Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 7, Issue 2, pp: (17-22), Month: March - April 2020, Available at: www.noveltyjournals.com

THE OCCURENCE OF LACTIC ACID BACTERIA IN FUFU SOLD IN EKPOMA

Helen A. Obiazi and Grace I. Okwu

Department of Microbiology, Faculty of Life Science, Ambrose Alli University, Ekpoma.

E-mail: obiazihelen@gmail.com

Abstract: **The present study was designed to isolate and identify Lactic acid bacteria associated with Cassava and fufu products sold in Ekpoma market. The isolates were confirmed as *Streptococcus, Enterococcus, Leuconostoc weisalle, Lactococcus* by cultural and staining characteristics, motility, biochemical test and standard microbiological procedures. The results showed that *Streptococcus, Lactobacillus, Leuconostoc weisalle, Lactococcus* were identified. The conclusions are based on short read so, a much more detailed study is necessary to determine if any of the Lactic acid bacteria that was detected might confer health benefits or even successfully assimilate into the human microbiome.**

Keywords: **Lactic acid bacteria, fufu products, health benefits, Ekpoma market.**

1. INTRODUCTION

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 and 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 shows table properties, and they 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). The symbiotic effect of these strains with Bifidobacterium sp., another probiotic strain, has also been reported (Kailasapathy and Chin, 2000). Probiotic bacteria have been extensively studied, and this has led to the development of a variety of probiotic foods, especially those involving dairy milk (Ukeyima *et al*. 2010). While it is fermented dairy foods which have been conventionally associated with probiotics, cereal-based products have also been developed, mainly through the combined use of probiotics, prebiotics and dietary fibres (Lamsal and Faubion 2009; Sanni *et al*. 2013). 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 behaviour towards pathogenic strains, either by producing antimicrobial agents (bacteriocins, organic acids, etc) or by reducing the adhesion of pathogenic bacteria

(Gareau *et al*. 2010). Cassava (*Manihot esculenta*, Crantz) is a very important crop in many African, Asian and South American countries, and according to Food and Agriculture Organization data, it is the fourth most consumed crop worldwide (Ferraro 2016). Nigeria is the primary world producer of cassava, with a production of 37.5 million tons reported in 2010 (Ishola *et al*. 2013). Indeed, many traditional Nigerian dishes are based on and/or derived from cassava, generating wastes in various forms, both solid and liquid. Lactic acid bacteria (LAB) are commonly associated with fermented dairy products and LAB make up high percentage of bacteria that provide probiotic properties. Therefore, the study aimed to examine lactic acid bacteria in fufu.

2. MATERIALS AND METHODS

AREA OF STUDY

The study was done in Ekpoma and its environs. Ekpoma is located in Esan West senatorial district of Edo State o latitude $6^{o}45N$, longitude 6^{o} 60E and altitude 332m above sea level. Ekpoma has a general hospital and many private hospitals which are used to cater for the health of the inhabitants. Ekpoma and Irrua are moderately populated with most occupants being farmers, traders, civil servants and students (Edo State of Nigeria, 1992).

MATERIALS AND REAGENTS

The materials and reagents used during the course of this research include, weighing balance, beakers, conical flasks, autoclave, petri-dishes, 70% ethanol, non-absorbent cotton wool, aluminium foil, test tubes, wire loops, incubators, microscope, nutrient agar, mona-rossa agar (MRA), peptone water, distilled water, and biochemical reagents.

LABORATORY PREPARATION OF FUFU

The laboratory fermentation of cassava tubers and maize grains to produce fufu was carried out by simulating the traditional methods of processing which involves cassava tubers peeling, tuber washing, steeping/fermentation (72 hours) at ambient temperatures, marching, sieving, pressing and finally wet fufu (cassava starch) followed by cooking.

SAMPLE COLLECTION

Freshly harvested cassava root tubers (Manihot esculenta) were obtained from the Ekpoma market, Edo State, Nigeria. They were brought in sterile polythene bags to the laboratory for immediate processing.

MICROBIOLOGICAL ISOLATION

Twenty five millilitre portions was aseptically removed at different stages of fermentation processes: raw water used for steeping cassava roots; steep waters (sampled each day for 72 h); water used for mashing steeped cassava; the steep water used further fermentation of the final products of fermentation. Each sample was homogenized with 200 ml sterile 0.1% peptone water (Oxoid, UK). This was then serially diluted and 0.1 ml from appropriate dilutions will be spread plated on MRS agar plates (Oxoid, UK) in duplicates and incubated in Gas Pak jars (GasPak System, BBL) at 30˚C for 72 h. Colonies with distinct morphological differences such as colour, size and shapes were randomly picked from MRS agar plates as presumptive lactic acid bacteria isolates and repeatedly streaked on fresh MRS agar plates to purify the isolates. They were then maintained on appropriate slants at 4˚C.

CHARACTERIZATION AND IDENTIFICATION

Each of the lactic acid bacteria isolate was initially examined for colonial and cell morphologies, cell arrangement, spore formation and motility. Only the Gram positive, catalase negative and non-spore forming isolates will be then characterized by phenotypic and biochemical tests. An overnight culture (inoculums) of each isolate in MRS broth was used for all tests incubated anaerobically (GasPak System, BBL) at 30˚C. The lactic acid bacteria isolates were tested for fermentation of the following carbohydrates (Sigma, Germany): D-Glucose, lactose, sucrose, galactose, maltose, mannitol, sorbitol, mannose, L-arabinose, D-xylose, cellobiose, dulcitol, inositol, raffinose, rhamnose, inulin and salicin. Bromocresol purple broth base was used as basal medium. One percent filter- sterilized sugar solution using 0.2 µm Millipore filter(Corning) was added aseptically into sterilized bromo-cresol purple broth base before inoculation with 18 - 24 h old culture of each lactic acid bacteria strain. The results were assessed with reference to an uninoculated control after anaerobic incubation at 30˚C for 5 d. Tubes in which bromocresol purple colour changed to yellow indicated utilisation of sugar or acid production. The various lactic acid bacteria strains were then identified by reference to the

Bergey's Manual of Systematic Bacteriology [2002] and The Genera of Lactic Acid Bacteria (2003) based on the results of the various tests. The identity of the lactic acid bacteria isolates were further confirmed by using the API 50 CHL tests and the Computer Program APILAB Plus (BioMerieux, France).

MICROSCOPIC EXAMINATION/BACTERIOLOGICAL EXAMINATION

GRAM STAINING

The growth on the culture plate was carefully placed on a sterile, grease-free microscope slide and allowed to air-dry. It was fixed by passing it over the pilot flame of the Bunsen burner three times. The fixed smear was flooded with Crystal violet for 30 seconds before washing off with tap water. Lugol's iodine was added and washed up after about 30 seconds and subsequently decolourised rapidly using acetone and washed off immediately. Neutral red (Counter stain) was then be added and washed off after about 60 seconds. The slides were then placed in a draining rack for the smear to air dry. After drying, a drop of immersion oil was applied on the smear and viewed microscopically using oil immersion objectives (Cheesbrough, 2006).

BIOCHEMICAL TESTS

Motility Test

This test is used for the determination of motile organism. A hollow ground slide and a cover slip were used to observe the organism from a peptone water culture. The slide will be then examined with a microscope to check for organisms that are motile. Those seen moving are motile while those that are not moving are non- motile (Ochei and Kolhatkar, 2000).

Coagulase test

Human plasma was diluted 1 in 10 in saline. Into each of the three test tubes for each sample, 0.5ml of the diluted sample will be added. Five (5) drops of 18-24 hour broth culture of the test organisms were added to the 1st test tube. Five (5) drops of 18-24 hour broth culture of *Staph*. *aureus* were added to test tube 2 and five (5) drops of sterile broth were added to test tube 3. They were all mixed gently and incubated in a water bath and examined for clot after one hour, two hours and at 30 minutes interval for up to 6 hours. The tubes were observed for clot. The positive control produced clot within 1 hour and the negative control did not produce any fibrin clot (Ochei and Kolhatkar, 2008).

Catalase Test

Few colonies of the organism were emulsified in distilled water on a clean grease free slide placed in a petri dish. 2 drops of H_2O_2 will be added to the colony and the petri dish covered. Gas bubbles were observed for some and will be not observed in others (Ochei and Kolhatkar, 2000).

Citrate Test

A light suspension of the organism was made in saline and inoculated into Koser's citrate medium with a straight wire. Growth (of bluecolour) indicated by turbidity in Koser's medium in Simmons agar indicates positive result meaning that citrate has been utilized (Ochei and Kolhatkar, 2000).

Oxidase Test

A few drops of oxidase reagent were added to a few colonies on the culture plate. Colour change of blue to deep purple was looked out for within 5-10 seconds (Ochei and Kolhatkar, 2000).

Indole Test

The organism was grown in peptone water overnight. A few drops of Kovac's reagent will be added to the overnight peptone water culture. Colour change was looked out for. Red colouration indicates positive indole production (Ochei and Kolhatkar, 2000).

STATISTICAL ANALYSIS

Results obtained from the study was displayed in tables and data analyzed using excel.

International Journal of Novel Research in Life Sciences
Vol. 7, Issue 2, pp: (17-22), Month: March - April 2020, Available at: www.noveltyjournals.com

3. RESULTS

Table 1 showed the isolated lactic acid bacteria from various forms of cassava sold in Ekpoma markets in which *Streptococcus, Enterococcus, Leuconostoc weisalle* and *Lactococcus* were identified with Raw cassava having the highest frequency of 37.5%, 25% and 12.5% for *Lactobacillus, (Streptococcus and Leuconostoc weisalle)* and *Lactococcus* respectively. While the Half Done cassava had the next highest frequency of 33.4%, 2.7%,23.4%, for *Enterococcus, Streptococcus, Leuconostoc weisalle* and *Lactococcus* respectively. For the already done fufu the frequency of occurrence include *Enterococcus* (30%), *Streptococcus* (26.7%), *Leuconostoc weisalle* (23.40%) and *Lactococcus* (20%)

Table 2 showed the colony forming unit count of various forms of cassava sold in Ekpoma markets in which raw cassava was highest 1.92 x 10^6, followed by Raw Cassava (2.72 x 10^4), Half Done Cassava (1.28 x 10^4).

Table 1: Isolated lactic acid bacteria from various forms of cassava sold in Ekpoma market

Cassava form	Number Examined	ISOLATES			
		***Streptococcus* (%)**	***Lactobacillus* (%)**	***Leuconostoc weisalle* (%)**	***Lactococcus* (%)**
Raw Cassava	40	10(25)	15(37.5)	10(25)	5(12.5)
Half Done Cassava	30	8(26.7)	10(33.4)	7(23.4)	5(16.7)
Already done fufu	30	8(26.7)	9(30)	7(23.4)	6(20)
Total	100	26	34	24	16

Table 2: Colony forming Unit count of various forms of cassava sold in Ekpoma market

Cassava form	Number examined	Dilution	Average CFU/g
Raw Cassava	40	10^{-8}	2.72 x 10^4
Half Done Cassava	30	10^{-8}	1.28 x 10^4
Already done fufu	30	10^{-8}	1.92 x 10^6
Total	100		

Table 3: Biochemical Test of isolated lactic acid bacteria from fermented cassava on MRS agar

Microorganism	Gas from glucose	Growth at 6.5% Nacl	Growth at 10°C	Growth at 45°C	Growth in broth at pH 9.6
Lactobacillus spp	(-)	(+)	(+)	(+)	(+)
Lactococcus spp	(-)	(-)	(+)	(-)	(-)
Streptococcus spp	(-)	(-)	(-)	(+)	(-)
Leuconostoc	(+)	(-)	(+)	(+)	(-)

Keys: (-): absent; (+): present

4. DISCUSSION

The present study was designed to isolate and identify Lactic acid bacteria associated with Cassava and fufu products sold in Ekpoma Market. The isolates were confirmed as *Streptococcus, Lactobacillus, Leuconostoc weisalle, Lactococcus* by cultural and staining characteristics, motility, and biochemical test. Furthermore, this study has shown the differences in prevalence of Lactic acid bacteria sold associated with Cassava and fufu products sold in Ekpoma Market**.** The result from this study showed that *Streptococcus* were highest for Halfdone Cassava and Already done fufu with 26.7% frequency while the raw cassava 10(25%). *Lactobacillus* were highest for Raw Cassava (37.5%) followed by Half Done Cassava (33.4%) and lest in Already done fufu (30%). *Leuconostoc weisalle* were highest for already done fufu (25%), followed by Half Done Cassava and Already done fufu which were 23.4%. *Lactococcus* were highest for already done fufu (20%) while half Done Cassava was next for 16.7% and raw cassava was least with 12.5%. Humans continuously and intimately interact with microorganisms. This study demonstrates that spontaneous, or traditional, fermentation promotes a diversity of lactic acid bacteria, including some *Lactobacillus* strains that may potentially interact with human and environmental microbes during production and consumption. Spontaneous fermentation and consumption of its product can be a microbial exchange between the environment and the human microbiome that is mediated by human

behavior, abiotic factors, and random chance. In turn, ferments by lactic acid bacteria are consumed and become a potential source of microbes for the human body. While not all LAB confer benefits, many Lactobacilli have been positively associated with human health. (Dethlefsen, McFall-Ngai & Relman, 2007; Costello *et al*., 2009; Spor, Koren & Ley, 2011; Human Microbiome Project Consortium, 2012; Linnenbrink *et al*., 2013). Lactic-acid bacteria are found in association with nutrient rich environments on animals and plants. While some strains produce biogenic amines that can be detrimental to human health (Hal´asz *et al*., 1994), other research highlights positive effects of consuming LAB in moderate amounts. In the human intestinal tract, high rates of adhesion to the mucus membrane allow for direct interface with the human intestine, and have been shown to protect against pathogens, modulate immune response, and promote mucus secretions to soothe the intestinal lining. In addition, lactic-acid bacteria provide digestion assistance, improving vitamin and mineral bioavailability while degrading antinutrients and other phytotoxins such as cyanide (Campbell-Platt, 1994;Westby, Reilly & Bainbridge, 1997; Aro, 2008; Chelule,Mokoena & Gqaleni, 2010; Turpin *et al*., 2010).

5. CONCLUSION

The conclusions are based on short reads so a much more detailed study is necessary to determine if any of the *Lactic acid bacteria* that was detected might confer health benefits or even successfully assimilate into the human microbiome. The isolates were confirmed as *Streptococcus*, *Lactobacillus*, *Leuconostoc weisalle*, *Lactococcus* by cultural and staining characteristics, motility, and biochemical test.

REFERENCES

[1] 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.

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

[3] Campbell-Platt, E., B. and de Kruijff. (1994). Review: the lantibiotic nisin, a special case or not? *Biochemica et Biophysica Acta* 1462: 223-234.

[4] 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.

[5] 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.

[6] 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.

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

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

[9] 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.

[10] Halász, A., Baráth, Á. and Holzapfel, W.H. (1994). The influence of starter culture selection on sauerkraut fermentation. *Zeitschrift für Lebensmittel Untersuchung und Forschung A*, 208: 434-438.

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

[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.

[13] 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.

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

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

[16] 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.

[17] Sathyabama, P., Sneath, P.H. and Holt, J.G. (2014). Bergey's Manual of Systematic Bbacteriology, 2nd edition. VOl 1, p. 64. A Waverly Company, Williams & Wilkins, Springer-Verlag, NY, USA.

[18] 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.

[19] 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.

[20] 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.

[21] Westby, C., Reilly.H., and Bainbridge, G. (1997). Cassava: the nature of the tuber and use as raw material. Encyclopedia of Food Science, Food Technology and Nutrition. *Academic Press, San Diego, California*, 734-743.