionally prepared by boiling for 8 to 12 hours, depending on the testa's strength and the effectiveness of the seeds' dehulling. According to Achi et al. (1992), boiling in 0.1 M Na2CO3 shortens the cooking time to 4 hours and boosts the de-hulling effectiveness to 80%. The testa can be extracted by mashing it with your bare foot or with a mortar and pestle. If not, you could want to add some clean sand to help remove the testa. The seeds are next rinsed under running water or in a big container of water using a traditional sieve known as a "ajere," which is constructed of perforated calabash. The popularity of fermentation is likely due to the fact that people in these regions are more

used to the natural organoleptic properties of food items than they are to artificial seasonings or imported spices (Liman et al., 2010). In most African nations and India, where protein calorie malnutrition is a serious issue, fermented food condiments add a nice scent to soups, sauces, and other prepared foods (Liman et al., 2010). Similar to fermented locust beans (Parkia biglobosa), dawdawa is a common condiment used by the Hausa tribe of Nigeria to flavor dishes like stew and soup made from soybeans. In the savannah area of West/Central Africa, it is also a valuable culinary seasoning, accounting for 1.4% of daily caloric intake and 5% of total protein consumption. To make condiments, oil seeds such soybean, melon, castor oil, mesquite, and African locust bean are fermented. The majority of the water samples used for "iru" processing came from surface water sources rather than pipes, which might have contaminated the peeled cotyledons. The study identifies several critical hazard points that are important for public health, such as the use of feet to dehull seed cotyledons, the frequent use of calabashes for fermentation, the addition of salt in open areas, and the wrapping of leaves. Teak (Tectona grandis) leaves are typically picked from the ground near shedding trees and can act as contaminants. Therefore, food safety and microbiological precautions are required. To increase the shelf life of home-produced foods like locust beans (iru), it is preferable to dehydrate, salt, and package them in plain plastic bags. This work involved the isolation and identification of certain bacterial and fungal species linked to certain regionally fermented products found in locust beans. To protect the manufacturing of this food condiment intended for human consumption, research on the microbial risks connected to fermented locust beans was also increased (Hesseltine, 1983).

African oil bean seeds, often known locally as "Ugba" (Pentaclethra macrophylla), are members of the mimosa cease leguminous family. It is often grown in forested places. When the pods reach maturity, they burst and release the seeds. According to Enujiugha and Agbede (2000), the uncooked seed has the ability to provide eatable protein, energy, and fatty acids. After that, the seed is let to ferment before being eaten. Ugba is an alkaline, fermented dish that is traditionally made with crude machinery. It is occasionally added to or served as part of the main course. Nonetheless, it is a significant component of the diets of the people who live in South Eastern Nigeria. Healthy

gastrointestinal function depends on probiotic microorganisms. Probiotics' beneficial effects on intestinal flora include a number of important advantages for the host, including improved nutrition and colon health, immunity system development, and defense against infections (Umesaki and Setoyama, 2000).

The milky sap of several tropical palm trees is naturally fermented to generate palm wine, an alcoholic beverage (Santiago et al., 2021). According to Erukainure et al. (2019), common examples are the date palm (Phoenix dactylifera), raffia palm (Raphia hookeri), coconut palm (Cocos nucifera), nipa palm (Nypa fruticans), and oil palm (Elaeis guineensis). The two main sources of palm sap in Nigeria are oil palms and raphia palms (Ezeronye and Legras, 2009). Because of its abundance in sugar molecules, proteins, alcohol, organic acids, minerals, vitamins, and direct-fed microbial products (yeast, lactic acid-forming bacteria (LAB), and acetic acid-forming bacteria (AAB), which have been shown to have probiotic potential, sap is consumed widely throughout most of West Africa and other parts of the world (Karamoko et al., 2016). According to reports, the sap contains significant amounts of sugar molecules that can support weight increase and profitability in native pigs found across Southeast Asia (Cano et al., 2016). Probiotics and organic acids from palm wine are sources in the creation of fermented foods: Due to its rich nutritional content (sugars, proteins, amino acids, vitamins, and minerals) and high concentration of living microorganisms, including yeast, LAB, and AAB, palm wine from various sources has been extensively researched for its possible health advantages to humans (Djeni et al., 2020). These microorganisms have been linked to the formation of organic acids, the fermentation of sugar, and probiotic qualities. Probiotic and organic acid supplementation in traditional foods has grown in popularity as a means of encouraging fermentation and producing antimicrobial compounds that can effectively colonize the gut and preserve the balance of microbes in the intestinal tract (Dowarah et al., 2017). Distilled palm wine, also known as ogogoro or Kaikai, is a spirit-like beverage made from fermented palm wine. Prior to distillation, palm wine typically ferments for seven days. The distillate has a high alcohol concentration of 35.2% v/v and is referred to locally as "ogogoro" (Goma, 1989).

2.2 MICROORGANISMS ROLE IN THE FERMENTATION PROCESS

LAB is used in the fermentation of food items, together with a variety of other bacteria like pathogens, yeast, and mold. LAB are found naturally in food, particularly in fermented items, and are crucial to the fermentation of nearly all foods and beverages (Nur, 2005). These bacteria are found naturally in soil, water, manure, waste, and plants. They can also produce organic acids that extend the shelf life of fermentation products. These bacteria are also found in parts of the mucous membrane, such as the intestines of humans and animals, the mouth, skin, urinary tract, and genitalia. It is likely that these bacteria have a positive effect on these organs. Following its discovery, LAB showed great promise in a wide range of uses, such as probiotics, medicines, food and feed fermentation starters, biological control agents, and probiotics (Wedajo, 2015).

Probiotics (Hill et al., 2014), antioxidants (Meira et al., 2012), fibrinolytic activity (Kotb, 2012), peptide production (De Mejia and Dia, 2010), poly-glutamic acid (Chettri and Tamang, 2014), degradation of antinutritive compounds (Babalola, 2014), and other functional properties of microorganisms in fermented foods may be important selection criteria for starter culture(s) to be used in the production of functional foods (Badis et al., 2004). Some microbe genera and species are commercially exploited as food fermentation starters, and some of the products are promoted and sold worldwide as nutraceuticals, health foods, functional foods, and therapeutic foods (Thapa and Tamang, 2015). Fermentation of food and beverages using LAB Since the beginning of human civilization, people have included fermented foods into their diets and have utilized them for more than 6,000 years as a way to increase their nutritional content, safety, shelf life, and digestibility (Georgala, 2013). Using microorganisms and its enzymes, lactic acid food fermentation is a method that turns fermentable sugars in food substrates primarily into lactic acid and other restricted products. Both in homes and the food industry, fermentation is frequently employed to preserve food and create a range of food products (Guarner et al., 2008). Due to its low cost, this food preservation method is crucial economically for underdeveloped nations (Nout and Motarjemi, 1997). The microflora that naturally exists on substrates can be the microorganisms that cause fermentation, or they can be added as starter cultures once the

food or drink has been cooked or prepared. Traditional food fermentation is mostly done by LAB, which has been granted the "generally regarded as safe" (GRAS) designation. Foods that have undergone fermentation have better organoleptic qualities and are more palatable. Fermented milks, sour porridges, and alcoholic and non-alcoholic drinks are common fermented food products in Africa (Oyewole, 1997). Worldwide, cereals constitute a staple diet. They can be fermented to obtain desired changes in acidity, flavor, taste, and food digestibility (Nout and Motarjemi, 1997).

Typical samples of the wide variety of traditional fermented foods and drinks found throughout the African continent are given. Apart from diverse fermented plant products, fermented meats and fish are included in the diets of Africans and people around the globe (Anihouvi et al., 2012), however they may not be as prevalent as they once were. However, when used correctly, fermented foods can provide as sufficient sources of probiotic bacteria. Meat that has been infected with a microbial starter culture during processing under regulated circumstances to produce desired qualities is referred to as fermented meat products. Another way to induce meat fermentation is to let the natural flesh microbial flora do the fermenting on their own. The use of cutting-edge technology and microbial cultures in the creation of functional fermented meats is covered in a recent review. According to (Zakpaa et al., 2009), LAB species, Streptococcus species, Staphylococcus species, and Micrococcus species make up the microbial makeup of fermented meats from Ghana. LAB species and Streptococcus spp. are classified as probiotics, while Streptococcus and Staphylococcus spp. are considered harmful. The fermentation techniques used are often native to the area and can be adapted to the local way of life. Nonetheless, the safety and quality of fermented foods in Africa are jeopardized by the absence of uniformity in processing techniques and hygienic standards. Therefore, further study is required to find answers to these problems. Depending on the raw materials that are accessible and the customs in the area, various manufacturing processes, raw ingredients, and microorganisms are used to manufacture fermented meals. Nonetheless, lactic acid, acetic acid, alkali, and alcoholic fermentation are the four primary types of fermentation. While bacteria are responsible for most of these fermentations, yeasts are the main microorganisms employed in the fermentation of alcohol to produce ethanol-containing drinks like

wines and beers, with LAB having a minor part in this process (Nout & Motarjemi, 1997). The majority of fermented foods and drinks are produced with the help of LAB, but little is known about the precise health advantages that these foods offer to their users or the traits of the bacterial strains that are employed in their creation. Furthermore, there is a dearth of research on probiotic bacteria from various fermented foods in Africa that have been used in the food and pharmaceutical sectors (Zakpaa et al., 2009). Variety of traditional fermented foods and drinks from Africa and the bacteria that go along with them: The Nigerian fermented foods that have been marketed include ogi, iru, and akpu; the remaining dishes are still made at home. A fermented food made from millet, sorghum, or maize grains is called ogi. According to Girum et al. (2005), akpu is a fermented cassava product made by shredding fresh roots into mush and putting them in bags to ferment. Iru is a fermented food made from African locust beans. The capacity to keep and transport the product is a prerequisite for the production of these traditional fermented meals. The most efficient way to get strong and genetically stable strains of microbes for industrially significant food items has been to isolate and screen them from natural sources (Adnan, 2007). Nevertheless, more trustworthy instruments like next-generation sequencing and other molecular approaches are still required to define the microbial diversity of the various fermented foods from various African locales. This will guarantee the safety of the food, drink, and pharmaceutical goods as well as allow for the selection of more resilient, probiotic microorganisms with desired traits to be employed as starting cultures.

2.3 PROBIOTIC MICROORGANISMS

According to Hill et al. (2014), probiotics are living bacteria that give the host health benefits when given in sufficient concentrations. According to Saad et al. (2013), probiotic organisms employed in food products need to be able to withstand exposure to bile, stomach secretions, and be able to multiply and colonize the digestive system. Probiotics are widely used as bio-ingredients in various functional fermented foods (Chávarri et al., 2010), and their positive impacts on human health and nutrition are continuously growing (Monteagudo-Mera et al., 2012). Although yeasts and other microorganisms have also been discovered as possible probiotics in recent years, the most often utilized

probiotic bacteria are members of the genera Bifidobacterium and the diverse group of LAB (Lactobacillus, Enterococcus) (Ouwehand et al., 2002). Bacillus coagulans BC30, marketed by Ganeden Biotech, Inc., Cleveland, OH, USA; Lactobacillus acidophilus NCFM, Lactobacillus rhamnosus HN001 (DR20), and Bifidobacterium lactis HN019 (DR10), marketed by Danisco (Madison, WI, USA); L. casei strain Shirota and B. breve strain Yakult, marketed by Yakult (Tokyo, Japan); L. fermentum VRI003 (PCC) marketed by Institut Rosell (Montreal, QC, Canada); Streptococcus oralis KJ3 marketed by Oragenics, Inc. (Alachua, FL, USA); and Saccharomyces cerevisiae (boulardii) marketed by Biocodex (Urushibata et al., 2002). Foods and supplements are often products that contain probiotic microorganisms (Varankovich et al., 2015). The most conventional source of probiotic strains of lactobacilli is fermented milk and beverage products (Shah, 2015); nevertheless, probiotics from commercial sources have also been added to meat products, snacks, and fruit juice. In 2010 Ranadheera et al.

2.4 SOURCES OF PROBIOTIC

Probiotics can be found in cereal-related goods. Lactic acid bacteria (LAB), bifidobacteria, and other microbes derived from fermented foodstuffs have been utilized for millennia in this context. In some parts of Africa or mongolia, spontaneous milk fermentation has a long history, and using advantageous bacteria in fermented foods has been a long-standing custom (Yu et al., 2011). These traditional fermented goods serve as a helpful source of probiotic strains due to their diverse compositions of lab species. Thus, it is not unexpected that the major microbial populations in a recent study that recovered 148 lab strains from kurut, a traditional naturally fermented yak milk from china, were l. Delbrueckii subsp bulgaricus and streptococcus thermophilus (Sun et al., 2011). Furthermore, probiotic-rich yeasts and lactobacillus strains have been identified in kefir grains, Masai milk, and Koumiss, a fermented milk beverage. These bacteria have the capacity to impact immunological responses (Romanin et al., 2010). In order to assess traditional fermented food as possible natural sources of probiotic bacteria, recent investigations were carried out. According to Will et al. (2011), the majority of bacteria found in fermented items are generally members of the Lactobacillus genus.

Remarkably, a Weisella strain was identified from fermented foods in Nigeria and chosen for its probiotic properties in a recent study (Ayeni et al., 2011). One dairy product that may help introduce probiotic bacteria into the human gut is cheese. Italian, Argentinean, and Bulgarian cheeses have all yielded L. plantarum strains that have been isolated (Zago et al., 2011). It's interesting to note that even when collected aseptically, breast milk was found to be non-sterile. This suggests that breast milk may include a naturally occurring bacterial inoculum (West et al., 1979). It has long been believed that the bacteria in breast milk are a result of fecal or skin infection. Despite the fact that the lactobacilli found in human milk differ genetically from those found in skin It has only recently been acknowledged that breast milk is an intriguing source of probiotic LAB and bifidobacteria for inclusion in infant formulas and foods targeted to both pre-term and full-term infants (Martin et al., 2009), and the LAB strains present in breast milk were also observed in the faeces of the corresponding infants (Martin et al., 2003). (Arboleya et al., 2012).

Furthermore, compared to formula-fed infants, breastfed newborns have been found to have less allergies and gastrointestinal tract infections; as a result, the intestinal microbiota of breastfed children may be regarded as being in optimal condition (Solis et al., 2010). The most common bacteria utilized in food and feed fermentation, as well as in the production of probiotics, belong to the diverse LAB genus Lactobacillus. Moreover, during fermentation, Lactobacillus, a kind of Gram-positive bacteria, produce lactic acid as the end product (Giraffa et al., 2010; Chilton et al., 2015). Probiotic bacterial strains can be used in a variety of food products, such as condensed milk containing Lactobacillus acidophilus and Lactobacillus reuteri bacteria, fermented beverages containing inulin from Bifidobacterium animalis and Lactobacillus bacteria, chocolate products containing Lactobacillus paracasei bacteria, and locus beans containing Lactobacillus rhamnosus bacteria (Aragon-Alegro et al., 2007). Furthermore, these strains are also employed to improve human health in both direct and indirect ways. These health benefits include mucosa defense, normal microflora repair, infection prevention, food allergy defense, blood cholesterol reduction, cariogenic activity, mucosal immune system

modulation, improved digestion, and intestinal microflora balance maintenance (Fioramonti et al., 2003).

Probiotics are not just isolated from the human digestive system. Probiotics may be found in the stomachs of a variety of animals, including rats, pigs, and even chickens (Audisio and Benıtez-Ahrendts, 2011). According to Audisio and Benı§tez-Ahrendts (2011), L. johnsonii CRL 1647, which was isolated from the stomach of Apis mellifera L. bees, has been proven to have a positive impact on honeybee colonies. Probiotic strains have also been isolated from the digestive systems of freshwater and marine fish, including rainbow trout, shrimp, and Carassius auratus gibelio (Chu and Zhu, 2011). (Hill and Barnes, 2009). According to other research, probiotic bacteria may also be detected in fermented nondairy substrates (Gallardo-Navarro and Rivera-Espinoza, 2010). Certain bacterial strains isolated from fruits (Lactobacillus plantarum, L. paracasei, and Staphylococcus carnosus) and meat (Lactosepi, L. curvatus, and L. sakei) have been shown in vitro experiments to exhibit functional and metabolic properties similar to those of human intestinal bacteria (Haller et al., 2001). Furthermore, a recent study (Abriouel et al., 2011) reported the isolation of a Lactobacillus strain from brines of naturally fermented Aloren،a green table olives. Additionally, L. buchneri P2, isolated from pickled juice, showed antibacterial activity, tolerance to acid and bile, and a decrease in cholesterol (Zeng et al., 2010).

2.4.1 ANTIMICROBIAL PROPERTIES

Because they produce antimicrobial chemicals like nisin and bacteriocin, several species of LAB that have been isolated from fermented cereal and beverage items show antimicrobial activity (Grosu-Tudor and Zamfir, 2013). According to Chang et al. (2008), some LAB strains isolated from ogi generate antimicrobial substances such bacteriocin by L. lactis BH5 and L. citreum GJ7. In addition to producing bacteriocin, microorganisms used as protective cultures, such as those that create bacteriocin, may also improve the product's taste, texture, and nutritional value (Gaggia et al., 2011). Numerous LAB strains isolated from kimchi are known to generate antimicrobial substances, including pediocin

(produced by P. pentosaceus) and bacteriocin (produced by L. lactis BH5 (Hur et al., 2000) and L. citreum GJ7 (Chang et al., 2008). Strong antibacterial activity against Salmonella typhimurium, E. coli, Staphylococcus aureus, and Listeria monocytogenes has been demonstrated by LAB species isolated from kimchi (Lee et al., 2009). Gram-positive and Gram-negative microorganisms are susceptible to the antibacterial activity of Weissella cibaria, which was isolated from fermented maize base product (Patel et al., 2014). Nisin Z, which is produced by Lactococcus lactis isolated from Indian curd dahi, inhibits both S. aureus and L. monocytogenes (Mitra et al., 2010). Antimicrobial activity against L. monocytogenes, E. coli, Salmonella, and Bacillus has been demonstrated by many LAB species isolated from traditional fermented fruits and vegetables in Romania (Grosu-Tudor and Zamfir, 2013). In addition to producing bacteriocin, microorganisms used as protective cultures, such as those that create bacteriocin, may also improve the product's taste, texture, and nutritional value (Gaggia et al., 2011).

2.4.2 ANTIOXIDANT ACTIVITY

Fermented foods have been found to contain antioxidant activities such as total phenol content (TPC) estimation, reducing power assay, 2,20 -azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid; ABTS) radical scavenging activity, and 1,1-diphenyl2-picryl hydrazyl (DPPH) radical scavenging activity (Liu and Pan, 2010; Abubakr et al., 2012). Numerous fermented foods from Asia, such as natto (Japan), chungkokjang and jang (Korea), douchi (China), kinema (India and Nepal), bekang and tungrymbai (India), thua nao (Thailand), and tempe mold-fermented food (Indonesia) (Nurrahman et al., 2013), are foods that have been fermented by Bacillus. Yogurt and kimchi have also been shown to have antioxidant properties (Park et al., 2011).

2.4.3 PEPTIDE PRODUCTION

Proteolytic microbes produce bioactive peptides when food ferments (De Mejia and Dia, 2010). Peptides found in fermented foods offer a variety of useful characteristics, including immunomodulatory, antithrombic, and antihypertensive effects (Qian et al., 2011; Phelan and Kerins, 2011). Certain Bacillus species are engaged in the enzymatic hydrolysis of amino acids and peptides

that produce proteins and are thought to have health advantages (Nagai and Tamang, 2010). Angiotensin converting enzyme (ACE) inhibitory characteristics have been investigated in a variety of fermented products (Quiros et al., 2005).

2.4.4 PRODUCTION OF ENZYMES BY MICROORGANISMS

Fermenting food is also a great way to encourage the production of highly helpful enzymes by microorganisms. In several Asian fermented soybean cuisines, Bacillus spp. create enzymes during food fermentation that break down complex chemicals to simple bio-molecules for many biological functions, including proteinase, amylase, mannase, cellulase, and catalase (Chettri and Tamang, 2014). Various carbohydrases, including α-amylase, amyloglucosidase, maltase, invertase, pectinase, ß-galactosidase, cellulase, and hemi-cellulase, as well as acid and alkaline proteases and lipases, are produced by common genera of mycelial fungi found in fermented foods and beverages, such as Actinomucor, Amylomyces, Aspergillus, Monascus, Mucor, Neurospora, and Rhizopus (Nout and Aidoo, 2002). Industry employs tata-amylase A (TAA), an enzyme generated by Aspergillus oryzae in koji, extensively. (Suganuma et al., 2007). High amylase activity yeasts, Saccharomycopsis fibuligera, S. capsularis, and Pichia burtonii, are found in dry, solid, cake-like mixed amylolytic starters used in the Himalayan region to produce alcohol (Tamang et al., 2007). According to Mine et al. (2005) and Kotb (2012), Bacillus subtilis subsp. natto in natto generates nattokinase with fibrinolytic activity. B. subtilis and B. amyloliquefaciens, Vagococcus carniphilus, V. lutrae, Enterococcus faecalis, E. faecium, E. gallinarum, and P. acidilactici, as well as Virgibacillus halodenitrificans SK1-3-7 isolated from the fermentation of fish sauce, are among the bacteria isolated from fermented foods that produce fibrinolytic enzymes (Montriwong et al., 2012)

.2.4.5 DEGRADATION OF ANTI-NUTRITIVE COMPOUNDS

Certain bacteria found in fermented foods have the ability to break down anti-nutritive compounds, turning the substrates into edible products (Tamang, 2015). Processing techniques used to reduce residual cyanide concentration in African fermented cassava products, such as peeling, washing,

grating fermentation, dewatering, and roasting, include fufu and gari (Babalola, 2014). In the traditional method of producing gari and fufu, bitter cassava tuber varieties contain cyanogenic glycosides called linamarin and lotaustralin. These glycosides are detoxified by species of Leuconostoc, Lactobacillus, and Streptococcus, resulting in hydrocyanic acid (HCN), which has a low boiling point and escapes from the dewatered pulp during toasting, making the product safe for human consumption (Lambri et al., 2013). Rhizopus oligosporus converts indigestible oligosaccharides as verbascose and stachyose into absorbable monosaccharides and disaccharides in tempe by eradicating flatulence (Sanchez, 2008). It has been documented in kinema that B. subtilis degrades anti-nutritive substances (Sarkar et al., 1997). Indian fermented grain foods like rabadi and idli have lower levels of phytotic acid during fermentation (Gupta et al., 1992).

2.5 CRITERIA FOR SELECTING POTENTIAL PROBIOTICS

According to Casey et al. (2004) and Yadav and Shukla (2017), a successful probiotic should be safe, able to withstand bile salt and gastric juice, able to attach to and colonize gut epithelial cells, phenol tolerant, active against pathogenic strains, and free of hemolytic activity

.2.5.1 Acidic pH and Bile Tolerance

In the gastrointestinal system, the presence of acid and bile salt serves physiological objectives in the body but can also be toxic to bacteria that cannot adapt to these circumstances, leading to their demise (Ruiz et al., 2013). These characteristics control the probiotics' capacity to survive and carry out their tasks in the small intestine (Ruiz et al., 2013). Probiotic strains that are tolerant to bile and acid have also demonstrated resistance to other stressors (Margolles et al., 2003), demonstrating their adaptability to a range of stressors (Sanchez et al., 2012).

2.5.2 Antibacterial Activity

Probiotics are consumed nowadays in an effort to inhibit pathogenic microbes and prolong the shelf life of food items. Antimicrobial compounds such as hydrogen peroxide, lactic acid, and bacteriocin are

released by these probiotics. Additionally, they compete with pathogens, which strengthens the host's defenses against infection (Soccol et al., 2010).

2.5.3 Antibiotic Resistance

A increasing ecological concern brought on by the indiscriminate and extensive use of antibiotics in veterinary medicine, human healthcare, cattle agriculture, and other industries is the creation of antibiotic-resistant bacteria. Using probiotics rather than antibiotics to treat specific illnesses in people and animals may help lessen the selection pressure on microbes that results in antibiotic resistance. It's crucial to remember, nevertheless, that the probiotic bacterial strains utilized in these applications also carry some danger since they can include genes that cause antibiotic resistance, which would propagate the resistance (Imperial and Ibana, 2016).

2.5.4 Phenol Tolerance

Dietary proteins include aromatic amino acids, which the gut flora can convert into phenols. These phenolic compounds have the ability to impede probiotic development. Thus, probiotics' survival in the digestive system depends on their capacity to withstand phenols (Yadav and Shukla, 2017).

2.5.5 Sodium Chloride Tolerance

It has been demonstrated that eating a diet high in salt affects the kinds of bacteria that make up the gut microbiome. LAB species are an essential component of a healthy human microbiome, and studies have indicated that high salt diets specifically decrease their population (Schroeter et al., 2013).

2.5.6 Haemolytic Activity

It is advisable to select a probiotic strain that does not exhibit hemolytic activity since this suggests that the bacteria are not pathogenic. Additionally, the lack of hemolysin keeps these strains from becoming opportunistic virulent (Perez et al., 2014).

2.6 HEALTH BENEFITS OF FERMENTED FOODS

According to Shin and Jeong (2015), ethnic cuisines contain built-in mechanisms that function as both food and medicine to satisfy hunger and heal illnesses. In addition to active physical activity, a healthy environment, happiness, and other various factors, the Okinawa prefecture in Japan is known for having the longest lifespans. Traditional and cultural foods like natto, miso, tofu, shoyu, fermented vegetables, cholesterol-free, low-fat, and high bioactive-compounded foods are also likely contributing factors (Willcox et al., 2004). It has been suggested that Korean kimchi possesses health-promoting properties (Park et al., 2014). Additionally, kimchi possesses anti-aging properties (Kim et al., 2002). High concentrations of nattokinase, isoflavones, saponins, vitamin K, unsaturated fatty acids, probiotics, and immunomodulating properties, mostly generated by B. subtilis, are only a few of the health advantages of natto (Nagai, 2015). Additionally, kinesema has certain health-promoting properties (Tamang, 2015). Famous fermented milk drink dahi from India possesses anti-carcinogenic qualities (Arvind et al., 2010). Kimchi's lactic acid may help treat obesity-related cardiac problems and prevent fat from accumulating (Park et al., 2008). Clinical experiments have shown that wine has anti-aging properties due to the presence of melatonin, which controls the body clock; anti-obesity benefits have also been observed in kimchi (Park et al., 2012) and doenjang (Kwak et al., 2012). Although scientific studies and confirmation of the health benefits claims of nearly all naturally fermented foods and drinks of the world need to be examined, ethnic groups have a traditional belief in the therapeutic properties of several of their ethnic cuisines, including fermented foods and beverages.

2.6.1 SYNTHESIS OF NUTRIENT

During food fermentation, vitamins, vital amino acids, and bioactive substances are added to substrates (Thapa and Tamang, 2015). Rhizopus oligosporus is found to increase the contents of folic acid, niacin, riboflavin, nicotinamide, and pyridoxine in tempe, an Indonesian soybean food fermented by mold (Astuti, 2015). On the other hand, nonpathogenic strains of Klebsiella pneumoniae and

Citrobacter freundii synthesize vitamin B12. (Keuth and Bisping, 1994). Idli, a fermented rice-legume dish popular in India and Sri Lanka, has higher levels of thiamine, riboflavin, and methionine throughout the fermentation process (Ghosh and Chattopadhyay, 2011). Similar to this, it has been shown that the fermentation of pulque, a Mexican alcoholic beverage derived from cactus plants, increases the contents of iron, lysine, and tryptophane. Additionally, it has been observed that several Asian fermented foods manufactured with Bacillus bacteria have higher levels of niacin and riboflavin (Nagai, 2015). It was discovered that L. mesenteroides and L. sakei manufacture riboflavin and folic acid in kimchi (Jung et al., 2013). Vitamin B12 is produced by the yeasts Saccharomyces cerevisiae, Candida tropicalis, Aureobasidium sp., and Pichia manschuria that are identified from fermented cereal dishes of Pakistan and India, such as idli and jalebi (Syal and Vohra, 2013). Fermented soybean meals have higher levels of free amino acids (Dajanta et al., 2011).

2.6.2 PREVENTION OF HYPERTENSION AND HEART DISEASE

Clinical experiments and animal models have been used to validate the antihypertensive characteristics of numerous fermented milk products (Seppo et al., 2002). According to Liu and Pan (2010), consuming fermented foods such as soybeans and fermented milks, along with probiotic bacteria, can reduce the risk of heart disease. The blood levels of LDL cholesterol, hypertriacylglycerolaemia, hypertension, coronary heart disease, insulin resistance, and hyperhomocysteinemia can all be lowered by eating whole grain meals that have undergone fermentation (Anderson, 2003). Certain fermented foods, such as tempe, kefir, and fermented soybean meals, lower cholesterol levels when consumed (Otes and Cagindi, 2003). Because mevinolin citrinin is present, Monascus purpureus, which is locally known as "angkak" in China, prevents the production of cholesterol by obstructing the enzyme HMG-CoA reductase (Pattanagul et al., 2008). Chinese fermented tea has been shown to protect against heart disease (Mo et al., 2008). Antihypertensive qualities have been noted in natto (Nagai, 2015) and tempe (Astuti, 2015), two Asian fermented soybean foods. Doenjang, a fermented soybean dish from Korea, contains isoflavone, which is crucial in avoiding heart disease (Shin et al., 2015). Consuming fermented whole grains may help prevent the

onset of diabetes and heart disease (Anderson, 2003). It's healthy to consume fermented drinks in moderation (Walker, 2014). Red wine's polyphenols likely work in concert with ascorbic acid (Vitamin C) and tocopherol (Vitamin E) to prevent lipid peroxidation (Feher et al., 2007). Patients with Type 2 diabetes and hypertension who regularly consume Korean fermented soybean foods see improvements in cardiovascular risk factors and a decrease in the hypocholesterolemic impact (Jung et al., 2014). (Lim et al., 2014). Food-derived ACE inhibitory peptides are utilized to treat hypertension (Jakubczyk et al., 2013). Rich in fibrinolytic enzymes, fermented foods can help prevent quickly developing cardiac problems by thrombolytic treatment (Singh et al., 2014).

2.6.3 PREVENTION FROM CANCER

Antimutagenic and anticarcinogenic properties have been found in some LAB-fermented foods (Lee et al., 2004). Cancer patients are treated with kefir (Otes and Cagindi, 2003). German fermented vegetables, such as sauerkraut, contain s-methylmethionine, which lowers the incidence of stomach cancer (Kris-Etherton et al., 2002). ß-glucuronidase, azoreductase, and nitroreductase (which accelerate the conversion of procarcinogens to carcinogens) are decreased, procarcinogens are likely removed, and consumers' immune systems are activated when fermented milk products containing live L. acidophilus cells are consumed (Macouzet et al., 2009). Indian dahi possesses anti-carcinogenic properties as well (Mohania et al., 2013). There have been reports of W. cibaria and L. plantarum's ability to prevent cancer in kimchi (Kwak et al., 2014). Yogurt consumption has been shown to lower the risk of bladder, colon, and cervical cancer (Chandan and Kilara, 2013).

2.6.4 PROTECTION AGAINST GASTROINTESTINAL DISORDERS

According to Verna and Lucak (2010), lactic acid bacteria found in fermented foods may reduce the frequency, duration, and severity of some gastrointestinal diseases. According to Orel and Trop (2014), the administration of certain Lactobacillus strains improves ulcerative colitis, paucities, and inflammatory bowel disease. According to Chung et al. (2014), L. rhamnosus GG is useful in treating acute diarrhea, and giving L. helveticus-fermented milk to healthy older persons improved their

cognitive performance. Live bacteria found in fermented milk products have the ability to modulate the immune system and treat diarrhea (Balamurugan et al., 2014). Kimchi from Korea can help manage inflammatory bowel conditions (Lim et al., 2011).

2.6.5 ANTI-ALLERGIC REACTIONS

According to Hong et al. (2010), *Lactobacillus kefiranofaciens* M1, which is isolated from kefir grains, possesses anti-allergic properties. As fermented milk products mature, casein digestion has been demonstrated to aid in the loss of allergic reactivity, increasing tolerance (Alessandri et al., 2012). According to Lee et al. (2014), Chongkokjang contains anti-allergic properties that include thicker dermis, thinner ears, auricular lymph nodes, and mast cell infiltration. According to research conducted by Won et al. (2011), lactobacillus species isolated from kimchi have the capacity to treat atopic dermatitis and food allergies by modulating the Th1/Th2 balance through the production of substantial amounts of IL-12 and IFN-γ. Omega-3 polyunsaturated fatty acid-rich fermented fish oil can lessen allergy sensitivity (Han et al., 2012).

2.6.6 PROTECTION FROM DIABETES AND OSTEOPOROSIS

Consuming high-fiber meals may improve insulin sensitivity in those without diabetes and reduce the need for insulin in those with diabetes (Meyer et al., 2000). (Fukagawa et al., 1990; Anderson, 2003). According to Yadav et al. (2007), a probiotic-supplemented diet considerably reduces the risk of diabetes by delaying the development of glucose intolerance, hyperglycemia, hyperinsulinemia, oxidative stress, and dyslipidemia. Chungkokjang increases insulin resistance when used daily, controlling diabetes (Tolhurst et al., 2012). Natto contains vitamin K2, which promotes bone growth and helps elderly Japanese women avoid osteoporosis (Yanagisawa and Sumi, 2005). According to Chandan and Kilara (2013), minerals including calcium, phosphorus, potassium, magnesium, and protein found in yogurt work in concert to support the development of strong bones.

2.6.7 ALLEVIATION OF LACTOSE MALABSORPTION

A deficiency of ßD-galactosidase causes lactose, the main carbohydrate in milk, to not be fully broken down into glucose and galactose, a disease known as lactose malabsorption (Shah, 2015). Lactobacillus delbrueckii subspecies bulgaricus and Streptococcus thermophilus, which are used to produce yogurt, contain significant amounts of ß-D-galactosidase, which helps lactose intolerant individuals with their lactose malabsorption symptoms (Shah et al., 2013). It has been shown that consuming fresh yogurt (with live yogurt cultures) improves lactose absorption and digestion more than consuming a pasteurized product (Pedone et al., 2000). Kefir provides an additional supply of β-galactosidase, which can help reduce the symptoms of lactose intolerance (Hertzler and Clancy, 2003).

2.7 HEALTH RISK OF FERMENTED FOODS

The presence of biogenic amines in fermented foods is one of the major health hazards. According to Zhao et al. (2012), biogenic amines are low molecular weight organic compounds that are produced either by microbial decarboxylation of their precursor amino acids or by amino acid transaminases transaminating aldehydes and ketones. These compounds can be found in some fermented foods like fish products, cheese, wine, beverages, dry sausages, and sauerkraut (Visciano et al., 2014). Major producers of biogenic amines in food include Enterobacteriaceae and Enterococci (Nout, 1994). It is possible to classify foods high in biogenic amines as harmful (Latorre-Moratalla et al., 2010). Human health may be negatively impacted by high concentrations of histamine and tyramine (>100 mg/kg) (Rauscher-Gabernig et al., 2009). The amount of biogenic amines in sauerkraut may be decreased by fermenting cabbage with certain lactic starters, such as Lactobacillus casei subsp. casei, Lactobacillus plantarum, and Lactobacillus curvatus (Rabie et al., 2011). Small amounts of histamine in food have little effect on healthy people, but in those who have diamine oxidase activity impairment (due to genetic predisposition, gastrointestinal disorders, or monoamine oxidase inhibitor medication), it can cause histamine intolerance (Maintz and Novak, 2007). A safe quantity of histamine for ingestion in food is indicated by a maximum limit of 100 mg/kg (Halász et al., 1994).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection and preparation

Samples of Ogi (Akamu), iru (*Pakia biglobosa*), ogiri-egusi (*Cucumeropsis mannii*), ugba ((*Pentaclethra macrophylla*), tuwo (*Oryza sativa*), ogogoro (Ethyl alcohol), fufu (*Manigot esculenta*) and palm-wine (*Palmyra*), were all purchased from Benin city, Edo state. They were collected in a polythene bag and taken to the laboratory immediately.

3.2 Sterilization of materials

All materials used for the experiments such as the glass-wares, conical flasks, beakers, pipette and measuring cylinders were thoroughly washed with detergent and rinsed. Then they were sterilized in an autoclave at 160°C for 1 hr. The inoculating loop was dipped into ethanol and flamed each time it was used. Bench tops were disinfected with ethanol to ensure a contamination free area.

3.3 Preparation of culture media

The media used were prepared according to the manufacturer's instructions. The media used were MRS Agar, Nutrient Agar and Mueller-Hinton Agar.

3.3.1 Preparation of Nutrient Agar

Twenty eight grams of nutrient agar (NA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 45-50°C and then dispensed aseptically into sterile Petri dishes.

3.3.2 Preparation of MRS (De Man, Rogosa and Sharpe) Agar

Sixty six point seventy three grams of MRS agar was suspended in 1 litre of distilled water in a conical flask, it was heated to dissolve completely. Then sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 50°C, mixed well and poured aseptically into sterile Petri dishes.

3.3.3 Preparation of Mueller Hinton Agar

Thirty eight grams of Mueller Hinton Agar was suspend in 1 litre of distilled water in a conical flask and heated to dissolve completely. Then it was sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 50°C, mixed well and poured aseptically into sterile Petri dishes.

3.4 Preparation of Nutrient Agar

Twenty eight grams of nutrient agar (NA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 min. The medium was cooled to 45-50°C and then dispensed aseptically into sterile Petri dishes.

3.4.1 Isolation of bacteria

One gram of each sample was weighed and dissolved in 9 ml of sterilized water. Aliquot volumes of serially diluted food samples were transferred to sterile petri plate, The nutrient agar plates were inoculated at a dilution faction of 10^{-6} while the De Man, Rogosa and Sharpe plates were inoculated at a dilution factor of 10^{-4}. The prepared nutrient agar and MRS agar (for bacteria growth) was poured in aseptically using pour plate method and incubated at 37°C for 24 hr. After successful growth of microorganisms, the colonies were counted with a colony counter and the results per dilution count

were recorded. The number of colony forming unit per gram was calculated with the formula (Aneja, 2018).

$$\text{Cfu/g} = \frac{number\ of\ colonies}{volume\ plated\ x\ dilution\ factor}$$

3.4.2 Pure culture

One single colony was identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate and incubated at 37°C for 24 h.

Cultural characteristics

Each colony morphology e.g., size, shape, margin, elevation, consistency, color, transparency was determined.

3.5 Identification of Bacterial Isolates

3.5.1 Cultural characteristics and Morphological Characteristics

Each colony characteristic including size, shape, margin, elevation, consistency, color, and transparency.

3. 5.1.1 Gram staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective (x100) (Atlas, 1986).

3. 5.2 Biochemical Tests

3. 5.2.1 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive (Garrity, 2012).

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3. 5.2.2 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30sec indicates a positive oxidase test (Garrity, 2012).

3. 5.2.3 Urease Test

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea broth and incubated at 37°C for 24-48 hr. Urease positive cultures produced a red-pink colour due to changes in the colour of the indicator (Kirsop and Doyle, 1991).

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

3. 5.2.4 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37ºC for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result (Garrity, 2012).

3. 5.2.5 Hydrogen Sulphide (H2S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe2+) or lead (Pb^{2+}) ion as H_2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produced reacts with sulphur metal salt (ferrous sulphate) forming a visible insoluble black sulphide precipitate (Kirsop and Doyle, 1991).

3. 5.2.6 Indole Test

Inoculate broth with the test organism and incubate for 18 – 24 hr at 37^0C. Add 5 ml of Kovac's reagent down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of indole and is a positive test wile absence is negative (Rao, 1999).

3. 5.2.7 Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. The growth medium comprised of peptone water, sugar (1%) and the indicator (bromocresol purple). The mixture was dispensed into test tubes and sterilized by autoclaving at 121°C for 15 min. After sterilizing, tubes were allowed to cool and then inoculated with the isolates and incubated at 37°C for 24 hr. Acid and gas production or acid only were observed after about 24 hr of incubation. Acid production was indicated by the change of the medium from purple to yellow colour indicated a positive test (Rao, 1999).

Sugars used are: lactose, sucrose, glucose, fructose, maltose, starch and sorbitol

3.6 Determination of Acid Tolerance

De Man, Rogosa and Sharpe broth was prepared and pH adjusted to pH 3 with 1 M HCl or NaOH and autoclaved at 121°C for 15 min. Standardized culture were inoculated into the sterilized MRS broth at pH 3 and placed in the incubator. A 100 µL of the 10fold serially diluted isolates were pour plated using MRS agar at 1 and 3 hr intervals and incubated 37°C for 24 hr. Plates were observed for growth and counted after incubation period (Singhal *et al.,* 2010; Desai, 2008).

3.7 Determination of Bile tolerance

According to Liong and Sha (2005) method, isolates were inoculated into MRS broths containing 0.3% bile and incubated at 37°C for 24 hr. After incubation period 100 µl of cultures were transferred to MRS agar by pour plate method at 1 and 3 hr then incubated at 37°C for 24 hr. The growth of isolates on the agar plate was used to confirm isolates as bile salt tolerant

3.8 Determination of Antibacterial activities

The inhibitory effects of bacterial isolates were test against selected clinical test strains using the agar well diffusion method (Nowroozi *et al.,* 2012). Three Indicator organisms (entero-pathogens) namely *Escherichia coli, Salmonella* sp. and *Klebsiella* sp. obtained from University of Benin Teaching Hospital were streaked on nutrient agar plates with an L-shaped glass rod. Then holes (6 mm in diameter) were aseptically punched with a cork borer. 100 µl overnight De Man, Rogosa and Sharpe broth cultures of the isolates were introduced into the holes and incubated at 37°C for 24 hr. After incubation period, inhibitions were observed as clear zones extending laterally from the border of the isolates and diameter was measured using a meter rule.

3.9 Determination of Antibiotic susceptibility

Test organisms were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion method on a Nutrient agar media. The isolates were tested against 14 antibiotics which include pefloxacin (10µ), gentamycin (30 µg), amplicox (30 µg), zinnacef (20 µg), amoxicillin (30 µg), rocephin (25 µg), ciprofloxacin (30 µg), streptomycin (30 µg), septrin (30 µg), erythromycin (10 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), augmentin (10 µg), and tarivid (10 µg). The antibiotic discs were carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates were inverted and incubated for 37°C for 24 hr. The diameters of the zone of inhibition were measured

in millimeters (mm) using a meter rule. The experiments were carried out in triplicates to minimize probability of error (Joseph *et al.,* 2020).

3.9.1 Determination of Tolerance to sodium chloride (NaCl)

Test organisms were subjected to 2% sodium chloride. A volume of 5 ml of nutrient broths (supplemented with 0.5% dextrose) containing 2% NaCl concentration (w/v) and 1ml of bromecresol purple indicator was added to 50ml broth and sterilized. The tubes were inoculated with 1% overnight cultures and then incubated at 37ºC for 24h. The change of the colour from purple to yellow indicated acid production (Lee *et al*., 2013).

3.9.2 Determination of Tolerance to Haemolytic activity

The test organisms were streaked on 5% sterile defibrinated blood agar and incubated at 37 °C for 24 hr. The blood was obtained aseptically from the Faculty of Veterinary Medicine Animal Research Laboratory, University of Benin, Edo State. Isolates that formed a green zone around the colony were designated as alpha hemolytic while those that formed a clear zone were denoted as beta hemolytic and also those without lysis nor clear zone were denoted as gamma hemolytic (Anand *et al*. 2000).

CHAPTER FOUR

RESULTS

A total of 13 isolates were obtained from the fermented foods. The mean heterotrophic bacterial counts of all fermented foods ranged from 5.00±0.28 (ogiri) – 8.70±0.42 $\times 10^7$ cfu/g (iru) while the lactic acid bacteria counts ranged from 0.80±0.42 (iru)-5.00±0.42$\times 10^4$cfu/g (ogogoro) as shown in Table 1.

The cultural, morphological and biochemical characteristics of all bacterial isolates revealed the presence of *Citrobacter* sp., *Streptococcus* sp., *Lactobacillus* sp*., Lactobacillus fermentum* and *Bacillus* sp. in ogi, *Klebsiella pneumoniae* in fufu, *Bacillus subtilis* in iru, ugba and ogiri-egusi, *Escherichia coli* in ogogoro, *Lactobacillus fermentum* in tuwo and *Lactobacillus* sp in palm wine as shown in Table 2..

The distribution pattern of bacterial isolates is shown in Table 3. The percentage frequency of occurrence of bacterial isolates showed that *Eshcherichia coli*, *Klebsiella pneumoniae, Citrobacter* sp, and *Bacillus* had the lowest frequency of (8.00**%**), followed by *Streptococcus* sp., *Lactobacillus* sp. and *Lactobacillus fermentum* with the frequency of (15.00%) while *Bacillus subtilis* had the highest frequency (23.00**%**) as shown in table 4.

The result of the acid tolerance tests performed on the bacterial isolates revealed that percentage survivability ranged from 55.6% (*Streptococcus* sp.) – 130.00% (*Lactobacillus fermentum*.) as shown in

Table 5. Percentage survival of bacterial isolates to bile salt concentration of 0.3% revealed that 148.00% (*Streptococcus* sp) had the least tolerance while 462.50 % (*Escherichia coli*) had the highest tolerance as in shown in Table 6.

The antibiotic sensitivity pattern of bacterial isolates using ten tips multiple disc are recorded in Table 7. The diameter of zones of inhibition of bacteria isolated from fermented foods against test pathogens ranged from 2 mm-10 mm. *Escherichia coli, Klebsiella pneumonia* and *Citrobacter* sp showed no antibacterial activity against the test organisms. However, *Bacillus subtilis*[2], *Bacillus subtilis*[3], *Lactobacillus fermentum2* and *Streptococcus* sp had zones of inhibition of 10mm each against *Salmonella* sp. and *Escherichia coli* respectively.

Table 1: Total heterotrophic bacterial counts of fermented foods

Sample	**Heterotrophic bacterial counts (10^7cfu/g)**	**Lactic acid bacterial counts (10^4cfu/g)**
Ogi	6.00±0.28	3.30±0.99
Fufu	8.40±0.57	1.30±0.42
Iru	8.70±0.42	0.80±0.28
Ugba	7.70±0.42	1.80±0.28
Tuwo	7.00±0.58	2.20±0.28
Ogo	6.10±0.71	5.00±0.42
Palm	6.40±0.57	1.10±0.42

Ogiri-egusi	5.00±0.28	2.60±0.57

Key O=Ogi F=Fufu I= Iru U= Ugba T=Tuwo Og=Ogogoro P=Palm-wine E= Ogiri-egusi

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolate

	T1	T2	T3	T4	T5	T6	T7	T8	T9	B10	B11	T12	T13
Shape	Round	Irregular	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
Size	Small	Small	Medium	Medium	Small	Small	Medium	Small	Small	Small	Medium	Medium	Medium
Elevation	Flat	Flat	Raised	Raised	Convex	Flat	Raised	Raised	Flat	Flat	Flat	Flat	Flat
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Margin	Entire	Undulate	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Agar 1 (NA)	Cream	Cream	Off white	Cream	Cream	Cream							
Agar 2 (MRSA)							Cream	Cream	Cream	Cream	Cream	Cream	Cream
Gram stain	+	+	-	-	-	+	+	+	+	+	+	+	+
Cell type	Rod	Rod	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell Arrangement	Single	Chain	Pair	Pair	Single	Pair	Chain	Chain	Single	Pair	Chain	Single	Chain
Urease	-	-	-	+	+	-	-	-	-	-	-	-	-
Indole	-	-	+	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	-	+	+	-	-	+	-	-	+	-	-
Catalase	+	+	+	+	+	-	-	+	-	-	+	-	-
H_2S	-	-	-	-	+	-	-	-	-	+	-	+	-
Oxidase	-	-	-	-	-	-	-	-	-	-	+	-	-
Lactose	+	-	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	-	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	+	+	-	-	-	-	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	-	+	+	+	+	+	+	+
Probable Organism	*Bacillus subtilis*	*Bacillus subtilis* 2	*Escherichia coli*	*Klebsiella pneumonia*	*Citrobacter* sp.	*Streptococcus* sp. 1	*Lactobacillus* sp. 1	*Bacillus ubtilis* 3	*Lactobacillus* sp. 2	*Lactobacillus fermentum* 1	*Bacillus* sp	*Lactobacillus fermentum* 2	*Streptococcus* sp. 2

Table 3a: Distribution of bacterial isolates in fermented foods

Bacterial Isolates	Ogi	Fufu	Iru	Ugba	Tuwo	Ogo	Palm	Ogiri egusi
Bacillus subtillis	-	-	+	+	-	-	-	+
Escherichia coli	-	-	-	-	-	+	-	-
Klebsiella pneumonia	-	+	-	-	-	-	-	-
Citrobacter sp.	+	-	-	-	-	-	-	-
Streptococcus sp.	+	-	-	+	-	-	-	-
Lactobacillus sp	+	-	-	-	-	-	+	-
Lactobacillus fermentum sp	+	-	-	-	+	-	-	-
Bacillus sp.	+	-	-	-	-	-	-	-

Key: + = present - = absent Ogo= Ogogoro Palm = palm wine

Table 3b: Frequency of occurrence of bacterial isolates

Bacteria	Number of Isolates	Percentage (%)	Occurrence
Bacillus subtilis	3	23%	
Escherichia coli	1	8%	
Klebsiella pneumoniae	1	8%	
Citrobacter sp.	1	8%	
Streptococcus sp.	2	15%	
Lactobacillus sp	2	15%	
Lactobacillus fermentum	2	15%	
Bacillus sp	1	8%	
Total	**13**	**100%**	

Table 4a: Acid tolerance of bacterial isolates to pH (3)

Bacterial Isolates	Viable Bacterial Counts ($\times 10^4$cfu/g)		
	1hr	**3hr**	**Percentage Survival (%)**
Bacillus subtilis 1	2.80	3.10	110.70
Bacillus subtilis 2	2.70	3.20	118.50
Escherichia coli	1.50	2.20	146.70
Klebsiella pneumoniae	0.80	0.50	65.50
Citrobacter sp. I	0.90	1.80	200.00
Streptococcus sp. 1	0.90	0.50	55.60
Lactobacillus sp. 1	2.60	3.30	126.90
Bacillus subtilis 3	2.70	2.10	77.80
Lactobacillus sp 2	2.20	3.30	150.00
Lactobacillus fermentum 1	0.40	3.00	125.00
Bacillus sp.	3.10	4.00	129.00
Lactobacillus fermentum 2	2.70	3.50	129.60
Streptococcus sp. 2	0.60	0.40	66.70

Table 4b: Tolerance of bacterial isolates to bile salt (0.30%)

Bacterial Isolates	**Viable Bacterial Counts ($\times 10^4$cfu/g)**		
	1hr	**3hr**	**Percentage Survival (%)**
Bacillus subtilis 1	2.20	4.00	181.80
Bacillus subtilis 2	2.00	4.40	220.00
Escherichia coli	0.80	3.70	462.50
Klebsiella pneumoniae	2.00	3.20	160.00
Citrobacter sp. I	2.00	3.20	160.00
Streptococcus sp. 1	1.80	4.30	238.90
Lactobacillus sp. 1	2.00	4.10	205.00
Bacillus subtilis 3	1.90	3.40	179.00
Lactobacillus sp 2	1.90	4.00	211.00
Lactobacillus fermentum 1	2.00	4.10	205.00
Bacillus sp.	2.80	5.25	185.70
Lactobacillus fermentum 2	2.00	4.90	245.00
Streptococcus sp. 2	2.30	3.40	147.80

Table 5: Antibacterial activity of bacterial isolates

	Diameter of zone of inhibition (mm)		
ISOLATES	*Escherichia coli*	*Salmonella* sp.	*Klebsiella* sp.
Bacillus subtilis[1]	-	4.00	6.00
Bacillus subtilis[2]	4.00	10.00	4.00
Escherichia coli	-	-	-
Klebsiella pneumoniae	-	-	-
Citrobacter sp.	-	-	-
Streptococcus sp.[1]	8.00	-	6.00
Lactobacillus sp.[1]	6.00	2.00	-
Bacillus subtilis[3]	10,00	-	4.00
Lactobacillus sp.[2]	8,00	-	2.00
Lactobacillus fermentum[1]	6.00	4.00	4.00
Bacillus sp.	4.00	-	2.00
Lactobacillus fermentum[2]	10.00	2.00	4.00
Streptococcus sp.[2]	10.00	400	8.00

The antibiotics susceptibility patterns of the bacterial isolates were determined, it showed that, *Bacillus subtilis*, *Lactobacillus* sp. were susceptible to pefloxacin, ampliclox and erythromycin, while *Lactobacillus fermentum* and *Lactobacillus* sp were resistant to gentamycin, ampliclox, zinnacef, ciprofloxacin and streptomycin.

The acid tolerance tests performed on the bacterial isolates revealed that percentage survivability ranged from 55.6% (*Streptococcus* sp.) – 200.0% (*Citrobacter* sp.) as shown in Table 4a. Percentage survival of bacterial isolates to bile salt concentration of 0.3% revealed that 147.80% (*Streptococcus* sp.) had the least tolerance while 462.5 % (*Escherichia coli*) had the highest tolerance as in shown in Table 4b.

The tolerance to NaCl of the isolates test result in the presence of 2% sodium chloride showed that *Streptococcus* sp[1] and *Streptococcus* sp[2] produced no colour change, which make them not capable of growing in a salt concentration, while *Escherichia coli* showed a week colour change.

The haemolytic reaction carried out showed *Lactobacillus* species appeared to be safe since they do not degrade mucus and did not exhibit β-haemolysis.

Table 6: Antibiotics sensitivity test of bacterial isolates

Isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	RI
Bacillus subtilis[1]	18(S)	2(R)	24(S)	0(R)	18(S)	0(R)	16(I)	0(R)	16(I)	20(S)	4
Bacillus subtilis[2]	20(S)	12(I)	20(S)	6(R)	14(I)	10(R)	18(S)	0(R)	14(I)	18(S)	3
Streptococcus sp.[1]	20(S)	10(R)	18(S)	16(I)	14(I)	12(I)	16(I)	12(I)	0(R)	18(S)	2
Lactobacillus sp.[1]	22(S)	18(S)	0(R)	0(R)	16(I)	0(R)	0(R)	4(R)	6(R)	24(S)	6
Bacillus subtilis3	14(I)	18(S)	18(S)	0(R)	20(S)	14(I)	22(S)	18(S)	0(R)	16(I)	2
Lactobacillus Sp.[2]	16(I)	0(R)	8(R)	12(R)	14(I)	0(R)	4(R)	0(R)	12(I)	26(S)	5
Lactobacillus Fermentum[1]	12(I)	6(R)	6(R)	8(R)	14(I)	10(R)	2(R)	0(R)	0(R)	18(S)	7
Bacillus sp.	20(S)	24(S)	18(S)	6(R)	18(S)	12(I)	16(I)	10(R)	12(I)	14(I)	2
Lactobacillus fermentum[2]	22(S)	4(R)	0(R)	0(R)	8(R)	14(I)	8(R)	6(R)	20(S)	20(S)	6
Streptococcus sp.[2]	18(S)	14(I)	16(I)	16(I)	18(S)	12(I)	4(R)	14(I)	10(R)	16(I)	2

Gram positive disc

Gram negative disc

Isolates	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	RI
Escherichia coli	2(R)	6(R)	16(I)	10(R)	0(R)	2(R)	4(R)	0(R)	12(I)	0(R)	8
Klebsiella Pneumoniae	10(R)	4(R)	18(S)	6(R)	2(R)	6(R)	24(S)	18(S)	20(S)	0(R)	6
Citrobacter sp.	16(I)	24(S)	22(S)	0(R)	2(R)	4(R)	8(R)	4(R)	12(I)	14(I)	5

Key: 0 – 10mm = Resistant (R), 11 – 16mm = Intermediate (I), 17mm & Above= Susceptible (S), *RI = Resistance Index

Table 7: Tolerance of bacterial isolates to salt (2% sodium chloride)

Isolates	Colour change
Bacillus subtilis[1]	Yellow
Bacillus subtilis[2]	Yellow
Escherichia coli	Weak yellow
Klebsiella pneumoniae	Yellow
Citrobacter sp.	Yellow
Streptococcus sp.[1]	-
Lactobacillus sp.[1]	Yellow
Bacillus subtilis[3]	Yellow
Lactobacillus sp.[2]	Yellow
Lactobacillus fermentum[1]	Yellow
Bacillus sp.	Yellow
Lactobacillus fermentum[2]	Yellow
Streptococcus sp.[2]	-

eys:

+ = present,

- = absent

-

Table 8: Tolerance of bacterial isolates to haemolysis

Isolates	Reaction
Bacillus subtilis[1]	β
Bacillus subtilis[2]	β
Escherichia coli	β
Klebsiella pneumoniae	γ

Citrobacter sp.	γ
Streptococcus sp.[1]	β
Lactobacillus sp.[1]	α
*Bacillus subtilis*3	β
Lactobacillus sp.[2]	α
Lactobacillus fermentum[1]	α
Bacillus sp	β
Lactobacillus fermentum[2]	α
Streptococcus sp.[2]	β

Keys:

S= single	**P = pairs**	**C = chains**	**+ = positive**
α = alpha	**β = beta**	**γ = gamma**	**- = negative**

CHAPTER FIVE

DISCUSSION

The major constituents of fermented foods are proteins, fats and carbohydrates, the organisms responsible for fermenting them must be capable of utilizing these three constituents (Szutowska and Gwiazdowska, 2021). An increase in the average heterotrophic bacteria colony counts of 8.70±0.42 x 10^7 cfu/g was observed in iru, with the lowest heterotrophic bacterial colony counts of 5.00±0.28 x 10^7 cfu/g in ogiri egusi; while, ogogoro has the highest lactic acid bacteria colony count of 5.00±0.42 x 10^4 cfu/g and the lowest lactic

acid bacterial colony count of 0.80±0.42 x 10^4 cfu/g among the food samples were lower compared to that of Ajayi who got iru samples from towns in Ondo state (Ajayi, 2014). The increase observed in this result as compared to that of Ajayi could be due to the use of local calabash and some other utensils (Ajayi, 2014).

A total of 13 species of bacteria were isolated from the samples, These isolates include *Bacillus* sp,, *Citrobacter* sp., *Lactobacillus* sp.[1], *Lactobacillus fermentum*[2] and *Streptococcus* sp.[2] isolated from ogi, *Bacillus subtilis*[3] and *Streptococcus* sp.[1] isolated from the ugba, *Klebsiella pneumoniae* isolated from fufu, *Lactobacillus fermentum*[1] isolated from tuwo, *Bacillus subtilis*[1] isolated from iru, *Lactobacillus* sp.[2] isolated from palm-wine while *Bacillus subtilis*[2] isolated from ogiri-egusi and *Escherichia coli* isolated from ogogoro. This result is similar to that of Akin and Musa (2017), who isolated *Bacillus subtilis* from a sample of fermented condiments obtained from different towns in Kogi State. The fermentation of food by different types of microorganisms is normal and does not pose any threat of health hazard, especially when none of the microorganisms is pathogenic (Oyeyiola, 2002).

Bacillus subtilis was the most predominant isolates in all fermented condiments with 23% frequency of occurrence. It was isolated from samples of iru, ugba and ogiri. These bacteria are known for their ability to break down oil (Forgarty *et al.,* 1974). This result is confirmed by the works of Antai and Ibrahim (1986) and Achi (1992) who also stated that *Bacillus subtilis* were the predominant strains responsible for the fermentation of iru and *Prosopis africana* respectively. It was observed that *Lactobacillus* species and *streptococcus* species were also predominant with 15% frequency of occurrence and it was isolated from ugba and tuwo. This corresponds with the result of Oranusi *et al.* (2013) in which *Lactobacillus* species and other microorganisms such as *Proteus* spp., *Aspergillus niger*, and *Stapylococcus epidermides* were isolated from ugba and 0giri. According to Anosike *et al.* (2022), *Lactobacillus* and other non-pathogenic bacteria are safe for consumption as the

harmful effect of these organisms on the host are not common. Alo *et al.* (2014), Chaminda *et al.* (2017), and Mallappa *et al.* (2012) have all documented the beneficial effects of these *Lactobacillus* species which include improvement of lactose intolerance, regulation of immunity and treatment of gastrointestinal disease

In contrast to the popular belief that *Escherichia coli* is a pathogenic microorganism, a particular strain, *Escherichia coli* Nissle, has been discovered to possess some probiotic properties. According to research conducted by Benson *et al.* (2013), *Escherichia coli* Nissle was among the earliest strains employed as a probiotic. This strain was initially isolated in 1917 from a healthy German soldier, in contrast to his comrades who fell ill due to infections caused by *Shigella*. Also in a study by Pradhan and Weiss (2020), stem cell-derived human intestinal organoid tissues were used to assess the safety and protective qualities of Nissle against pathogenic *Escherichia coli* bacteria. The results indicated that Nissle was safe for human intestinal tissues and conferred protection against the harmful effects of pathogenic *Escherichia coli*. Instead of eradicating the pathogenic strain, Nissle appeared to activate the body's natural defenses.against isolates shows antibiotics resistance and hence the need to protect these fermented foods preparation under better hygienic processes.

In the selection of probiotics, acid-bile tolerance is one of the major factors that must be put into consideration (Nwagu *et al.,* 2010). In this study, the bacterial isolates were tested for acid and bile tolerance at 1hr and 3hr. *Streptococcus* sp. had the lowest acid tolerance with a percentage survival of 55.60%. while *Citrobacter* sp. had the highest survival rate of 200.00% survival. Similarly, *Streptococcus* sp. had the lowest bile tolerance with a percentage survival of 125.90% and *Escherichia coli* had the highest tolerance of 462.50% survival. This result shows that most of the organisms were tolerant to acidic pH and bile salt concentration. A similar research by David *et al.* (2016) examined the probiotic properties of different strains of *Bacillus* species isolated from iru woro and iru pete, they reported that

most of the strains could not survive an acidic pH of 3 except *Bacillus subtilis* and *Bacillus lichenformis*. They also reported that most of the strains could not survive salt concentration of 0.4% except *Bacillus subtilis*, *Bacillus cereus* and *Bacillus megaterium*. However, all the *Bacillus* species in this study had more than 100% survivability for acidic pH and bile salt concentration. Anosike *et al.* (2022) also reported that *Lactobacillus* species can survive low gastric juice condition. This corresponds with the result of this study where *Lactobacillus* sp. 1 and 2 had 126.90 and 118.20% survivability for acidic pH of 3 respectively while *Lactobacillus fermentum* had acidic survivability of 125.00%.

The presence of acid and bile salt in the gastrointestinal tract have their physiological benefits in the body but at the same time, it can be toxic to microorganisms that do not have the ability to adapt to the gastrointestinal tract conditions, which can lead to the destruction of such microorganism (Ruiz *et al.,* 2013). These properties determine the ability of probiotics to survive in the small intestine, and consequently their capacity to play their functional roles (Ruiz *et al,.* 2013). On some other occasions, acid and bile tolerant strains have also displayed cross-resistance to other stress factors (Margolles *et al.,* 2003). This shows the ability of probiotics to respond to various stress conditions (Sanchez *et al.,* 2012). Probiotics have developed some mechanisms to tolerate acid and bile salt. As reported by Ali *et al.* (2020), Pfeiler and Klaenhammer (2009), Wang *et al,* (2021), Gaucher *et al.* (2019) and Pfeiler *et al.* (2007), these mechanisms include increasing the extrusion of the bile acid that accumulate in the cytoplasm by activating bile acid efflux pumps (ATCC), hydrolysis of conjugated bile acids that enter the cytoplasm by using catalysis of intracellular bile salt hydrolases, changes in envelope structure by producing capsular exopolyssacharides or S-layer protein, they activate molecular machinery to counteract oxidative acid stressed by sensing bile acid and salt presence using two component regulatory systems.

This study also examined the susceptibility of these isolates to antibiotics. The isolates were tested against fourteen antibiotics which include pefloxacin, gentamycin, amplicox, zinnacefm amoxacillin, rocephin, ciprofloxacin, streptomycin, septrin, erythromycin, chloramphenicol, sparfloxacin, augmentin, and tarivid. *Bacillus subtillis* (E2) showed the highest susceptibility to antibiotics, while *Escherichia coli* was the least susceptible. This report is in contrast to that of David *et al.* (2016) which showed that most of the *Bacillus subtilis* species isolated from iru woro and iru pete had high resistance to antibiotics. It also corresponds with the reports of Abderrahmen *et al.* (2014); Ravi *et al.* (2007) and Dai *et al.* (2010). Antibiotic susceptibility test was carried out against fourteen antibiotics which include pefloxacin, gentamycin, amplicox, zinnacefm amoxacillin, rocephin, ciprofloxacin, streptomycin, septrin, erythromycin, chloramphenicol, sparfloxacin, augmentin, and tarivid, the test result showed that *Bacillus subtillis* species and *Streptococcus* species the highest susceptibility to the antibiotics, while *Escherichia coli* was the least susceptible. This report is in contrast to that of Das *et al*. (2019) that the Bacillus species had high susceptible to antibiotics.

This study also included evaluation of the antimicrobial activity of the isolates. The isolates were tested against three clinical pathogens which are *Escherichia coli*, *Salmonella* sp and *Klebsiella* sp. The result shows that *Bacillus subtilis*[2], *Bacillus subtilis*[3] and *Lactobacillus fermentum*[2] had the highest antibacterial activity with inhibition zones of 10mm each against *Salmonella* sp, *Escherichia coli* and *Escherichia coli* respectively. This corresponds with the report of Lim and Im (2009), they reported that Lactobacillus plantarum was shown to inhibit the growth of *Helicobacter pylori*. *Lactobacillus* sp. and *Lactobacillus* sp. exhibited the least antimicrobial activity with inhibition zones of 2mm each both against *Salmonella* sp*., Escherichia coli, Citrobacter* sp. *and Citrobacter* sp. had no antibacterial activity therefore, they cannot be used a probiotics. In a similar research by Mitra *et al.*

(2010), *Lactococcus lactis* isolated from dahi (Indian curd), generates nisin Z, which has the ability to hinder the growth of *Listeria monocytogenes* and *Stapylococcus aureus*. Antibacterial agents have the ability to kill or inhibit the growth of bacteria and this test is important in drug discovery and production, epidemiological studies and predicting treatment effectiveness (Mounyr *et al.,* 2016). Production of bacteriocin or organic acid is one of the ways by which these organisms carry out their antibacterial activity (Mariyappan *et al.,* 2022).

The test result of sodium chloride subjected to 2% NaCl concentration showed that Lactobacillus spp. isolated from ogi change halotolerant than its counterpart. Pundir et al. (2013) reported tolerance of lactic acid bacteria isolates to 1–6.5% NaCl concentration. Also, tolerated 1–9% NaCl (Hoque *et al*., 2010). The haemolytic reaction carried out showed *Lactobacillus* species appeared to be safe since they do not degrade mucus and did not exhibit β-haemolysis. This was in line with the result obtained by Meji and Hassouna, that *Lactobacillus* isolates does exhibit β-haemolysis.

5.1 CONCLUSION AND RECOMMENDATION

CONCLUSION

Escherichia coli had the highest tolerance for bile salt, it was also tolerant for acid, however, its antibiotic resistance index was too high and it also had no antibacterial activity against pathogens, it was therefore suspected to as contaminant and cannot be recommended as a probiotic. Due to the possibility of contamination of these foods,

certain measures should be taken by both producers and consumers to minimize the rate of invasion as well as ingestion of contaminants.

RECOMMENDATION

These measures include following proper hygienic, production and storage processes, while consumers should ensure proper cooking of these foods before consumption. On the other hand, *Bacillus subtilis* was able to pass all the screening tests and can therefore be recommended as a potential probiotic, however, it should be subjected to other screening tests like lectinase activity and others to ensure its viability as a probiotic.

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APPENDIX 1: VARIOUS AFRICAN TRADITIONAL FERMENTED FOODS AND BEVERAGES

Fermented product	Raw substrates	Microorganisms implicated
Iru	African locust bean	*Bacillus*, *Staphylococcus* spp
Fufu	Cassava	. Gari West Africa Cassava L
Tuwo	Cassava, maize, sorghum, millet	*Lactobacillus* spp., *Pediococcus pentosaceus, Weissella confusa*, *Issatchenkia orientalis*, *S. cerevisiae*, *Candida pelliculosa* and *C. tropicalis*

(Oyewole, 1997; Kostinek *et al*., 2005, Nyambane *et al*., 2014)

APPENDIX 2: CONCENTRATION OF ANTIBIOTICS DISCS

KEY: POSITIVE DISC			KEY: NEGATIVE DISC		
Abbreviation	Antibiotics	Concentration	Abbreviation	Antibiotics	Concentration
PEF	Pefloxacin	10µg	SXT	Septrin	30µg
CN	Gentamycin	30µg	CH	Chloranphenicol	30µg
APX	Ampliclox	30µg	SP	Sparfloxacin	10µg
Z	Zinnacef	20µg	CPX	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	AM	Amoxacillin	30µg
R	Rocephin	25µg	AU	Augmentin	10µg
CPX	Ciprofloxacin	30µg	CN	Gentamycin	30µg
S	Streptomycin	30µg	PEF	Pefloxacin	30µg
SXT	Septrin	30µg	OFX	Tarivid	10µg
E	Erythromycin	10µg	S	Streptomycin	30µg