In Vitro Studies of the Probiotic Properties of Lactic Acid Bacteria Isolated from Akamu – A Nigerian Weaning Food

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Abstract Akamu is a popular fermented nutritive porridge made from cereals and is mostly eaten at infancy as a weaning food. Lactic acid bacteria contribute towards the safety, nutritional value, shelf life and acceptability of a wide range of cereal based foods and have been reported to have probiotic potential against gastrointestinal microorganisms, thus in vitro studies of the probiotic properties of lactic acid bacteria isolated from akamu produced with sorghum and maize grains were carried out using standard analytical methods. The pH of the cereal slurries decreased while the titratable acidity and the total lactic acid bacterial counts increased during the period of the studies. The lactic acid bacteria isolated were *Lactobacillus delbrueckii* subsp *bulgaricus*, *L. fermentum*, *L. brevis*, *L. plantarum*, *L. amylovorus*, *Pediococcus acidilactici* acid, *P. Pentosaceus*. The isolates grew optimally at pH 4.0 and NaCl concentration of 3.0% and survived in fresh bovine bile. The bacteria except *Lactobacillus brevis* adhered to the intestinal mucosa as shown by the cell surface hydrophobicity assay and were resistant to most of the antibiotics used. This study indicated that the lactic acid bacteria isolated from raw akamu have probiotic characteristics and that raw akamu will be effective in the prevention and treatment of gastrointestinal diseases.

Keywords Kamu, Lactic Acid, Bacteria, Probiotic, Sorghum, Maize

1. Introduction

The fermentation process has developed over the years such that organic substrates are now converted into more desirable substances by *Lactobacillus plantarum* and *Candida tropicalis*, the actions of enzymes or other

microorganisms under controlled conditions [1]. Lactic acid bacteria are a group of gram positive, non-spore forming rods or cocci which produce lactic acid as the major end product from fermentation of carbohydrates. Majority of microorganisms used as probiotics belong to the lactic acid bacteria and *Bifidobacteria*.

Lactobacillus species are the most commonly utilized group of microorganisms within the group of lactic acid bacteria as probiotics. The antagonistic activity of such bacteria is known to inhibit a large number of enteric and urinary pathogenic bacteria. Lactic acid bacteria such as *Lactobacillus* and *Bifidobacteria* are found throughout the gastrointestinal tract and they have been reported to cause a reduction in lactose intolerance and alleviation of some diarrhoea as well as an increase in immune response [2].

Nigeria is endowed with a wide range of fermentable indigenous staple foods that serve as raw materials for Agro-allied cottage industries. These industries utilize small scale equipment and provide alternatives for rural communities while adding value to such local porridge [3]. One common example of indigenous fermented foods in Nigeria is Akamu which is prepared and consumed by the Ibos and other tribes in Nigeria. This cereal product is common in seemingly poor and impoverished communities across the developing countries and is produced largely from maize and sorghum. Its production is often by small scale enterprise undertaken by unskilled female attendants [4].

Spontaneous fermentation of cereal-based foods is borne out of competitive activities of endogenous or contaminating microorganisms and its initiation may take 24 - 48 hours. At the early stages of fermentation, contaminating microorganisms may increase slowly in number and compete for nutrients in order to produce metabolites [5]. The results are products of variable attributes in terms of quality and safety [6].

Lactic acid bacteria and yeasts occur as part of the

natural microbial population in spontaneously fermented foods and as starter cultures in the food and beverage industry [7]. Amylolytic lactic acid bacteria breakdown starches to simple sugars which are favourable for lactic acid bacteria growth. The simple sugars are then converted to organic acids that create the acid environment which is known to improve product stability and safety.

The antibiosis mediated by lactic acid bacteria has been attributed to the production of acids, hydrogen peroxide and antibiotics. The production of organic acids reduces the pH to below 4.0, making it difficult for some spoilage organisms that are present in cereals to survive [8]. Lactic acid bacteria lack the enzyme catalase to breakdown the hydrogen peroxide generated, therefore it can accumulate and become inhibitory to some microorganisms [9].

Fermented products having lactic acid bacteria also have viricidal and anti tumor effects [10]. This work was therefore aimed at determining the in vitro probiotic properties of lactic acid bacteria isolated from the Nigerian staple food Akamu prepared with sorghum and maize.

2. Materials and Methods

2.1. Collection of Sorghum and Maize Samples

Maize and sorghum grains were purchased at Eke Awka Market in Awka, Anambra State, Nigeria and transported to the laboratory in sterile polythene bags for processing.

2.2. Preparation of Akamu

Akamu was prepared using the traditional method described by Odunfa [11]. The grains were sieved to remove pebbles and dirt and subsequently soaked in water for three days, with the water changed daily after which they were mashed, milled and sieved to obtain the slurries using a sieve and cotton material. The slurries were bagged, dewatered and stored for three days to produce the edible food – Akamu.

2.3. Determination of the pH of the Slurries with Time

The pH was determined as described by Akinrele [12] using a Pye – Unicam pH meter. One gram of the sample was dissolved with 24ml of deionised water to produce a 25ml solution. The pH meter was standardized using pH buffers 4.0 and 7.0. The electrode was inserted into the solution contained in a test tube and the pH was taken when the meter reading stabilized. The pH was determined at twenty four hours intervals for three days.

2.4. Determination of the Titratable Acidity of the Slurries with Time

This was determined as described by Onuorah et al. [13].

One gram of the slurry was dissolved with 24ml of deionised water to give a 25ml solution. The solution was titrated with 0.1N NaOH solution using phenolphthalein as the indicator till the colour of the solution changed to pink. The titratable acidity was determined at twenty four hours intervals for 72hours. Triplicate determinations were made for each sample.

2.5. Determination of the Total Lactic Acid Bacterial Counts of the Slurries with Time

The total lactic acid bacterial counts of the samples were determined as done by Guesh et al. [14]. Aliquots of serially – diluted samples were inoculated into Mann Rogosa Sharpe (MRS.) agar plates containing 0.5% nystatin to inhibit the growth of gram negative bacteria and fungi. The samples were distributed uniformly over the medium using a sterile glass rod spreader. The plates were incubated in an inverted position at 37°C for 48 hours and the discrete colonies that grew were counted and recorded. The cultures were purified several times on MRS agar plates and stored on MRS agar slants for characterization and identification.

2.6. Characterization and Identification of the Lactic Acid Bacterial Isolates

The morphological and biochemical characteristics of the lactic acid bacterial isolates were determined. Gram staining, catalase test, sugar fermentation test, coagulase test, indole test, methyl red test, spore staining, citrate utilization test, voges proskauer test and motility test were carried out as described by Bonade et al.[15]. The isolates were identified using 16S ribosomal RNA sequencing using the FASTA algorithm.

2.7. Growth of the Isolates at Varying pH Levels

The test was performed as described by Tambekar and Bhutada [16]. The isolates were inoculated into sterile tomato juice broth tubes of varying pH (2.5, 3.0, 4.0 and 5.0). The pH was adjusted using hydrochloric acid. The tubes were incubated at 37°C for 48hours. 0.1ml inoculum from each tube was inoculated into Mann Rogosa and Sharpe (MRS) agar plate using the spread plate method. The plate was incubated at 37°C for 48hours.

2.8. Salt Tolerance of the Lactic Acid Bacteria

This was performed as described by Hyronimus et al. [17]. Isolated lactic acid were inoculated into MRS broth in test tubes containing 3.0%, 6.5% and 10.0% (w/v) of NaCl and incubated at 37°C for 24 hours. The tubes were thereafter examined for turbidity which was a positive reaction.

2.9. Survival of the Lactic Acid Bacteria in Fresh Bovine Bile

This was carried out using the agar well diffusion method as described by Vinderola et al. [18]. Sterile Mann Rogosa Sharpe agar was mixed with 0.2ml of a 24 hour culture of each isolate. Wells of 10mm in diameter were made in each agar plate and 0.2ml of fresh bovine bile was placed in each well. The plates were incubated anaerobically at 37°C for 36 hours. Diameters of zones of inhibition around the wells were measured and recorded.

2.10. Cell Surface Hydrophobicity Assay

The assay was carried out as described by Dunne et al.[19]. Fresh cultures of the isolates were centrifuged at 4000rpm for 10 minutes. The cells were thereafter washed three times with phosphate buffered saline (PBS) and suspended in 1.2ml of PBS. 0.6ml of xylene was added to 3.0ml of the cell suspension. The mixture was thoroughly vortexed for 2 minutes and allowed to stand for the xylene to separate completely. The aqueous phase was carefully removed with a springle and the remnant was transferred to a cuvette and the absorbance values were taken at 560nm using the spectrophotometer.

$$\% \text{ hydrophobicity} = \frac{A_0 - A}{A_0} \times 100$$

A_0 = Absorbance values of the mixture before the addition of xylene

A = Absorbance values of the mixture after the addition and removal of xylene.

2.11. Determination of Antibiotics Susceptibility Profile of the Isolates

The method used by Onuorah et al.[20] was adopted. The isolates were inoculated into tomato juice broth individually and incubated at 37°C for 24hours. 0.1ml of each isolate was thereafter spread evenly on sterile solidified MRS agar plates using sterile glass rods. Antibiotics discs namely Amoxicillin(30µg), Septrin (30µg), Ciprofloxacin (10µg), Gentamycin (10µg), Streptomycin (30µg), Perfloxacin (10µg), Ampiclox (30µg), Erythromycin (10µg), Zinnacef (20µg) and Rocephin (25µg) were placed upside down on top of the agar plates. The plates were incubated at 37°C for 24hours and examined for zones of inhibition. Inhibition zone diameters were measured using a meter rule.

3. Results

The changes in pH and titratable acidity of the sorghum slurry with time are shown in Table 1. The pH decreased from 4.44 to 3.81 while the titratable acidity increased from 0.7 to 1.6.

Table 1. Changes in pH and titratable acidity of the sorghum slurry with time

Time (hrs)	pH	Titratable acidity
0	4.44	0.7
24	4.03	0.9
48	3.88	1.0
72	3.81	1.6

Table 2 showed the changes in pH and titratable acidity of the maize slurry with time. The pH of the slurry decreased from 4.41 to 3.85 while the titratable acidity increased from 0.6 to 1.2.

Table 2. Changes in pH and titratable acidity of the maize slurry with time

Time (hrs)	pH	Titratable acidity
0	4.41	0.6
24	3.97	0.8
48	3.91	0.9
72	3.85	1.2

The total lactic acid bacterial counts of the sorghum and maize slurries with time are presented in Table 3. The lactic acid bacterial populations of the sorghum slurry increased from 80cfu/g to 143cfu/g while those of the maize increased from 85cfu/g to 150cfu/g.

Table 3. Total lactic acid bacterial counts of the sorghum and maize slurries with time

Time (hrs)	Sorghum (cfu/g)	Maize (cfu/g)
0	80	85
24	108	114
48	124	135
72	143	150

The morphological and biochemical characteristics of the lactic acid bacteria from the sorghum and maize slurries with time are presented in Table 4. The bacteria were *Lactobacillus delbrueckii* subsp *bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus amylovorus*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus*.

Table 4. Morphological and biochemical characteristics of the lactic acid bacteria from the sorghum and maize slurries

Morphological and biochemical characteristics	<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus brevis</i>	<i>Lactobacillus plantarum</i>	<i>Lactobacillus amylovorus</i>	<i>Pedococcus acidilactici</i>	<i>Pedococcus pentosaceus</i>
Appearance	Rod	Rod	Rod	Rod	Rod	Coccus	Coccus
Gram reaction	+	+	+	+	+	+	+
Catalase test	–	–	–	–	–	–	–
Coagulase test	–	–	–	–	–	–	–
Motility test	–	–	–	–	–	–	–
Spore test	–	–	–	–	–	–	–
Indole test	–	–	–	–	–	–	–
Methyl red test	+	–	+	+	–	+	+
Voges proskauer test	–	–	–	–	–	–	+
Citrate utilization test	–	–	+	–	–	+	+
Lactose fermentation test	+	–	+	+	+	–	+
Glucose fermentation test	+	+	+	+	+	+	+
Sucrose fermentation test	–	–	+	+	+	–	+

+=Positive reaction

_ =Negative reaction

The distribution of the lactic acid bacteria in the sorghum and maize slurries is shown in Table 5. All the isolates except, *P. acidilactici* were isolated from the sorghum slurry while all the isolates were isolated from the maize slurry.

Table 5. Distribution of the lactic acid bacteria in the sorghum and maize slurries

Lactic acid bacteria	Sorghum	Maize
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	+	+
<i>Lactobacillus fermentum</i>	+	+
<i>Lactobacillus brevis</i>	+	+
<i>Lactobacillus plantarum</i>	+	+
<i>Lactobacillus amylovorus</i>	+	+
<i>Pedococcus acidilactici</i>	—	+
<i>Pedococcus pentosaceus</i>	+	+

+ = detected

— = not detected

Table 6 showed the frequency of occurrence of the lactic acid bacteria in the sorghum and maize slurries. *Lactobacillus delbrueckii* subsp *bulgaricus* occurred most frequently (33.3%) in the sorghum slurry while *Lactobacillus plantarum* had the lowest frequency of occurrence of 6.7%. *L. delbrueckii* subsp *bulgaricus* also had the highest frequency of occurrence of 26.0% in the maize slurry while *L. amylovorus* had the lowest frequency of occurrence of 9.4%. Generally, *L. delbrueckii* subsp *bulgaricus* occurred most frequently (29.0%) in the slurries while *P. acidilactici* had the lowest frequency of occurrence of 5.8%.

Table 6. Frequency of occurrence of the lactic acid bacteria in the sorghum and maize slurries

Lactic acid bacteria	Sorghum (n)	Maize (n)	Total (n)
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	100 (33.3%)	111 (26.0%)	211 (29.0%)
<i>Lactobacillus fermentum</i>	55 (18.3%)	85 (19.9%)	140 (19.3%)
<i>Lactobacillus brevis</i>	45 (15.0%)	53 (12.4%)	98 (13.5%)
<i>Lactobacillus plantarum</i>	20 (6.7%)	46 (10.8%)	66 (9.0%)
<i>Lactobacillus amylovorus</i>	38 (12.7%)	40 (9.4%)	78 (10.7%)
<i>Pedococcus acidilactici</i>	0 (0.0%)	42 (9.8%)	42 (5.8%)
<i>Pedococcus pentosaceus</i>	42 (14.0%)	50 (11.7%)	92 (12.7%)
Total	300 (100.0%)	427 (100.0%)	727 (100.0%)

n = number of isolates.

The growth of the lactic acid bacteria from the sorghum and maize slurries at varying pH levels is shown in Table 7. The isolates showed growth at the different pH levels but grew optimally at pH 4.0 and minimally at pH 2.5.

Table 7. Growth of the lactic acid bacteria from the sorghum and maize slurries at varying pH levels

Lactic acid bacteria	pH levels			
	2.5	3.0	4.0	5.0
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	+	++	++++	++++
<i>Lactobacillus fermentum</i>	+	++	++++	+++
<i>Lactobacillus brevis</i>	+	++	++++	++++
<i>Lactobacillus plantarum</i>	+	++	++++	+++
<i>Lactobacillus amylovorus</i>	+	++	++++	+++
<i>Pedococcus acidilactici</i>	+	++	++++	+++
<i>Pedococcus pentosaceus</i>	+	++	++++	+++

+ = minimal growth

++ = moderate growth

+++ = heavy growth

++++ = very heavy growth

Table 8 showed the salt tolerance of the lactic acid bacteria. All the isolates were tolerant to 3.0% concentration while all except *Lactobacillus brevis* and *Pedococcus acidilactici* were tolerant to 6.5% salt concentration. However, all the lactic acid bacteria did not grow at 10.0% salt concentration.

Table 8. Salt tolerance of the lactic acid bacteria

Lactic acid bacteria	Salt tolerance		
	3.0%	6.5%	10.0%
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	+++	++	—
<i>Lactobacillus fermentum</i>	++	+	—
<i>Lactobacillus brevis</i>	++	—	—
<i>Lactobacillus plantarum</i>	++	+	—
<i>Lactobacillus amylovorus</i>	++	+	—
<i>Pedococcus acidilactici</i>	+	—	—
<i>Pedococcus pentosaceus</i>	+++	++	—

+ = minimal tolerance

++ = moderate tolerance

+++ = high tolerance

— = No tolerance

The survival of the lactic acid bacteria in fresh bovine bile is presented in Table 9. All the isolates was resistant to the bile, hence there were no zones of inhibition.

Table 9. Survival of the lactic acid bacteria in fresh bovine bile

Lactic acid bacteria	Zone of Inhibition (mm)
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	0.0
<i>Lactobacillus fermentum</i>	0.0
<i>Lactobacillus brevis</i>	0.0
<i>Lactobacillus plantarum</i>	0.0
<i>Lactobacillus amylovorus</i>	0.0
<i>Pedococcus acidilactici</i>	0.0
<i>Pedococcus pentosaceus</i>	0.0

Table 10. Cell surface hydrophobicity of the lactic acid bacteria

Lactic acid bacteria	Cell surface hydrophobicity (%)
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	59.34
<i>Lactobacillus fermentum</i>	16.03
<i>Lactobacillus brevis</i>	0.00
<i>Lactobacillus plantarum</i>	13.33
<i>Lactobacillus amylovorus</i>	25.11
<i>Pedococcus acidilactici</i>	14.00
<i>Pedococcus pentosaceus</i>	56.00

Table 11. Antibiotics susceptibility profile of the lactic acid bacteria from the sorghum and maize slurries

Lactic acid bacteria	Amo (mm)	Sep (mm)	Cip (mm)	Gen (mm)	Str (mm)	Pef (mm)	Amp (mm)	Ery (mm)	Zin (mm)	Roc (mm)
<i>Lactobacillus delbrueckii</i> subsp <i>bulgaricus</i>	0	0	19	0	0	0	0	20	19	0
<i>Lactobacillus fermentum</i>	17	17	17	15	14	10	0	18	20	17
<i>Lactobacillus brevis</i>	0	23	23	0	0	11	0	22	18	16
<i>Lactobacillus plantarum</i>	0	0	19	11	0	13	0	20	23	17
<i>Lactobacillus amylovorus</i>	0	18	18	13	18	13	0	18	17	18
<i>Pedococcus acidilactici</i>	0	12	17	0	0	14	0	18	16	18
<i>Pedococcus pentosaceus</i>	0	11	17	0	14	13	0	19	19	18

Values (mm) = Diameters of zones of inhibition

Amo = Amoxicillin, Sep = Septrin, Cip = Ciprofloxacin, Gen = Gentamycin, Str = Streptomycin, Pef = Perfloxacin, Amp = Ampiclox, Ery = Erthromycin, Zin = Zinnacef, Roc = Rocephin

Table 10 showed the result of cell surface hydrophobicity assay of the lactic acid bacteria which reflects their adherence potential to the intestinal mucosa. The percentage hydrophobicity ranged from 0.00% to 59.34%. *Lactobacillus delbrueckii* subsp *bulgaricus* exhibited the highest level of hydrophobicity of 59.34% while *Lactobacillus brevis* showed no hydrophobicity to the intestinal mucosa.

The antibiotics susceptibility profile of the lactic acid bacteria from the sorghum and maize slurries are presented in Table 11. All the isolates were sensitive to Ciprofloxacin, Erythromycin and Zinnacef while Ampiclox had no inhibitory effect on any of them.

4. Discussion

The pH of the sorghum slurry decreased while the titratable acidity increased during the period of fermentation (Table 1). While the pH ranged from 4.44 to 3.81, the titratable acidity ranged from 0.7 to 1.6 during the fermentation period. Similar results were obtained with the maize slurry. The pH decreased from 4.41 to 3.85 while the titratable acidity increased from 0.6 to 1.2 during the period of fermentation (Table 2). Nwachukwu et al. [21] however reported that there is no significant correlation between the total bacterial counts, pH and titratable acidity.

The total lactic acid bacterial counts of the sorghum and maize slurries increased throughout the period of fermentation (Table 3). This result indicated that the

nutrients status and environmental conditions prevalent in the slurries encouraged the growth of the bacteria. Seven lactic acid bacteria were isolated from the sorghum and maize slurries. They were *lactobacillus delbrueckii* subsp *bulgaricus*, *L. fermentum*, *L. brevis*, *L. plantarum*, *L. amylovorus*, *Pedococcus acidilactici* and *P. pentosaceus*. More gram positive rods were isolated from the slurries than the gram positive cocci (Table 4).

More lactic acid bacteria were isolated from the maize slurry than the sorghum slurry. While *Pedococcus acidilactici* was not detected from the sorghum slurry, all the isolates were isolated from the maize slurry (Table 5). *Lactobacillus delbrueckii* subsp *bulgaricus* occurred most frequently (33.3%) in the sorghum slurry while *Lactobacillus plantarum* had the lowest frequency of occurrence (6.7%) in the slurry. However, *L. delbrueckii* subsp *bulgaricus* also had the highest frequency of occurrence (26.0%) in the maize slurry while *lactobacillus amylovorus* had the least frequency of occurrence of 9.4% in the maize slurry (Table 6).

Obinna-Echem et al. [22] reported that the lactic acid bacterial population of a selected Nigerian traditional fermented maize food called akamu was found to be dominated by strains of *lactobacillus plantarum*, *L. fermentum*, *L. delbrueckii* subsp *bulgaricus* and *L. Helveticus*. The isolates differed in their tolerance to acidity and varying salt concentration (Tables 7 and 8). At pH of 2.5, all the isolates showed minimal growth but their growth increased with increase in acidity, with the optimum growth at pH of 4.0. Several lactobacilli have

been reported to retain viability when exposed to pH values of 2.5 – 4.0 but showed loss of viability at lower pH values [19].

Lactobacilli are usually more resistant to acidic conditions than the other lactic acid bacteria. This property enables them to continue to grow during natural lactic fermentations, when the pH has dropped too low for the other lactic acid bacteria to grow, indicating that the lactobacilli are often responsible for the final stages of many lactic acid fermentations [23,24]. All the lactic acid bacteria grew at 3.0% salt concentration while none grew at 10.0% salt concentration indicating that the high salt concentration was inhibitory to their growth.

All the isolates were resistant to bile (Table 9). The resistance of organisms to bile is a determining factor for probiotic strains given that the liver excretes about 1.0 litre of bile each day into the small intestine. The effect of bile salts on bacterial cultures is more adverse compared to low pH [25]. Unconjugated bile salts even at low concentrations can inhibit the in vitro growth of microorganisms.

Six of the isolates showed cell surface hydrophobicity (Table 10) confirming their abilities to adhere to the intestinal mucosa. However, *Lactobacillus brevis* showed no hydrophobicity. The adhesion of microorganisms to intestinal epithelial cells involves several mechanisms of which cell surface hydrophobicity is one of the physicochemical properties that facilitate the first contact between the microorganisms and the host cells [26]. This initial interaction precedes the subsequent adhesion process mediated by mechanisms involving cell surface proteins and lipoteichoic acids [27,28].

Several reports have been made on lactic acid bacteria surviving the gastrointestinal tract of humans and animals [29,30]. A study comparing the properties of lactic acid bacteria originating in fermented food with those in the gastrointestinal tract showed that strains are able to attach in vitro to human enterocyte – like epithelial cells (Caco-2 cell line) and survive low pH and the presence of bile [31].

The Lactic acid bacteria were resistant to Ampiclox but were inhibited by Ciprofloxacin, Erythromycin and Zinnacef. However, *Lactobacillus delbrueckii* subsp *bulgaricus* was more resistant to the antibiotics than the other lactic acid bacteria (Table 11). This result agreed with Halami et al. [32] who reported that lactic acid bacteria are normally more resistant to the principal types of antibiotics. Ammor et al. [33] however reported that the resistance to these antibiotics is usually intrinsic, hence resistance genes are not transferable.

5. Conclusions

This study indicated that the lactic acid bacterial species isolated are the principal bacterial species responsible for

the fermentation of sorghum and maize into the weaning food - Akamu, due to the relatively low pH and high acidity of the fermentation process. These properties should be exploited in the preservation of foods leading to increased shelf life and products of good and acceptable quality. In addition, the results of the study showed that these lactic acid bacteria from raw Akamu possess probiotic properties, therefore, raw Akamu can be used for the treatment and prevention of intestinal diseases.

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