Isolation and Screening of Antifungal Producing Lactic Acid Bacteria From Pro-Vitamin A Cassava

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Abstract
Lactic Acid Bacteria (LAB) are non-pathogenic bacteria reported to play essential role in preservation, fermentation and contribute to organoleptic and textural profile of food items. This study investigated antifungal producing LAB during fermentation of Pro-vitamin A and white cassava variety which were used against spoilage mould of cassava products (garri, lafun and fufu). One white cassava variety (IITA TMS IBA 30572 (ITI3)) and one yellow variety (IITA TMS IBA 011368 (ITI10)) were fermented for 96 hours. The pH and Total Titratable Acidity (TTA) of the fermenting medium and LAB counts of the supernatant and cassava mash were determined using standard procedures. The LAB were screened with Aspergillus niger to detect their antifungal ability. The LAB were identified as different species which are Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus mesenteroides, Lactobacillus casei and Lactobacillus acidophilus. These LAB isolates posses the potential to inhibit most of the isolated moulds used in this study. Quantities of lactic acid, diacetyl and hydrogen peroxide produced by the LAB were also determined using standard procedures. It was also observed that the pH and temperature of the growth medium have significant effect on the production of these antifungal metabolites by the isolated lactic acid bacteria. The proliferation of LAB at low pH and their inhibition against some moulds indicate their potential as bio-protective agents in cassava food products.

Keywords: Fufu, Lafun, Garri, Fermentation, LAB, Antifungal compounds

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1. INTRODUCTION
Cassava (Manihot esculenta Crantz) is an important root crop often processed into various food products in tropical countries. In West Africa the most widely known cassava derived food is garri. Several research works have been carried out on the processing, nutritional and microbiological characteristics and quality improvement of garri (Ogiehor and Ikenebomeh, 2006; Kostinek et al., 2005; Padonou et al. 2009). Cassava significantly contributes to food security, incomes and employment opportunities in the rural areas of sub-Saharan Africa (Githunguri et al., 2007). About 38% of the cassava produced in the coastal lowlands of Kenya is consumed at household level, and 51% of the farmers make dried chips for domestic use, sale to starch and feed factories or as an intermediate for production of flour (Kiura et al., 2005; Gaheru et al., 2016). As at date, new cassava varieties are being introduced to farmers for their agronomic benefits with little considerations for the quality of the end products.

Today, biofortification provides one of the best ways to achieve improvements in nutrition. Crop breeders at the National Root Crops Research Institute, Umudike in collaboration with the International Institute of Tropical Agriculture, and the Harvest Plus, have worked assiduously to develop cassava varieties that serve as sources of vitamin which led to the development of yellow fleshed cassava roots that have appreciable quantity of pro-vitamin A or carotenoids (through bio-fortification) (Akinwunmi, 2011). Vitamin A deficiency is a major public health issue and of global concern. According to the World Health Organization report (WHO, 2002; Olapade and Ogunade, 2014). The pro-vitamin A rich yellow cassava varieties are proposed to be used against spoilage mould of cassava products (garri, lafun and fufu). One white cassava variety (IITA TMS IBA 30572 (ITI3)) and one yellow variety (IITA TMS IBA 011368 (ITI10)) were fermented for 96 hours. The pH and Total Titratable Acidity (TTA) of the fermenting medium and LAB counts of the supernatant and cassava mash were determined using standard procedures. The LAB were screened with Aspergillus niger to detect their antifungal ability. The LAB were identified as different species which are Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus mesenteroides, Lactobacillus casei and Lactobacillus acidophilus. These LAB isolates posses the potential to inhibit most of the isolated moulds used in this study. Quantities of lactic acid, diacetyl and hydrogen peroxide produced by the LAB were also determined using standard procedures. It was also observed that the pH and temperature of the growth medium have significant effect on the production of these antifungal metabolites by the isolated lactic acid bacteria. The proliferation of LAB at low pH and their inhibition against some moulds indicate their potential as bio-protective agents in cassava food products.

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Microbial deterioration and unfavorable biochemical changes of cassava roots are caused by physiological reactions and activities of microorganisms, which enter cassava roots through bruises and cuts caused during harvesting. Traditional processing of cassava chips and flour is generally unhygienic. The chips are sun-dried in open surfaces such as on flat rocks, roads, flat rooftops, flat baskets, or bare ground (FAO, 2005). Storage conditions after drying may also be of high humidity, thus leading to an increase in moisture levels hence creating conducive environments for growth and proliferation of microorganisms. Unhygienic conditions during production (for instance lack of protective clothing, lack of hand washing areas, drying on dirty surfaces), storage and slow sun-drying especially during the rainy seasons, often results in bacteria and mould contamination (Chiona et al., 2014), with for instance Aspergillus species that produce aflatoxins which are a major health concern to humans and livestock (Manjula et al., 2009; Gacheru et al., 2016). Several millions of people in the African continent consume garri and it forms a significant part of the people’s diet especially in the West African sub
region (Edem *et al*., 2001; Ogiehor *et al*., 2007; Kostinek *et al*., 2005 and Oranusi *et al*., 2014).

A group of bacterial called lactic acid bacteria (LAB), a component of several spontaneously fermented foods including dairy products have long been consumed by humans. Lactic acid bacteria are the focus of intensive research for their essential role in most fermented foods. Representatives of this group of bacteria include Lactobacillus and *Bifidobacterium*. Lactic acid bacteria (LAB) are the most prominent non-pathogenic bacteria that play a prominent role in our everyday life such as fermentation, preservation and production of wholesome foods and vitamins to prevent certain diseases and cancer due to their antimicrobial action (Saranya *et al*., 2011). Lactic acid bacteria are the biological basis for the production of a great multitude of fermented foods (Lasagno *et al*., 2002).

Lactic acid bacteria (LAB) have been exploited for centuries in food fermentations, preservation and are also source of promoting good human health (Oloyede and Afolabi, 2013). First sign of LAB instrumentation date back to 6,000BC, describing the fermentation of milk, fermentation of meat (1,500 BC) and vegetable products (300 BC) (Bhattacharyya, 2009). LAB have been widely used as starter cultures for the manufacturing of various fermented foods such as dairies, alcoholic beverages, meat and vegetables etc (Harun-ur-Rashid *et al*., 2009; Antara *et al*., 2009). They play important role in food fermentation, primarily by causing the characteristic flavor changes and contributing a preservative effect on the fermented product. Some species of LAB have been used on industrial scale as starter cultures and also as probiotics. Nowadays, LAB and their food products are brought to confer a variety of important nutritional and therapeutic benefits on humans and animals (Boaventura *et al*., 2012).

Reservative efforts to find microorganisms which produce antibiotic against wide range of pathogens have been pointed out to lactic acid bacteria. The fermented food is a source of various bacteria especially lactic acid bacteria (Anto *et al*., 2005). The problem of selection of resistant microorganisms to antimicrobials and the increasing demand for safe foods, with less chemical additives, has increased the interest in replacing these compounds by natural products, which do not injure the host or the environment (Kapil, 2005). Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products as well as their technical application in food. Foods that have been processed could be spoilt during storage in certain period of time, even though they have been designated free from harmful microorganisms. This condition has pushed food producers to frequently use high dose of dangerous chemical preservative in order to extend the shelf life of the products. (Anto *et al*., 2005).

Fungi have a profound biological and economic impact as food spoilage agents, decomposers, plant and animal pathogens (Oranusi *et al*., 2013). Moulds and yeasts are common spoilage microorganisms of food products and are responsible of 5 to 10 % of world’s food lost (Schnurer and Magnusson 2005). A few studies have reported the antifungal activity of some lactic acid bacteria (LAB) through the production of phenylactic acid (Lavermicocca *et al*., 2003), cyclic dipeptides, 3-hydroxy fatty acids and peptides (Storm *et al*., 2002; Sjogren *et al*., 2003). The inhibition of growth of spoilage organisms may also be due to the production of many metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, diacetyl and bacteriocins (Ennahar *et al*., 2000). The incorporation of these compounds as biopreservative ingredient into model food has been shown to be effective in the control of pathogenic and spoilage microorganisms (Mc Auliffe *et al*., 2001). For this reason, LAB have received much attention for use as natural or biopreservatives in food in recent years (Savadogo *et al*., 2004).

Therefore, the study is aimed at isolating and characterizing antifungal producing LAB present in fermenting pro-vitamin A and assessing the activity of the antifungal metabolites produced against spoilage moulds of cassava products by these bacteria.

**2. MATERIALS AND METHODS**

**2.1. Collection of samples**

Samples were collected randomly from cassava root and cassava products. Fresh roots (5kg each) of white root variety; IITA TMS IBA 30572 and yellow root variety; IITA TMS IBA 011368 were obtained in a clean sack from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state, Nigeria and brought to the Postgraduate Laboratory, Microbiology Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Three different cassava products namely: “Fufu”, “Lafun” and “Garri” were produced in the laboratory from the obtained cassava roots. The cassava effluent was obtained from fermentation of the cassava root in the laboratory.

**2.2. Samples Preparation**

Pro-vitamin A and white cassava were fermented using the submerged fermentation technique. Five hundred grams (500 g) of the three varieties of the cassava were separately peeled, cut in to smaller sizes, washed and soaked in an air tight container with 1500ml of water and fermented for 96hours at room temperature (28±2°C). The fermentation supernatant and mash samples were collected at 12 hrs interval for microbiological analysis. “Fufu”, “Lafun” and “Garri” were produced in the laboratory from the obtained cassava roots. About 1.5 kg each of the cassava root varieties were used for the production of the three cassava products. Roots of each variety were
separately peeled, cut into small sizes and washed. The products were all produced using traditional methods.

2.3. Determination of pH
The pH change of the fermenting medium was monitored at 12 hours intervals using a pH meter. The method described by Wakil and Osamwonyi (2012) was used. Ten milliliters of the fermenting gruel was aseptically removed from the fermenting medium and its pH was determined with the aid of a pre-standardized pH meter (HANNA instrument) by inserting the pH electrode inside the samples and taking the reading on the monitor.

2.4. Titratable acidity
The procedure described by Wakil and Osamwonyi (2012) was used. The titratable acidity (expressed as percentage lactic acid) was determined by shaking the fermenting vessel to enable mixing of fermentation water and fermenting cassava. The samples were collected at 12 hours interval. Twenty five milliliters (25 ml) of the fermentation supernatant from the two varieties of cassava were collected separately in different conical flasks and labeled accordingly. The collected fermentation supernatant was titrated against 0.1M NaOH using 1ml of phenolphthalein as indicator. The total titratable acidity was calculated as lactic acid (% w/v).Each milliliter of 1N NaOH is equivalent to 90.08 mg of lactic acid. The total titratable acidity was then calculated as stated in A.O.A.C (2000).

2.5. Isolation and characterization of Lactic Acid Bacteria
Lactic acid bacteria were isolated from the fermentation supernatant and cassava mash generated from the fermentation of the two varieties of cassava roots. One milliliters of the fermentation supernatant and 1 g of the mash were aseptically removed from the fermenting vessels for serial dilution at 12 hours and plated out on De Mann Rogosa and sharpe (MRS) agar at 37ºC for 48 hours.. Pure cultures were maintained on MRS agar slants and stored at 4ºC for further studies. The LAB isolates were characterized phenotypically and were identified using Bergey’s Manual of Determinative Bacteriology (Wakil and Osamwonyi, 2012). Further characterization was carried out using molecular methods.

2.6. Screening of lactic acid bacteria for antifungal activity
The LAB isolates were assayed for antifungal activity using modified overlay method described by Rouse et al., 2008 as an initial screening step. On MRS agar plates, LAB after growing at 30ºC overnight in MRS broth were streaked. The plates were allowed to grow anaerobically at 37ºC for 48 hrs. The plates were then overlaid with PDA that contained mould spores per ml of indicating organism. After 24 -48 hrs of aerobic incubation at 28ºC, inhibition zone around the LAB were measured. The inhibition was graded according to the size of inhibited growth area. Inhibition test was performed in triplicate. The degree of inhibition was calculated as area of inhibited growth in relation to the total area of the Petri dish and the scale was the following: - = no visible inhibition, + = no fungal growth on 0.1–3 % of plate area, ++ = no fungal growth on 3–8 % of plate area, +++ = no fungal growth on >8 % of plate area.

2.7. Production of Metabolites by the Lactic acid bacteria
The antifungal producing isolates with inhibition greater than 8% were cultivated in MRS broth and incubated anaerobically for 24 hrs at 30ºC. The broth culture was centrifuged at 5000 rpm for 15 mins and cell-free supernatants (CFS) (filtrate) were used as inoculums (Wakil and Osamwonyi, 2012).

2.8. Detection of antifungal activity by agar well diffusion method
Antifungal assay was performed by using the agar well diffusion method. Potato Dextrose Agar medium seeded with each test mould strains was added the added to sterile Petri dishes. The plates were allowed to dry and a sterile cock borer of diameter 5.00 mm was used to dig wells of equal distance seeded plates. Before the assays, the isolated LAB strains were twice pre-cultured in MRS broth, for 24 hrs at 37ºC. Afterwards, each well was filled with 50 µl of each CFS and one well was filled with sterile distilled water to serve as a negative control. All the assays were carried out in triplicates (for each spoilage moulds). The plates were left for sometimes to allow the test materials to diffuse in the agar and then incubated at 37ºC for 48 hrs. The antifungal activity was assayed by measuring the diameter of the clear zone of inhibition formed around the well. The diameter (mm) of the zone of inhibition of 1.5 mm and above was considered as a significant inhibition (Muhialdin, 2013)

2.9. Production of Antifungal Metabolites by Lactic Acid Bacteria
2.9.1. Quantitative Estimation of Lactic Acid: The quantity of lactic acid produced by bacteria isolates was carried out using the method described by Wakil and Osamwonyi (2012). The quantity of lactic acid produced by antimicrobial producing isolates at 24 hrs, 48 hrs, 72 hrs and 96 hrs was determined by transferring 25 ml of broth cultures of test organisms into 100 ml flasks. This was titrated with 0.1M NaOH and 1 ml of phenolphthalein
indicator. The titratable acidity was calculated as lactic acid (% w/v). Each milliliter of 1 M NaOH is equivalent to 90.08 mg of lactic acid. The titratable acidity was then calculated as stated in A.O.A.C (2000).

\[
\text{Lactic acid produced} = \frac{\text{ml NaOH} \times \text{M NaOH} \times \text{M.E}}{\text{Weight of sample}} \times 100
\]

Where;
ml NaOH = volume of NaOH used
M NaOH = molarity of NaOH
M.E = equivalence factor (90.08/mg)

2.9.2. Quantitative Estimation of Diacetyl: The quantity of diacetyl produced by bacteria isolates was carried out using the method described by Wakil and Osamwonyi (2012). Diacetyl production at 24 hrs, 48 hrs, 72 hrs and 96 hrs was determined by transferring 25 ml of broth cultures of test organisms into 100 ml flasks. Hydroxylamine solution (7.5 ml) of 1 molar was added to the flask and to a similar flask for residual titration. Both flasks were titrated with 0.1 M HCl to a greenish yellow end point using bromothymol blue as indicator. The equivalence factor of HCl to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated using the A.O.A.C. (2000).

\[
\text{AK} = \frac{(b-s)(100E)}{W}
\]

Where;
AK = % of diacetyl
b- s = volume of HCl used
E = equivalence factor (21.52/mg)
W = volume of broth
100 = constant

2.9.3. Quantitative Estimation of Hydrogen Peroxide: The quantity of hydrogen peroxide produced by bacteria isolates was carried out using the method described by Wakil and Osamwonyi (2012). Hydrogen peroxide production at 24 hrs, 48 hrs, 72 hrs and 96 hrs was determined by measuring 25 ml of broth cultures of the test organisms into a 100 ml flask. To this was added 25 ml of freshly prepared 0.1M H₂SO₄. This was then titrated with 0.1M potassium permanganate (KMnO₄). Each milliliter of 0.1 N KMnO₄ is equivalent to 1.701 mg of H₂O₂. A decolorization of the sample was regarded as the end point. The volume of H₂O₂ produced was then calculated (A.O.A.C; 2000).

\[
\text{H₂O₂ produced} = \frac{\text{ml KMnO₄} \times \text{NKMnO₄} \times \text{M.E} \times 100}{\text{ml H₂SO₄} \times \text{Volume of sample used}}
\]

Where;
ml KMnO₄ = volume of KMnO₄
N KMnO₄ = Normality of KMnO₄
ml H₂SO₄ = Volume of H₂SO₄ used
M.E = Equivalence factor (1.701/mg)

2.10. Determination of the effect of initial pH on the production of antimicrobial substances
One hundred milliliters of composed MRS Broth was adjusted to initial pH values of 4.5, 5.5, 6.5 and 7.5, using either 1M HCl or 1M NaOH. Each medium was inoculated with an overnight culture of antimicrobial producing organisms and incubated at 30°C for 48 hrs; the quantity of antimicrobials produced was estimated using the method of A.O.A.C (2000) as described above for the quantification of antimicrobial substances (Wakil and Osamwonyi, 2012).

2.11. Determination of the effect of initial temperature on the production of antimicrobial substances
A modified method of Wakil and Osamwonyi (2012) for the effect of pH on antimicrobial production and Adesokan et al., (2009) were used. One hundred milliliters of composed MRS Broth was prepared in triplicates. Each medium was inoculated with an overnight culture of antimicrobial producing organisms and incubated at 15°C, 30°C and 45°C for 48 hrs respectively; the quantity of antimicrobials produced was estimated using the method of A.O.A.C (2000) as described above for the quantification of antimicrobial substances.

2.12. Statistical analysis: All experiments were performed in triplicate and the results are expressed as mean ± standard deviation and Duncan Multiple Variance analysis (p<0.05). Data analysis was performed using SPSS 19.0 (IBM Corp., Armonk, NY, USA).
3. RESULTS
3.1. Changes in pH during fermentation: Changes in pH during the fermentation of the cassava varieties are presented in Table 1. The pH ranges from 3.65±0.56 to 6.40±0.06, 3.50±0.02 to 6.40±0.06 for ITI0 and ITI3 respectively. The pH decreased progressively with fermentation time for the two cassava varieties.

3.2. Changes in TTA during fermentation: The result of TTA (g/L) during the fermentation of the cassava varieties with respect to fermentation time is presented in Table 2. TTA increases with fermentation time for all the cassava varieties. TTA of the variety ITI0 increased from 0.22±0.00 g/L at 0 hrs to 1.73±0.10 g/L at 96 hrs while the TTA of ITI3 increased from 0.22±0.00 g/L at 0 hrs to 2.83±0.11 g/L at 96 hrs.

3.3. Isolation and Enumeration of LAB from the Fermentation Water and Cassava mash: Table 3 and 4 show the plate count obtained on MRS agar for LAB isolated from fermentation supernatant and cassava mash respectively. There was no count detected at 0hrs of fermentation. The LAB count in fermentation supernatant ranged from 1.2±0.24x10^3 CFU/ml at 12 hrs to 7.5±0.06x10^4 CFU/ml at 96 hrs in ITI0 and 1.0±0.08x10^3 CFU/ml at 12 hrs to 2.1±0.13x10^5 CFU/ml at 96 hrs. The LAB count in fermented cassava mash ranged from 1.0±0.28x10^3 CFU/g at 12 hrs to 1.8±0.08 x10^4 CFU/g at 96 hrs in ITI0 and from 1.2±0.02x10^5 CFU/g at 12 hrs to 1.6±0.23x10^5 CFU/g at 96 hrs for ITI3.

3.4. Screening of lactic acid bacteria for antifungal activity: A total of 20 LAB isolates were isolated from both fermentation supernatant and cassava mash of the two cassava varieties on MRS agar at 37°C under anaerobic condition. The LAB isolates were screened against selected mould indicator to detect their antifungal activity and the result is shown in Table 5. Out of the 22 isolated LAB, 5 did not show visible inhibition, 3 inhibited fungal growths between 0.1-3% of plate area, 4 inhibited fungal growth between 3-8% of plate area while the remaining inhibited fungal growth at a value greater than 8% of the plate area.

3.5. Identification and Characterization of the isolated LAB: Lactic acid bacterial isolates that shows antifungal activity to the isolated moulds were identified using various physiological and biochemical tests. The LAB isolates were identified as five different genera namely L. casei, L. mesenteroides, L. plantarum, L. acidophilus and L. fermentum. Figure 1 shows the percentage occurrence of the isolated LAB which shows that Lactobacillus plantarum and Lactobacillus mesenteroides are dominant in occurrence with 25% of occurrence while Lactobacillus acidophilus showed the least occurrence with 10% of occurrence.

3.6. Determination of antifungal activity of LAB by agar well diffusion method: The antifungal activity of the selected lactic acid bacteria that inhibited greater than 8% against growth of spoilage mould of the three cassava products was determined by agar well diffusion method and the results are presented in Table 6. The zones of inhibition were the diameter of the circle formed as a result of the inhibitory activity of LAB isolates against spoilage moulds of cassava products excluding the diameter of the cork borer used (5.00 mm). The zones of inhibition ranged from 1.00 mm to 17.30 mm in diameter. The highest zone of inhibition (17.30 mm) was from LAB isolate L. plantarum (WLW103) against mould isolate WFE101 while the lowest zone of inhibition (1.00 mm) was from the LAB isolate L. fermentum (YLW102) against the mould isolate YFE103. Aspergillus niger showed the highest susceptibility and Rhizopus sp. showed the least susceptibility to the LAB isolates.

Out of the 9 LAB isolates, only isolates L. plantarum (YLM102), L. plantarum (WLW107) and L. acidophilus (WLW101) showed inhibition against more than 50% of the mould isolates. Isolates L. acidophilus (WLW101) and linhibited 19 mould isolates out of 21 accounting to 90.47%. Mould isolates YFF102 and YFL102 are the least inhibited. YFF102 was only inhibited by LAB isolate L. plantarum (WLW107) while YFL102 was inhibited by only by L. plantarum (WLW103). It was also observed that LAB isolates from ITI3 cassava variety were greatly active against moulds isolated from ITI0 while LAB isolate from ITI0 were mildly active against moulds of other cassava variety as they showed better activity against the mould isolated from them.

3.7. Molecular Characterization of LAB: Molecular techniques was also used to identify the LAB with highest and lowest antifungal activity. The LAB isolates were sequenced and BLAST to be Lactobacillus fermentum NBRC15885 (YLM102), Lactobacillus plantarum NRRLB-14768 (YLW104), Lactobacillus plantarum NBRC15891 (YLM102), Lactobacillus acidophilus VPI6032 (WLW105) and Lactobacillus plantarum NBRC15891 (WLW107).

The antifungal metabolites were quantified by titration. The quantity (g/L) of antifungal metabolites responsible for the antifungal activities of the LAB isolates against spoilage moulds is shown in Table 7.
3.8. Quantitative estimation of antifungal metabolites: The antifungal metabolites detected were lactic acid, diacetyl and hydrogen peroxide. The highest quantities of the metabolites were produced at 48 hrs of incubation after which there was a general reduction in the quantity of metabolites produced at incubation time higher than that.

The peak lactic acid produced at 48 hrs was 1.28 g/L by *Lactobacillus plantarum* (WLW103) while the least was 0.52 g/L by *Lactobacillus fermentum* (YLW102). The highest diacetyl quantity produced at 48 hrs was 1.92 g/L by *Lactobacillus plantarum* (WLW103) with the lowest being 0.88 g/L by *Lactobacillus plantarum* (WLW107). The highest quantity of hydrogen peroxide produced at 48 hrs was quantified as 0.039 g/L by *Lactobacillus mesenteroides* (YLW103) and *Lactobacillus mesenteroides* (YLW103) with the lowest produced by *Lactobacillus plantarum* (YLM102) of 0.043 g/L quantity.

3.9. The effect of pH and Temperature on antifungal metabolites production: The optimization of the production of antifungal metabolites with varying temperature and pH was also determined. The pH of the medium was varied to pH 4.5, 5.5, 6.5 and 7.5. The result of the optimization of antifungal metabolite quantification with varying pH is shown in Table 8. It was observed that all the antifungal metabolites were optimally produced in the acidic pH with highest performance at pH 5.5 and general reduction at pH greater than that. The highest quantity of lactic acid produced at pH 5.5 was quantified as 1.42 g/L by *Lactobacillus plantarum* (WLW107) with the lowest produced by *Lactobacillus casei* (YLM101) of 0.95 g/L quantity.

The peak diacetyl quantity produced at pH 5.5 was 2.86 g/L by *Lactobacillus acidophilus* (WLM101) with the lowest being 1.01 g/L by *Lactobacillus plantarum* (WLW102). The highest quantity of hydrogen peroxide produced at pH 5.5 was quantified as 0.085 g/L by *Lactobacillus mesenteroides* (YLW103) with the lowest produced by *Lactobacillus casei* (YLM101) of 0.021 g/L quantity. The quantification of the antifungal metabolites was done at 15°C, 30°C and 45°C. Table 9 shows the effect of temperature on the quantity of antifungal metabolites produced. It was observed that the production of the antifungal metabolites was optimally produced at 30°C. The highest quantity of lactic acid produced at 30°C was 1.32 g/L by *Lactobacillus fermentum* (YLW102) while the least was 0.88 g/L by *Lactobacillus plantarum* (WLW107). The peak diacetyl quantity produced at 30°C was 2.53 g/L by *Lactobacillus casei* (YLM101) with the lowest being 1.01 g/L by *Lactobacillus fermentum* (YLW102). The highest quantity of hydrogen peroxide produced at 30°C was quantified as 0.054 g/L by *Lactobacillus mesenteroides* (YLW103) with the lowest produced by *Lactobacillus plantarum* (YLW104) of 0.018 g/L quantity.

**Table 1: pH of the cassava varieties during fermentation**

| Cassava varieties | TI10                | TI13                |
|-------------------|---------------------|---------------------|
| 0                 | 6.40±0.06           | 6.40±0.06           |
| 12                | 5.20±0.12           | 5.10±0.10           |
| 24                | 4.45±0.03           | 4.20±0.33           |
| 36                | 4.05±0.03           | 3.95±0.10           |
| 48                | 3.95±0.08           | 3.88±0.12           |
| 60                | 3.80±0.15           | 3.75±0.09           |
| 72                | 3.76±0.13           | 3.72±0.22           |
| 84                | 3.75±0.76           | 3.70±0.08           |
| 96                | 3.65±0.58           | 3.50±0.02           |

**Table 2: TTA of the different cassava varieties during fermentation**

| Cassava varieties | TI10                | TI13                |
|-------------------|---------------------|---------------------|
| 0                 | 0.22±0.00           | 0.22±0.00           |
| 12                | 0.71±0.17           | 0.75±0.23           |
| 24                | 1.08±0.16           | 1.08±0.12           |
| 36                | 1.20±0.17           | 1.35±0.25           |
| 48                | 1.20±0.17           | 1.35±0.25           |
| 60                | 1.35±0.23           | 1.40±0.10           |
| 72                | 1.38±0.15           | 1.62±0.16           |
| 84                | 1.51±0.06           | 1.85±0.23           |
| 96                | 1.73±0.10           | 2.83±0.11           |
Table 3: Lactic Acid Bacteria count of fermentation supernatant during fermentation

| Microbial Count LogCFU/ml | ITI0   | ITI3   |
|---------------------------|--------|--------|
| Hours                     |        |        |
| 0                         | ND     | ND     |
| 12                        | 1.2± 0.24x10³ | 1.0±0.08 x 10³ |
| 24                        | 1.6±0.05x10³  | 2.1± 0.02x10³  |
| 36                        | 2.8±0.30x10⁴  | 3.0± 0.07x10⁴  |
| 48                        | 4.0±0.15x10⁴  | 4.3± 0.31x10⁴  |
| 60                        | 5.2±0.14x10⁴  | 5.8± 0.15x10⁴  |
| 72                        | 1.9±0.05x10⁵  | 7.2± 0.49x10⁴  |
| 84                        | 1.2±0.23x10⁵  | 1.1± 0.30x10⁵  |
| 96                        | 7.5±0.06x10⁴  | 2.1± 0.13x10⁵  |

Table 4: Lactic Acid Bacteria count of Cassava mash during fermentation

| Microbial Count LogCFU/ml | ITI0   | ITI3   |
|---------------------------|--------|--------|
| Hours                     |        |        |
| 0                         | ND     | ND     |
| 12                        | 1.0±0.28 x10³  | 1.2±0.02x10³  |
| 24                        | 1.4±0.21x10³  | 1.5± 0.07x10³  |
| 36                        | 3.0±0.16x10³  | 3.2± 0.08x10³  |
| 48                        | 4.1±0.40x10³  | 4.2± 0.06x10³  |
| 60                        | 1.6±0.25x10⁴  | 1.4± 0.04x10⁴  |
| 72                        | 1.7±0.25 x 10⁴ | 1.8± 0.04x10⁴  |
| 84                        | 1.2±0.04x10⁵  | 1.9±0.30x10⁴  |
| 96                        | 1.8±0.08 x 10⁵ | 1.6±0.23x10⁵  |

Table 5: Screening of lactic acid bacteria for antifungal activity.

| S/N | Variety | Isolate code | Activity |
|-----|---------|--------------|----------|
| 1   | ITI0    | YLW101       | -        |
| 2   | ITI0    | YLW102       | +++      |
| 3   | ITI0    | YLW103       | +        |
| 4   | ITI0    | YLW104       | +++      |
| 5   | ITI0    | YLW105       | +        |
| 6   | ITI0    | YLM101       | +++      |
| 7   | ITI0    | YLM102       | +++      |
| 8   | ITI0    | YLM103       | -        |
| 9   | ITI0    | YLM104       | +        |
| 10  | ITI0    | WLW101       | ++       |
| 11  | ITI0    | WLW102       | +        |
| 12  | ITI0    | WLW103       | +++      |
| 13  | ITI0    | WLW104       | -        |
| 14  | ITI0    | WLW105       | +++      |
| 15  | ITI0    | WLW106       | ++       |
| 16  | ITI0    | WLW107       | +++      |
| 17  | ITI0    | WLM101       | +++      |
| 18  | ITI0    | WLM102       | -        |
| 19  | ITI0    | WLM103       | ++       |
| 20  | ITI0    | WLM104       | -        |
| 21  | ITI3    | WLW101       | ++       |
| 22  | ITI3    | WLW102       | +        |
| 23  | ITI3    | WLW103       | +++      |
| 24  | ITI3    | WLW104       | -        |
| 25  | ITI3    | WLW105       | +++      |
| 26  | ITI3    | WLW106       | ++       |
| 27  | ITI3    | WLW107       | +++      |
| 28  | ITI3    | WLM101       | +++      |
| 29  | ITI3    | WLM102       | -        |
| 30  | ITI3    | WLM103       | ++       |
| 31  | ITI3    | WLM104       | -        |
Table 6: Diameter of zones of inhibition (mm) of Mould isolates by LAB isolates

| Mould Isolates | YFL101 | YFL102 | YFL103 | YFL104 | YFL105 | YFF101 | YFF102 | YFF103 | YFF104 | YFF105 | YFE101 | YFE102 | YFE103 | YFE104 | YFE105 | YFE106 | YFE107 | YFL101 | YFL102 | YFL103 | YFL104 | YFL105 | WFF101 | WFF102 | WFE101 | WFE102 | WFL101 | WFL102 | WFL103 |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                | -      | -      | 2.7    | -      | 3.0    | 4.7    | 9.3    | 4.0    | 8.0    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFF102         | -      | -      | -      | -      | -      | -      | -      | 4.3    | -      |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFF103         | -      | -      | 10.0   | 4.3    | 8.3    | 8.0    | 11.7   | 4.0    | 5.3    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFF104         | -      | -      | -      | -      | 4.0    | 5.3    | -      | 9.7    | 5.7    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFF105         | -      | 4.7    | 11.7   | 9.3    | 8.7    | 17.6   | -      | -      | 5.7    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFE101         | -      | -      | 12.0   | 5.0    | 10.0   | 5.3    | 3.3    | -      | 10.0   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFE102         | -      | -      | -      | -      | -      | 11.7   | 10.0   | -      | 4.0    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFE103         | 2.0    | 3.0    | -      | -      | -      | 6.3    | -      | 8.3    | 7.3    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFE104         | -      | 5.0    | -      | 10.0   | -      | -      | 4.0    | -      | 2.3    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFL101         | -      | -      | -      | -      | -      | 6.7    | -      | 12.0   | 10.0   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFL102         | -      | -      | -      | -      | -      | 2.3    | -      | -      | -      |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFL103         | 4.0    | 3.0    | -      | -      | -      | 6.0    | 3.0    | 5.0    | 2.0    | 4.0    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFL104         | -      | -      | -      | 3.0    | 5.0    | 2.0    | 5.0    | 2.0    | 6.0    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| YFL105         | -      | 6.3    | -      | 8.0    | -      | -      | 7.0    | -      | 5.7    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFF101         | -      | -      | -      | -      | -      | 10.3   | -      | -      | 10.3   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFF102         | -      | -      | -      | -      | 5.0    | 7.3    | 5.0    | 4.5    | 7.3    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFE101         | -      | -      | 3.0    | -      | -      | 17.3   | -      | 10.0   | 8.3    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFE102         | -      | -      | -      | -      | 4.3    | 14.3   | -      | 10.0   | 8.0    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFL101         | 7.7    | -      | -      | -      | -      | 10.7   | 10.0   | -      | 9.3    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFL102         | 9.0    | -      | -      | -      | 5.0    | 11.7   | -      | -      | 9.0    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| WFL103         | 9.7    | -      | -      | -      | 7.0    | 10.3   | -      | -      | 6.5    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
### Table 7: Quantity of Lactic Acid (g/L), Diacetyl (g/L) and Hydrogen peroxide (g/L) produced by the LAB isolates from the two cassava varieties at different incubation time

| S/N | Isolates code | Lactic acid (g/L) 24hrs | 48hrs | 72hrs | 96hrs | Diacetyl (g/L) 24hrs | 48hrs | 72hrs | 96hrs | Hydrogen peroxide (g/L) 24hrs | 48hrs | 72hrs | 96hrs |
|-----|---------------|-------------------------|-------|-------|-------|---------------------|-------|-------|-------|-----------------------------|-------|-------|-------|
| 1   | YLW102        | 0.17                    | 0.52  | 0.48  | 0.40  | 0.92                | 1.32  | 1.24  | 0.97  | 0.015                       | 0.023 | 0.018 | 0.014 |
| 2   | YLW103        | 0.25                    | 0.91  | 0.90  | 0.83  | 0.98                | 1.21  | 1.10  | 0.73  | 0.027                       | 0.039 | 0.015 | 0.008 |
| 3   | YLW104        | 0.46                    | 1.01  | 0.93  | 0.73  | 0.76                | 0.99  | 0.84  | 0.65  | 0.007                       | 0.016 | 0.012 | 0.009 |
| 4   | YLM101        | 0.22                    | 0.56  | 0.52  | 0.49  | 0.97                | 1.06  | 1.04  | 1.01  | 0.019                       | 0.025 | 0.018 | 0.012 |
| 5   | YLM102        | 0.26                    | 0.92  | 0.82  | 0.74  | 0.79                | 0.93  | 0.88  | 0.81  | 0.008                       | 0.014 | 0.014 | 0.011 |
| 6   | WLW103        | 0.69                    | 1.28  | 1.19  | 1.05  | 0.87                | 1.92  | 1.51  | 1.20  | 0.018                       | 0.029 | 0.021 | 0.019 |
| 7   | WLW105        | 0.64                    | 0.82  | 0.74  | 0.63  | 0.75                | 1.10  | 0.96  | 0.83  | 0.015                       | 0.022 | 0.019 | 0.014 |
| 8   | WLW107        | 0.72                    | 1.05  | 0.97  | 0.86  | 0.46                | 0.88  | 0.83  | 0.84  | 0.014                       | 0.021 | 0.015 | 0.009 |
| 9   | WLM101        | 0.26                    | 0.97  | 0.94  | 0.90  | 0.73                | 1.20  | 0.91  | 0.83  | 0.008                       | 0.028 | 0.022 | 0.013 |

### Table 8: The effect of initial pH on production of Lactic Acid (g/L), Diacetyl (g/L) and Hydrogen peroxide (g/L) by the LAB isolates

| S/N | Isolates code | Lactic acid (g/L) ph4.5 | ph5.5 | ph6.5 | ph7.5 | Diacetyl (g/L) ph4.5 | ph5.5 | ph6.5 | ph7.5 | Hydrogen peroxide (g/L) ph4.5 | ph5.5 | ph6.5 | ph7.5 |
|-----|---------------|-------------------------|-------|-------|-------|---------------------|-------|-------|-------|-----------------------------|-------|-------|-------|
| 1   | YLW102        | 0.35                    | 0.71  | 0.57  | 0.45  | 0.81                | 1.52  | 0.94  | 0.72  | 0.018                       | 0.026 | 0.021 | 0.021 |
| 2   | YLW103        | 0.45                    | 1.10  | 0.92  | 0.85  | 0.89                | 1.34  | 1.21  | 0.78  | 0.036                       | 0.085 | 0.046 | 0.035 |
| 3   | YLW104        | 0.75                    | 1.34  | 1.18  | 1.08  | 0.75                | 1.23  | 1.08  | 1.08  | 0.013                       | 0.025 | 0.015 | 0.013 |
| 4   | YLM101        | 0.53                    | 0.95  | 0.61  | 0.41  | 1.23                | 1.65  | 1.23  | 1.12  | 0.016                       | 0.021 | 0.019 | 0.019 |
| 5   | YLM102        | 0.51                    | 1.01  | 0.91  | 0.45  | 0.87                | 1.01  | 0.91  | 0.86  | 0.024                       | 0.056 | 0.028 | 0.021 |
| 6   | WLW103        | 0.91                    | 1.34  | 1.21  | 1.09  | 1.14                | 2.13  | 1.83  | 1.21  | 0.019                       | 0.036 | 0.024 | 0.017 |
| 7   | WLW105        | 0.54                    | 1.08  | 0.95  | 0.57  | 1.12                | 2.53  | 1.63  | 1.10  | 0.021                       | 0.044 | 0.029 | 0.021 |
| 8   | WLW107        | 0.97                    | 1.42  | 1.18  | 0.79  | 1.35                | 2.47  | 1.98  | 1.42  | 0.017                       | 0.036 | 0.013 | 0.028 |
| 9   | WLM101        | 0.54                    | 1.08  | 1.01  | 0.97  | 1.10                | 2.86  | 1.52  | 0.94  | 0.015                       | 0.028 | 0.019 | 0.019 |

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**Figure 1:** Percentage occurrence of LAB isolates from fermentation supernatant and cassava mash

### 4. DISCUSSION

The nutritional and organoleptic qualities of fermented products such as Ogi, Eko, Garri, Lafun and Fufu are usually as a result of the interactions between different organisms (Omemu et al., 2007). The interactions may be beneficial adding to the final product by means of desirable biochemical changes like the production of aromatic compounds and enzymatic activities (Viljoen, 2001). On the other hand, the interactions may be detrimental causing spoilage by inhibiting the growth of starter cultures and producing off-flavors, or discoloration (Omemu et al., 2007).
The varying value in pH and titratable acidity observed in this study have been reported by several authors to be a major characteristic of the fermentation of carbohydrate rich raw materials like tubers, roots and cereal which is in line with the findings of Adesokan et al. (2009) and Wakil and Osamwonyi (2012). The decrease in pH and increase in TTA is due to the activity of Lactic acid bacteria producing organic acid primarily lactic acid which is a common characteristic of fermenting wet mash (Raimbault, 1995; Wakil and Osamwonyi, 2012).

The high population of LAB in the fermentation of these cassava varieties indicates that they are predominant microorganisms responsible for the fermentation of the cassava roots and this is in agreement with the works of Obadina et al. (2006) and Krabi et al. (2016) who in their various studies recorded a high LAB count in the fermentation of cassava and cassava products. It is also an indication that the LAB proliferation is not affected by the low pH of these food products which means they can utilize the substrate content of the cassava roots.

The lactic acid bacteria were identified culturally, morphologically, physiologically and biochemically as L. casei, L. mesenteroides, L. plantarum, L. acidophilus and L. fermentum with L. plantarum being the predominant one and this agrees with the work of Adesokan et al. (2009) who reported predominant of this organism in spontaneous fermentation of cereal and roots. Species of Aspergillus are the most common isolates obtained from the spoil food samples. This is in agreement with the report of Aderiye et al. (2006) and Omemu et al. (2015) who in their various studies isolated mold in some stored fermented foods. The presence of mould such as Aspergillus niger, Aspergillus flavus, Rhizopus sp. and Penicillium sp. during the spoilage of Garri, Lafun and Fufu agrees with the findings of Ishola and Adebayo-Tayo (2012) and Rosales-soto (2012), where they isolated these organisms from fufu samples.

The increasing need for food safety and security has been a major concern in the public health sector. Many filamentous mould such as Aspergillus niger, Aspergillus flavus, Rhizopus sp. and Penicillium sp. have been implicated in food spoilage (Omemu et al., 2015). The traditional method of tackling this problem was the use of chemical antifungal which have resulted in the development of antifungal resistance strains of these spoilage organisms. Draksler et al. (2004) and Pounce et al. (2008) also reported that the inhibition of mould by LAB may be as a result of the low pH due to the organic acid production which can result in the increase of the production of hydrogen peroxide. The inhibition of the moulds by the isolates was at varying degrees which is in consonance with the work of Kalalou et al. (2004) and Toyi (2014) in their various studies where they observed varying degrees of inhibition of various food borne pathogens by CFS of LAB.

All the LAB isolates produced lactic acid, diacetyl and hydrogen peroxide with highest production at 48hrs of incubation. L. plantarum having highest production of lactic acid while Lactobacillus fermentum had the least. The highest diacetyl was also produced by Lactobacillus plantarum and the least produced by Lactobacillus plantarum also. The highest amount of hydrogen peroxide was produced by Lactobacillus plantarum and Lactobacillus mesenteroides with the lowest produced by Lactobacillus plantarum. These results were in agreement with the works of Afolabi et al. (2008), Adesokan et al. (2009), Wakil and Osamwonyi (2012) and Ishola and Adebayo-Tayo (2012) who in their various studies reported that antimicrobial metabolites of LAB are optimally produced at 48 hrs of incubation with L. plantarum producing the highest quantity of lactic acid. In similar studies, Wakil and Osamwonyi (2012) had reported high amount of hydrogen peroxide being produced by Lactobacillus mesenteroides and that Lactobacillus plantarum produced the least quantity of diacetyl in his studies. Ogunbanwo et al. (2004) also obtained a similar result for L. plantarum isolated from fufu, a traditional fermented cassava product.

The hydrogen peroxide produced adds to the antimicrobial activity of LAB and in some cases be a precursor for the production of other potent antimicrobial compounds such as super oxide (O2−) and hydroxyl (OH·) radicals (Wakil and Osamwonyi, 2012). The antimicrobial effect of hydrogen peroxide may result from the oxidation of sulphydryl groups causing denaturing of a number of enzymes, and from the peroxidation of membrane lipids thus they increased membrane permeability (Wakil and Osamwonyi, 2012). Nettes and Barefoot (1993) reported that hydrogen peroxide accumulates in cultures of Lactobacillus, Leuconostoc and Pediococcus species. Diacetyl which is mainly produced by lactic acid bacteria including strains of Leuconostoc, Lactococcus, Pediococcus and Lactobacillus is effective against bacteria (Schnurer and Magnusson, 2005). In fermented foods, LAB plays an important role in the rheological and flavoring quality of the fermented product (Olatunde, 2016). They are able to perform this role because of the production of diacetyl, which contribute to the typical flavor and taste of many foods, particularly dairy products and their antagonistic activity has been partly traced to the antimicrobial properties of diacetyl (Wakil and Osamwonyi, 2012).

The result of this research gives evidence that antifungal producing LAB are found in the fermentation of pro-vitamin A and white root cassava and these isolates have the ability to survive in a low pH. The effect of pH and temperature on the production of the lactic acid, diacetyl and hydrogen peroxide was investigated. The initial pH of the culture medium was observed to affect the production of antifungal substances by LAB. After varying the initial pH of the medium and allowing the same incubation time for all the isolates, it was evident that there was a difference in the amount of antifungal substances produced. The acidic range of pH was seen to favor production of antifungal substances.
The maximum production of antifungal substances occurred at pH 5.5 which is agreement with the results of Afolabi et al. (2008) and Wakil and Osamwonyi (2012). The optimum pH for the growth of LAB is 5.5 and at this pH the quantity of antifungal substances produced was highest. The production of antifungal metabolites in adjusted pH medium decreases with increasing pH greater than pH 5.5. These results indicate that the antifungal substances are greatly favoured by acidic medium with optimal pH of 5.5. Also, the antifungal substances are greatly produced at 30°C above which there is a decline. This is in agreement with the work of Adesokan et al. (2009) who reported similar cases in their various studies that optimum temperature and pH for antimicrobial metabolites production in LAB is 30°C and pH5.5 respectively. This also indicates that the quantity of antifungal produced is a function of the LAB to survive in the culture medium.

Five lactic acid bacteria isolates were selected based on their antifungal activities for molecular analysis using the 16S rRNA partial sequencing for DNA extraction. This is necessary in order to properly identify the LAB isolates involved in the fermentation of the three cassava varieties. Identification of LAB by 16s rRNA has been referred to as a very reliable method and has been used for their identification by several authors like kostinek et al., (2005) and Oguntoyinbo and Narbad, (2012). Molecular techniques, especially Polymerase Chain reaction (PCR) based methods are important for the specific characterization and detection of LAB strains (Adiguzel and Atasever, 2009; Lawalata et al., 2011 and Mohania et al., 2008). The 16S rRNA gene sequencing and BLAST searching at the NCBI identified the isolates as different strains of Lactobacillus plantarum, lactobacillus fermentum and Lactobacillus acidophilus. Many strains of these lactic acid bacteria have been reported by many authors to produce antifungal activities and have been isolated from fermented foods (Wakil and Osamwonyi, 2012; Adesokan et al. 2009 and Afolabi et al. 2008).

In conclusion, the research on the antifungal activities of LAB isolated from cassava fermentation especially on pro-vitamin A cassava is still novel as there were paucity of information on them as at the time of conducting this research. The application of the antifungal substances from LAB as bio-preservatives which are consumer friendly is highly significant because of their non toxic nature compared to the current chemical antifungal substances. Since LAB is among the Generally Regarded As Safe (GRAS) microorganisms, they may be exploited for the control of spoilage mould as indicated from these results. Lactic acid bacteria and their metabolites have been described to be more effective in numerous applications. Most antifungal substances produced by LAB are safe and effective natural inhibitors of food spoilage mould in various fermented foods. These isolated strains can positively have impact on use as starter cultures for traditional fermented foods, with a perspective to improve the shelf life, nutritional values and safety of such foods. There are mixed cultures of LAB species during the fermentation of the three cassava varieties used in this study. This research work has confirmed that Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus caesi and Lactobacillus acidophilus are good producer of lactic acid, diacetyl and hydrogen peroxide. Due to this fact, these microorganisms are among the microorganisms Generally Recognized As Safe (GRAS) for fermentation and bio-preservative applications. However, more species of bacteria should also be further analyzed for antifungal properties.

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