BERKELEY PUBLICATION
AND RESEARCH INTERNATIONAL
06.30.2023
Journal of Health, Metabolism & Nutrition Studies Vol. 22 No. 3

AN INVESTIGATION OF THE LACTIC ACID BACTERIA PRESENT IN READY-TO-EAT FUFU

OBI, CHISOM P. AND NDULUE GINIKA P.
Department of Science Laboratory and Technology (Microbiology option), Federal polytechnic, Oko.Anambra State. Nigeria.

Abstract

Lactic acid bacteria (LAB) are bacteria that produce lactic acid as their main byproduct of fermentation. Other LAB strains may be isolated from a number of sources, but traditional fermentation has been practiced for thousands of years and fermented foods, notably cassava, have been consumed all over the world. Investigation and isolation of Lactic acid bacteria associated with the ready-to-eat cassava (fufu) sold at the renowned oko market in Anambra State is the aim of this study. In this study, lactic acid bacteria were isolated using one-tenth serial dilution from ten (10) ready-to-eat fufu samples and then grown anaerobically on De Man, Rogosa, and Sharpe (MRS) agar for 48 hours. Investigations using biochemical and other common microbiological techniques were carried out. Four lactic acid bacteria isolates were collected; they included *Lactobacillus sp, Streptococcus sp, Enterococcus sp, and Leuconostoc sp*. They were also Gram

Introduction:

Consumer demand for food items with many health benefits, such as those containing probiotic bacteria, has increased as a result of consumers' growing knowledge of the need of leading a healthy lifestyle. By improving the properties of the natural microbiota, probiotics are live microbial cultures that, when consumed by people or animals (as fermented products or dehydrated cells), may enhance their health. The ongoing use of these substances as infection treatments has led to a rise in antibiotic resistance (Galán et al. 2013). Therefore, there is a need for microorganisms that are effective against such

ISSN: 1213-4406

positive, catalase negative, non-spore-producing rods and cocci. Fermented ready-to-eat fufu was a great source of lactic acid bacteria for a long-term healthy lifestyle because of its probiotic ability to support digestion, gut health maintenance, and economic development.

Keywords: Lactic acid bacteria, ready to eat fufu, probiotics, oko market

Illnesses yet not hazardous to human health. One theory put up is that good bacteria produce substances poisonous to infections or that they compete with them for nutrients and living space (e.g., by colonizing the intestinal cells of the colon). A particularly efficient method for acquiring useful and genetically stable bacterial strains has historically been the isolation and screening of microorganisms from natural sources (Adnan and Tan 2006). For instance, it is well known that foods that spontaneously ferment with different cultures may be utilized to create bacterial strains. Furthermore, because of the complex habitat from which they were removed, these bacteria are exceptionally able to endure stress factors. They have stable properties in different scenarios. Various probiotic bacteria fall within the extremely important microorganism type known as lactic acid bacteria (LAB), with *Lactobacillus spp.* being the most active and harmless (i.e., non-pathogenic) of these bacteria. These strains have been shown to coexist harmoniously alongside *Bifidobacterium sp.,* a different probiotic strain (Kailasapathy and Chin, 2000). Since probiotic bacteria have been extensively studied, a variety of probiotic meals, especially those including dairy milk, have been developed (Ukeyima *et al.*, 2010). Although fermented dairy products have historically been associated with probiotics, cereal-based products have also been made, often by combining bacteria, prebiotics, and dietary fibers (Lamsal and Faubion 2009; Sanni *et al.* 2013). For probiotic bacteria to be effective, they need to possess a variety of distinctive qualities. One such trait is the ability to survive in acidic and bile-containing fluids, which they must accomplish throughout their journey through the digestive system (Klaenhamer and Kullen 1999). Additionally, they must demonstrate antibacterial behavior against pathogenic strains in order to be helpful to human health, either by producing antimicrobial substances (bacteriocins, organic acids, etc.) or by

reducing harmful microbe adhesion (Gareau *et al.* 2010). According to figures from the Food and Agricultural Organization, cassava (Manihot esculenta, Crantz) is the fourth most consumed crop in the world and a staple in many African, Asian, and South American countries (Ferraro 2016). Nigeria produced 37.5 million tonnes of cassava in 2010, making it the world's largest producer. (2013) (Ishola *et al.*). In fact, a lot of traditional Nigerian dishes are made with or from cassava, which produces a variety of solid and liquid wastes. In general, fermented dairy products are associated with lactic acid bacteria (LAB), and LAB make up a sizeable fraction of bacteria that have probiotic properties. So, the study looked at whether lactic acid bacteria were present in ready-to-eat fufu.

Materials and Methods

Area of Study

South East Nigeria's Oko, Orumba North L.G.A., in addition to the surrounding areas, served as the study site. Latitude 6^{o} 05N, longitude 7^{o} 06E, and height 332m are the coordinates for this place. The study area is defined by an extensive alluvial plain and undulating terrain. The bulk of the vegetation in Oko is forest (tropical vegetation), which is a rain forest zone.

Components and Materials

The following equipment was used in this study: a weighing scale, conical flasks, autoclave, petri dishes, non-absorbent cotton wool, aluminum foil, test tubes, wire loops, incubators, microscope, nutrient agar, De Mann Rogosa and Sharpe (MRS) agar, peptone water, distilled water, and biochemical reagents.

Sample Gathering

The ready-to-eat fufu was acquired from a number of vendors at the busiest and most populated market in Oko, known as Eke Oko, and was then transported straight to the lab.

The Isolation of Microorganisms

With ten milliliters of sterile, 0.1% peptone water (Oxoid, UK), each sample was homogenized. Following serial dilution, this was spread out in triplicate

on MRS agar plates (Oxoid, UK) and incubated anaerobically for 72 hours. To purify the isolates, colonies with various morphological characteristics, such as color, size, and shape, were randomly chosen from MRS agar plates as potential lactic acid bacteria isolates. The isolates were then repeatedly streaked on fresh MRS agar plates. Then, they were maintained properly angled at 4 degrees Celsius.

Identification and Characterization

Each lactic acid bacteria isolate's colony and cell morphologies, cell organization, spore generation, and motility were first investigated. Following morphological and biochemical tests, the isolates that were Gram positive, catalase negative, and did not generate spores were characterized. Each isolate was cultured (inoculated) overnight in MRS broth for all anaerobically incubated experiments. The ability of lactic acid bacteria isolates to ferment D-glucose, lactose, sucrose, galactose, maltose, mannitol, sorbitol, mannose, and L-arabinose was examined. Bromocresol purple broth foundation was used as the basic medium. A one percent filter-sterilized sugar solution was aseptically added to a sterile bromo-cresol purple broth base before each lactic acid bacteria strain was inoculated with an 18–24 hour old culture. The results were compared to an uninoculated control after 5 days of anaerobic incubation at 30 degrees Celsius. Tubes that produced sugar or acid had purple bromocresol that had gone yellow. Bergey's Handbook of Systematic Bacteriology [2002] and The Genera of Lactic Acid Bacteria [2003] were used to identify the various lactic acid bacteria strains based on the results of the various tests. The identification of the lactic acid bacteria isolates was verified using the API 50 CHL tests and the computer application APILAB Plus (BioMerieux, France).

Microbiological and bacterial testing

Gram Staining

A clean, grease-free microscope slide was used to gently transfer the growth off the culture plate, and it was then allowed to air dry. Three passes over the Bunsen burner's pilot flame were used to fix it. The glued smear was soaked in Crystal violet for 30 seconds before being rinsed with tap water. Lugol's

iodine was administered and washed off after about 30 seconds, and then acetone decolorization and washing away came next. After that, neutral red (counter stain) was applied, and after about 60 seconds, it was washed off. After that, the slides were placed on a drying rack so that the smear could dry naturally. Following drying, a drop of immersion oil was added to the smear, which was then seen using oil immersion lenses under a microscope (Cheesbrough, 2006).

Biochemical Tests

Motility Test

An organism's motility may be assessed using this technique. Using a hollow ground slide and a cover slip, the organism from a peptone water culture was seen. After that, the slide was examined under a microscope to search for movable organisms. According to Ochei and Kolhatkar (2000), those that are seen moving are motile, whilst those that are seen fixed are not.

Test for coagulase

Saline was used to dilute human plasma. 1:10. For each sample, 0.5ml of the diluted sample was added to each of the three test tubes. Five (5) drops of the 18–24 hour broth culture of the test organisms were added to the first test tube. Test tube 2 received five (5) drops of *Staph. aureus* 18–24 hour broth culture, while test tube 3 received five (5) drops of sterile broth. A water bath was used to incubate them for one hour, two hours, and up to six hours at intervals of 30 minutes. Clots were checked for in the tubes. In comparison to the negative control, the positive control developed a fibrin clot within an hour (Ochei and Kolhatkar, 2008).

Catalase test

Several colonies of the organism were emulsified in distilled water on a grease-free slide in a petri dish. Two drops of H_2O_2 were added to the colony, and the petri dish was covered. In some instances, gas bubbles were discovered, but not in others (Ochei and Kolhatkar, 2000).

Citrate Test

The organism was injected into Koser's citrate medium while suspended in saline and using a straight wire. According to Ochei and Kolhatkar (2000),

turbidity in Koser's medium in Simmons agar suggests good growth (blue color), indicating that citrate has been consumed.

Test for Oxidase

A few colonies on the culture plate received a few drops of the oxidase reagent. The color transitioned from blue to deep purple after 5–10 seconds (Ochei and Kolhatkar, 2000).

Test for Indole

The organism developed in the peptone water over night. A few drops of Kovac's reagent were added to the overnight peptone water culture. Color changes were seen. Red's presence implies that indole production is ongoing (Ochei and Kolhatkar, 2000).

Statistical Investigation

The study's conclusions were laid out in tables, and Excel was used to analyze the data.

Results

The ready-to-eat fufu had the highest frequency of occurrence, with *Enterococcus* (40%), *Streptococcus* (20%), *Leuconostoc weisalle* (30%), and *Lactococcus (*10%). Table 1 displayed the isolated lactic acid bacteria from various ready-to-eat fufu sold in Oko markets, which included *Streptococcus, Enterococcus, Leuconostoc weisalle,* and *Lactococcus.*

Table 2 shows that ready-to-eat fufu sold in Oko markets had an average colony forming unit count of 2.5 x 10-1. The isolates from the fermented ready-to-eat fufu were then shown in tables III and IV to have probiotic properties and to have undergone biochemical testing.

Table I: Isolated lactic acid bacteria from the ready to eat fufu

Cassava form	Number Examined	Isolates			
Ready to eat cassava	10	*Lactococcus spp*	*Lactobacillus spp*	*Streptococus spp.*	*Leuconostoc spp*
		2(20%)	4(40%)	2(20%)	1(10%)

ISSN: 1213-4406

Table II: Colony forming unit count of ready to eat fufu (cfu\g)

Cassava form	Number Examined	Dilution factor	Average Cfu/g
Ready to eat cassava	10	10^{-1}	2.5×10^{-1}

Table III: Probiotic test of isolated lactic acid bacteria from fermented ready to eat cassava

Microoganisms	Gas from glucose	Growth at 6.5% NaCl	Growth at 10°C	Growth at 45°C	Growth in broth at pH 9.6
Lactococcus spp	-	-	+	+	-
Lactobacillu spp.	-	+	+	+	+
Streptococcus spp.	-	-	-	+	-
Leuconostoc spp.	+	-	+	+	-

Key:
- negative
+ Positive

Table IV: Biochemical Identification and characterization of the isolates

Biochemical tests Performed	Isolate code A	B	C	D	
Gram stain	+	+	+	+	
Cell morphology	rods	cocci	rods	cocci	
Catalase reaction	-	-	-	-	
Motility	-	-	-	-	-
Glucose fermentation		+	+	+	+
Gas from glucose	-	-	+	-	
Lactose fermentation		+	+	+	+

ISSN: 1213-4406

Sucrose fermentation	+	+	+	+
Fructose fermentation	+	+	+	+
Galactose fermentation	+	-	+	+

KEY

+ = positive
− = negative
A= *Lactococcus spp* ,B=*Lactobacillus spp* ,C =*Streptococcus* spp,D =*Leuconostoc* spp

Table V: Identification of LAB Isolates Based on Colony Morphology

Isolate Codes	Colony morphology on MRS plate
A	punctiform, smooth whitish colony
B	circular, flat off white colony
C	punctiform, smooth, cream colony
D	round, smooth, whitish colony

Key: A= *Lactococcus spp* ,B=*Lactobacillus spp* ,C =*Streptococcus* spp,D =*Leuconostoc* spp

DISCUSSION

The present study sought to isolate and characterize Lactic acid bacteria associated with the ready-to-eat fufu sold in the Oko market in Anambra state. The isolates were recognized as *Streptococcus, Lactobacillus, Leuconostoc weisalle,* and *Lactococcus* using morphological, cultural, staining, motility, probiotic, and biochemical assays. This study also identified changes in the prevalence of lactic acid bacteria linked to the Oko Market's ready-to-eat fufu. According to the study's results, *Streptococcus* (20%), *Leuconostoc weisalle* (30%), *Lactococcus* (10%), and *Lactobacillus* (40%) were the most common bacteria found in ready-to-eat fufu. This study demonstrates the wide community of lactic acid bacteria that spontaneous or conventional fermentation maintains, including certain *Lactobacillus* strains that could interact with humans and other microbes during production and consumption. By way of human activity, abiotic factors, and chance, spontaneous fermentation and product intake may be a microbial interaction between the environment and the human microbiome. In reaction, lactic acid

bacteria ferments are consumed, perhaps providing the body with microorganisms. While not all LAB are advantageous, a number of *Lactobacilli* have been associated with enhanced human health. Human Microbiome Project Consortium, 2012; Linnenbrink *et al.,* 2013; Dethlefsen, McFall-Ngai, and Relman, 2007; Spor, Koren, and Ley, 2011; Linnenbrink *et al.,* 2013). In nutrient-rich environments, lactic-acid bacteria are found on the surfaces of both plants and animals. Despite the fact that certain strains produce biogenic amines that might be dangerous to human health (Costello *et al., 2*009), other research indicates that consuming LAB in moderation has several advantages. High rates of adhesion to the mucus membrane in the human digestive tract enable direct contact with the human gut, which has been shown to modulate immunological response, enhance mucus secretions to calm the intestinal lining, and protect against infections. Additionally, according to many studies (Aro, 2008; Chelule, Mokoena, and Gqaleni, 2010; Turpin *et al*., 2010), lactic-acid bacteria improve vitamin and mineral absorption while eliminating antinutrients and other phytotoxins like cyanide.

Conclusion

This study's objective was to establish the prevalence of lactic acid bacteria in Nigeria's delicacy, ready-to-eat fufu. A number of isolates were identified as *Streptococcus, Lactobacillus, Leuconostoc weisalle,* and *Lactococcus* using motility, probiotic, and biochemical assays, along with morphological, cultural, and staining characteristics. These isolates are known to include lactic acid bacteria. Since numerous probiotic bacteria fall within the extremely significant lactic acid bacteria (LAB) group, it is commonly acknowledged that foods that spontaneously ferment may be utilized to produce bacterial strains that are important for human health.

Results and Suggestions

The study's findings revealed the existence of probiotic lactic acid bacteria, which are classified as present in ready-to-eat fufu. Probiotics are live microbial cultures that, when consumed by people or animals (as fermented products or dried cells), may enhance the beneficial properties of the body's natural microbiota.

As a result, i advise and propose that we include this fermented food, which has health advantages for all of humanity, in our diet.

References

Adnan, D.N. and Tan, K.M. (2006):.Enhanced thermal destruction of *Listeria monocytogenes* and *Staphylococcus aureus* by the lactoperoxidase system. *Applied and Environmental Microbiology*, 56: 2711-2716.

Aro, H. (2008). Transformation of Lactobacillus strains used in meat and vegetable fermentations. *Food Research International*, 25: 253-261.

Chelule, H., Mokoena, C. and Gqaleni, R.E. (2010). Influence of growth conditions on production and activity of mesenterocin 5 by a strain of Leuconostoc mesenteroides. *Applied Microbiology and Biotechnology*, 39: 166-173.

Costello, W.M., Mulders, J.W. Siezen, R.J. Hugenholtz, J. and Kuipers. O.P. (2009). Properties of Nisin Z and distribution of its gene, nisZ, in Lactococcus lactis. *Applied Environmental Microbiology*, 59: 213-218.

Dethlefsen, S.T., McFall-Ngai, J.A. and Relman, S.A. (2007). Effect of processing variables on the formation of calcium lactate crystals in Cheddar cheese. *Journal of Dairy Science*, 71: 1701-1710.

Ferraro, T. (2016). Homofermentative Lactobacillus species predominantly isolated from canine feces. *Journal of Veterinary Medical Sciences*, 58: 591-593.

Galán ,U.,Fuller, R., G.R. Gibson. (2013). Probiotics and probiotics: Microbiota management for improved gut health. *Clinical Microbiological Infectious*, 4: 477-480.

Gareau, L.J., H.P. Fleming, T.R. Klaenhammer. (2010). Characterization of two nisin- producing *Lactococcus lactis* subsp. *lactis* strains isolated from a commercial sauerkraut fermentation. *Applied Environmental. Microbiology*, 58:1477-1483.

HMPC (Human Microbiome Project Consortium), (2012). Cassava in tropical Africa. Reference Manual, IITA, Ibadan, Nigeria. Pp.12

Ishola , Jarvis, B and Jay, J.M. (2013). Resistance to nisin and production of nisin-inactiveting enzymes by several *Bacillus* species. *Journal of Genetic Microbiology*, 47: 33-48.

Kailasapathy A.H. and Chin, W.M. (2000). Genetic marking of Lactococcus lactis shows its survival in the human gastrointestinal tract. *Applied and Environmental Microbiology*, 61: 2771- 2774.

Klaenhammer, T. R. and Kullen (1999). Bacteriocins of Lactic Acid Bac- teria, *Biochemie*, 70(3): 337-349.

Lamsal, O.P. and Faubion J. (2009). Current strategies for improving food bacteria. *Research Microbiology*, 151,815-822.

Salminen, S. and VonWright, S. (1998) "Lactic Acid Bacteria in Health and Disease," In: S. Salminen and A. Van Wright, Eds., *Lactic Acid Bacteria*, Marcel Dekker Inc., New York, pp. 234-294.

Sathyabama, P., Sneath, P.H. and Holt, J.G. (2014). Bergey's Manual of Systematic Bacteriology, 2nd edition. VOl1, p. 64. A Waverly Company, Williams & Wilkins, Springer-Verlag, NY, USA.

Spor , T., Koren, K.H and Ley.K. (2011). Bio-enrichment: production of vitamins in fermented foods. In: Wood,B.J.B. (Ed.)., Microbiology of fermented foods. Blackie Academic and Professional. *London,* pp. 603-619.

Turpin, K.M., H.M. Probert, C.W. Smejkal, G.R. Gibson. (2010). Review: Using probiotics and probiotics to improve gut health. *Drug Discovery Today* 8: 692-700.

Ukeyima, N., K. Watanebe, A. Mike, Y. Tagami, R. Tanaka, M. Ohwaki, M. Morotomi. (2010). Survival of a probiotic, Lactobacillus casei strain Shirota, in the gastrointestinal tract: Selective isolation from faeces and identification using monoclonal antibodies. *International Journal of Food Microbiology*, 48: 51-57.