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Microbial succession and potential cyanide-degrading starter culture isolated from retted cassava

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Abstract

Cassava-derived products are widely consumed across the globe. Cassava, in its natural state, consists of high cyanide content, which is usually degraded during fermentation. Therefore, consumers of inadequately processed cassava products are at risk of cyanide exposure, which is highly toxic to human health. Accordingly, this study investigated the microbial succession of spontaneously fermented/retted cassava tubers to select a promising starter culture with cyanide-degrading potential. Fermentation, isolation, characterization, and tentative identification were done using standard microbiological methods involving the use of Nutrient Agar (NA), MacConkey Agar (MAC), DeMan Rogosa and Sharpe Agar (MRS), and Yeast Extract Agar (YEA). Cyanide content was assessed using the alkaline picrate method. Microbial counts ranged from 3.9×10^5 – 1.6×10^7; 1.0×10^7-8.3×10^9; 1.4×10^5-4.1×10^7 were recorded for Enterobacteriaceae, lactic acid bacteria, and yeast, respectively. The pH and titratable acidity also ranged from 7.1-5.3 and 0.0042-0.019, respectively. Additionally, tentative identification of isolated microorganisms revealed *Staphylococcus aureus, Bacillus* sp., *Corynebacterium* sp., *Shigella* sp., *Klebsiella* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Lactococcus* sp., *Saccharomyces cerevisiae, Escherichia coli*, *Leuconostoc* sp., *Enterobacter* sp., *Geotrichum* sp. and *Candida* sp. However, the highest survival rates throughout the spontaneous fermentation process were 18.10%, 10.95%, and 6.67% for organisms identified as *Lactobacillus* sp.2, *Candida* sp., and *Saccharomyces cerevisiae,* respectively. Furthermore, *Lactobacillus* sp. 2 survived throughout the fermentation process, and tested negative in pathogenicity assays.. Although, the spontaneous fermentation process was terminated after 72 hr, a 2.85 μg/mL of cyanide was recorded at 72 hr during the controlled fermentation, which was significantly reduced to 0.06 μg/mL at 120 hr. In conclusion, the findings suggest that *Lactobacillus* sp. 2 is a promising organism that may be employed for cyanide degradation, making it a potential starter culture for the fermentation of cassava.

Keywords: Cyanide-degradation, *Lactobacillus* sp., *Manihot esculenta*, spontaneous fermentation, starter culture

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Introduction

Cassava (*Manihot esculenta* Crantz) is a widely farmed tropical crop that constitutes a staple food for millions of people across Africa, the Caribbean, Latin America, Europe, North America, and Asia. Cassava is a primary source of carbohydrate, with a high starch content that can be sold fresh or processed into a variety of products such as garri, fufu, lafun, tapioca, abacha, and bread (Obi and Ugwu, 2019). Despite its many advantages, its use as a food source is usually associated with low protein content and probable toxicity (Dike, 2023). Cassava contains the cyanogenic glycosides linamarin and smaller amounts of lotaustralin, which are hydrolysed upon tissue disruption, releasing hydrogen cyanide (HCN) (Kandasamy *et al*., 2015; Mahendran *et al*., 2019). Consequently, cassava may be toxic when consumed raw or under-processed (Tamang *et al*., 2016). Cyanogenic compounds present in raw cassava can trigger cyanide poisoning, which can have adverse effects such as dizziness, abdominal pain, headache, nausea, vomiting, and in some cases, death (Adetunji *et al*., 2017). Furthermore, the consumption of food products with residual amounts of cyanogenic glycosides may lead to chronic disorders like neurosis, goitre, ataxic neuropathy, as well as diabetes (Ezemba *et al.,* 2021). The World Health Organization recommends that cyanide concentrations in cassava products should not exceed 10 mg HCN/kg in foods and feeds, respectively (Alvarado-Lopez *et al*., 2023). In developing countries like Nigeria, where adequate food preservation systems remain a major challenge, deterioration of cassava is usually observed within 3-5 days after harvesting due to the lack of proper storage and/or processing facilities, resulting in spoilage and the development of unpleasant odours, as a result of the breakdown of cyanogenic glycosides by microorganisms (Maiga *et al*., 2022). However, to overcome the various demerits associated with cassava processing, proper handling and fermentation techniques should be employed.

According to the literature, fermentation still remains the oldest food processing technique that started over 6000 years ago (Obi and Ugwu, 2019). The fermentation process can be performed in two major ways, namely: traditional (spontaneous) and starter (controlled) fermentation. In traditional fermentation, naturally occurring microorganisms present in the environment are employed. However, due to the adaptation period (lag phase) of naturally occurring microorganisms, fermentation usually takes a longer process, which in turn leads to the production of non-uniform and microbially unstable products (Umeh and Odibo, 2014). Therefore, due to the limitations associated with spontaneous fermentation, scientists have now developed another fermentation method known as starter or controlled fermentation in which well-defined microbial strain(s), that can either be mono or co-cultures, are employed for the fermentation process, resulting in the production of uniform and microbially stable food products (Obueh *et al*., 2017; Ozabor *et al*., 2024; Ozabor *et al*., 2026a). Starter culture(s) not only facilitate fermentation but may also contribute desirable functional properties such as the production of bioactive metabolites and flavour compounds, cyanide degradation potentials, amongst some other functional qualities (Ezemba *et al*., 2021; Ozabor *et al*., 2026b). Therefore, this study was designed to determine the microbial succession in spontaneously fermented cassava and to select potential cyanide-degrading microbial strain(s) that may be employed in future fermentation processes to develop microbially stable and uniform food products.

Methodology

Sample collection

A 500 g of fresh cassava tubers was purchased from Bodija market, Ibadan and placed inside Ziploc bags. The samples were immediately transported to the Food Microbiology laboratory, Department of Microbiology, University of Ibadan for further analysis.

Spontaneous fermentation of cassava

Using the modified method of Odunfa and Oyewola. (1998), the fresh cassava tubers were peeled, washed with potable water, and cut into uniform sizes of approximately 5cm. One hundred (100) g of the cut cassava tubers were weighed in triplicate and submerged into a 2 L Erlenmeyer flask, and allowed to ferment on the laboratory bench for 72 hr at room temperature (25 + 2 ^{0}C). Ten (10) g of the fermentate was aseptically taken every 6 hr, homogenized in 90 mL of distilled water, and filtered using the Whatman filter paper no 1. This was done to study the microbial succession and physicochemical properties (Umeh and Odibo, 2014).

Microbiological analysis

Isolation of organisms and assessment of colonial characteristics

One (1) g of the retted cassava was homogenized in 9 mL of sterile distilled water, filtered through Whatman filter paper (no 1), and serially diluted to $10^{-}10$. One hundred (100) µL of dilution factors 10^{-5}, 10^{-7}, 10^{-8} and 10^{-9} were aseptically plated on sterile Petri plates of Nutrient Agar (NA), MacConkey Agar (MAC), De Man Rogosa and Sharpe agar (MRS), and Yeast Extract Agar (YEA) for the isolation of Enterobacteriaceae, lactic acid bacteria (LAB) and yeast respectively using the spread plate technique. The inoculated plates were gently swirled to ensure even distribution of the inoculum. Thereafter, the NA and MAC plates were incubated aerobically at 35 ^{0}C for 18-24 hr, while MRS agar plates were incubated anaerobically for 24-48 hr at 35 ± 2 ^{0}C. Additionally, YEA plates were incubated aerobically at 30 ^{0}C for 3-5 days. All the inoculated plates were observed for visible growth and counted using a colony counter. The yeast isolates were subjected to morphological characterization using the fungi compendium as well as carbon and nitrogen assimilation tests (Umeh and Odibo, 2014). Representative colonies were randomly selected and purified by sub-culturing until pure isolates were obtained. Pure bacterial isolates were Gram-stained and thereafter characterized based on colony morphology (such as elevation, colour, opacity, shape, size, etc.), biochemical tests (such as oxidase, catalase, motility, starch hydrolysis, maltose, glucose, fructose, etc.).

Assessment of physicochemical properties

Determination of pH

The pH meter was calibrated with buffer solutions (pH 4, 7, and 9). One (1) mL of the retted cassava was measured and transferred into a 20 mL beaker, into which 10 mL of distilled water was added, and homogenized properly for 20 min. The pH meter probe was dipped into each mixture, and readings were taken. The probe was rinsed after each reading to prevent cross-contamination (AOAC, 2012).

Determination of Titratable Acidity (TTA)

The TTA was determined according to the method outlined in AOAC (2012). One (1) mL of the retted cassava filtrate was dispensed into 20 mL Erlenmeyer flask into which 20 mL of distilled water was added and homogenized properly for 20 min. Two drops of phenolphthalein indicator were added to the reaction mixture as the solution was titrated against 0.1 mol/L NaOH to a faint pink colour end-point. Blank titration was also carried out by dispensing 20 mL of distilled water into 50 mL Erlenmeyer flask. Two drops of phenolphthalein were added and titrated against 0.1N NaOH to a faint pink colour end point. TTA was calculated as follows:

$$\%\ \text{TTA} = 0.1 \times (\text{Vs} - \text{Vb}) \times 0.090 \times 100/\text{weight of sample}$$

Where: Vs = Volume of NaOH used for sample; Vb = Volume of NaOH used for blank.

Test for pathogenicity (production of hemolysis, DNase and gelatinase)

Test for hemolysis

All the isolated microorganisms were subjected to hemolysis production by aseptically streaking the organisms on sterile blood agar plates. The inoculated Enterobacteriaceae (NA & MAC), and yeast (YEA) plates were incubated aerobically at 35±2 ^{0}C and 30 ^{0}C for 18-24 hr and 48-72 hr respectively, while lactic acid bacteria plates (MRS) were incubated anaerobically at 35±2 ^{0}C for 24-48 hr (Ozabor *et al*., 2026b).

Test for DNase production

A loopful of 18-24 hr microbial broth cultures were aseptically streaked on sterile DNase agar plates, containing methyl green indicator. The inoculated Enterobacteriaceae (NA & MAC), and yeast (YEA) plates were incubated aerobically at 35±2 ^{0}C and 30 ^{0}C for 18-24 hr and 48-72 hr respectively, while lactic acid bacteria plates (MRS) were incubated anaerobically at 35±2 ^{0}C for 24-48 hr (Ozabor *et al*., 2026b).

Test for gelatinase production

A loopful of 18-24 hr microbial broth cultures were aseptically inoculated into sterile gelatin medium that was supplemented with 10% NA, MAC, MRS and YEA in 100 mL Erlenmeyer flasks.

The inoculated Enterobacteriaceae (NA & MAC), and yeast (YEA) plates were incubated aerobically at 35±2 ^{0}C and 30 ^{0}C for 18-24 hr and 48-72 hr respectively, while lactic acid bacteria plates (MRS) were incubated anaerobically at 35±2 ^{0}C for 24-48 hr (Ozabor *et al*., 2026b).

Preparation of starter culture

A 24 hr-old microbial culture was aseptically transferred into MRS agar plate and incubated for 18-24 hr. The microbial cells were harvested by centrifuging at 4000 x g for 10 min and rinsed with 1 × Phosphate Buffer Saline (PBS) three times. The washed cells were thereafter transferred into another 10 mL of PBS. The inoculum was standardized to approximately 1.5×10^8 CFU/mL corresponding to a 0.5 McFarland standard (Ozabor *et al.*, 2026a).

Determination of cyanide-degrading potential by the selected starter culture

The cyanide content of the fermented cassava was determined using the alkaline picrate method described by Okoko (2011). Ten (10) g of the fermented cassava slurry was mixed with 90 mL sterile distilled water, homogenized, and filtered through Whatman Filter paper (no 1). A 20 mL of the filtrate was measured into a test tube, after which 10 mL of alkaline picrate was added and homogenized again. The test tube was capped and placed inside a water bath, which had been preheated to 94 ^{0}C for 5 min. A blank was also prepared using distilled water substituted for the filtrate. The test tubes were removed after 5 min, cooled, and the absorbance was read with a UV-Vis spectrophotometer at 510 nm against a blank. Cyanide concentrations of the fermented dough were deduced from the standard curve (AOAC, 2012). All cyanide-containing solutions were handled following institutional laboratory safety guidelines.

Preparation of the standard cyanide curve

Pure potassium cyanide (KCN) was used as a standard to evaluate cyanide content. A 0.1 - 0.6 mg/mL solution of KCN was prepared, and 20 mL of distilled water was added to each test tube, followed by the addition of 10 mL of alkaline picrate. The test tubes were thoroughly homogenized, capped, and placed inside a water bath that had been preheated to 94 ^{0}C for 5 min. The test tubes were removed after 5 min, cooled and the absorbance was read with a UV-Vis spectrophotometer at 510 nm. The absorbances were plotted against the concentrations to get a standard curve (AOAC, 2012).

Statistical analysis

Bacteria counts were calculated in triplicate using the formula below:

$$N = \Sigma C / \{(1\ x\ n1) + (0.1\ x\ n2)\}\ x\ \mathrm{d}$$

where N = Number of colonies; ΣC = Sum of all colonies on all plates counted; n_1 = Number of plates in first dilution counted; n_2 = Number of plates in second dilution counted; d = Dilution from which the first counts were obtained. The 95% confidence interval (CI) for the proportion was estimated using the formula:

$$95\%\ \mathrm{CI} = \hat{p} \pm z(\alpha/2) \times \sqrt{(\hat{p}(1-\hat{p})/n)}.$$

where n = sample size; x = number of isolated organisms.; $\hat{p}$ = estimated proportion of isolated organisms (x/n); $z_{\alpha/2}$ = 95% CI ($z_{0.025}$ = 1.96). Microbial counts were reported in percentages.

Results

Microbial counts obtained during a 72 -hr spontaneous fermentation of cassava is presented in Table 1. The total bacterial count (TBC) increased from 5.8×10^7 CFU/mL at 6 h to 1.0×10^9 CFU/mL at the end of the fermentation period. Enterobacteriaceae Count (EC) was recorded to be 3.9×10^5 at the 6th hr, which thereafter increased to 1.6×10^7 at 54 hr, and no count was recorded between 60 – 72 hr.

The Total Lactic Acid Bacteria (TLAB) count increased from 1.0×10^7 at 18 hr to 8.3×10^9 at 72 hr, while the Total Yeast Count (TYC) increased from 1.4×10^5 at 24 hr to 4.1×10^7 at 72 hr. No detectable growth of lactic acid bacteria was observed between 6 and 12 hr. However, growth of yeasts was not detected between 6 and 18 hr of fermentation.

Table 1: Total microbial count during 72 hr of cassava spontaneous fermentation

Time (Hours)	Total Bacteria Count (CFU/mL)	Total Enterobacteriaceae Count (CFU/mL)	Total Lactic acid Bacteria Count (CFU/mL)	Total Yeast Count (CFU/ml)
6	5.8×10^{7a}	3.9×10^{5a}	-	-
12	5.4×10^{7a}	3.7×10^{5a}	-	-
18	3.6×10^{7a}	3.5×10^{5a}	1.0×10^{7a}	-
24	3.2×10^{7a}	3.1×10^{5a}	1.3×10^{7a}	1.4×10^{5a}
30	2.8×10^{7a}	2.9×10^{5a}	2.9×10^{7a}	1.2×10^{5a}
36	2.0×10^{7a}	2.8×10^{7b}	3.6×10^{7a}	1.4×10^{5a}
42	1.4×10^{9a}	1.9×10^{7b}	5.4×10^{7a}	2.0×10^{5a}
48	1.4×10^{8b}	1.6×10^{7b}	5.9×10^{7a}	2.1×10^{5a}
54	1.5×10^{8b}	1.6×10^{7b}	7.5×10^{7a}	3.0×10^{5a}
60	1.5×10^{9c}	-	7.7×10^{9a}	3.3×10^{7b}
66	1.2×10^{9c}	-	8.1×10^{9b}	3.5×10^{7b}
72	1.0×10^{9c}	-	8.3×10^{9b}	4.1×10^{7b}

Key:
-= No growth

Values with different letters down the column are significantly different at (P=0.05)

Figure 1 illustrates the changes in pH during the 72-h spontaneous fermentation of cassava. It was recorded that the pH ranged from 7.1 to 5.3 at 0 hr and 72 hr respectively, while the titratable acidity also ranged from 0.0042 to 0.019 at 0 hr to 72 hr respectively (Figure 2).

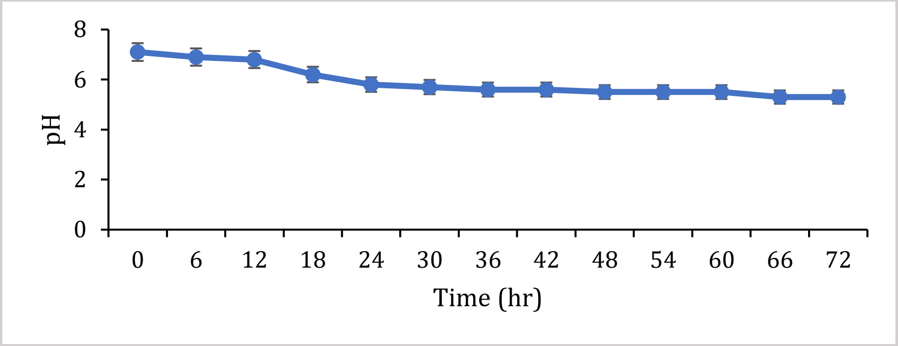

Figure 1. Changes in pH during 72 hr cassava spontaneous fermentation

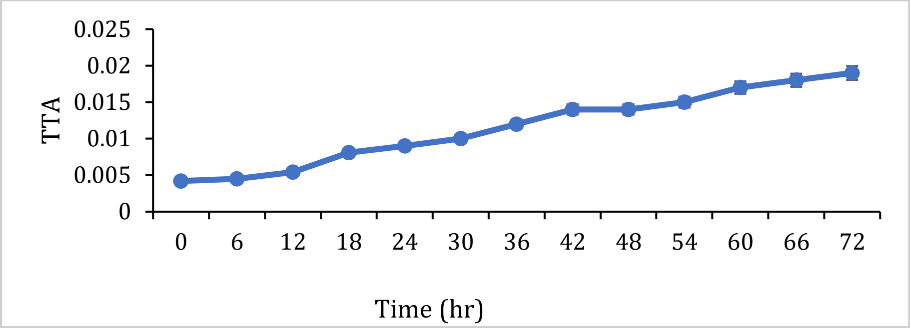

Figure 2. Changes in TTA during 72 hr cassava spontaneous fermentation

Morphological and microscopic characterization of isolated organisms from retted cassava

The colonies exhibited diverse morphological characteristics, including cell elevations that were either raised, flat, or embedded; colour was either cream, pink, white, or golden yellow; surface: smooth, dry mucoid, or shiny; edge: irregular or entire; opacity: opaque or translucent; shape: irregular, circular, or spindle; size: small, medium, or large. Microscopic examination revealed bacterial cells as either cocci, rods, or coccobacillus, while the yeast organisms had small budding cells, hyphae in chains, and budding cells with pseudo-hyphae.

Table 2 presents the Gram's staining and biochemical results of isolated bacteria and yeasts during a 72-hr cassava spontaneous fermentation. Based on Gram staining, morphological characteristics, and biochemical tests, fourteen microorganism taxa were tentatively identified as; *Staphylococcus aureus, Bacillus* sp., *Corynebacterium* sp., *Shigella* sp., *Klebsiella* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Lactococcus* sp., *Saccharomyces cerevisiae, Leuconostoc* sp., *Geotrichum* sp., *Escherichia coli*, *Enterobacter* sp. and *Candida* sp.

The percentage occurrence of tentatively identified organisms showed that the highest occurrences of 18.10%, 10.95%, and 6.67% were recorded in the isolates depicted as *Lactobacillus* sp.2, *Candida* sp., and *Saccharomyces cerevisiae,* respectively, while the lowest occurrences of 2.38%, 1.91% and 1.90% was recorded in *Bacillus* sp., *Shigella* sp. and *Staphylococcus aureus,* respectively (Table 3). *Lactobacillus* sp. 2 exhibited the highest occurrence frequency and persisted throughout the fermentation period (Table 4). Furthermore, it tested negative for hemolytic, DNase, and gelatinase activities, suggesting its potential safety for use as a starter culture. Cyanide concentration of 7.85 μg/mL was recorded in *Lactobacillus* sp. 2 at 0 hr, and significantly reduced to 0.06 μg/mL at 120 hr during the controlled (starter) fermentation process as cyanide activity of 2.85 μg/mL at 72 hr (Figure 3).

Table 2: Gram staining and biochemical characterization of isolated bacteria and yeasts during 72 hr of cassava spontaneous fermentation

Isolate code	Gram’ s reaction	Catalase	Oxidase	Motility	Citrate	Starch hvdrolvsis	Indole	Gelatin hvdrolvsis	MR test	VP test	Glucose	Lactose	Maltose	Sucrose	Fructose	Mannitol	Galactose	KNO_3	$NaNO_3$	Probable organism
C_1	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	+	-	ND	ND	*Staphylococcus aureus*
C_2	+	+	-	+	+	+	-	+	-	+	+	-	-	-	+	+	+	ND	ND	*Bacillus* sp.
C_3	+	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	ND	ND	*Corynebacterium* sp.
C_4	-	+	-	-	+	-	-	-	-	-	+	+	-	+	+	-	+	ND	ND	*Enterobacter* sp.
C_5	-	+	-	-	-	-	-	-	+	-	-	-	+	+	-	+	-	ND	ND	*Shigella* sp.
C_6	-	+	-	+	-	+	+	-	+	-	+	+	-	-	+	+	+	ND	ND	*Escherichia coli*
C_7	+	+	-	+	-	+	-	+	-	+	+	-	+	-	+	-	-	ND	ND	*Bacillus* sp.
C_8	+	+	-	+	-	+	-	+	-	+	+	-	-	+	+	+	+	ND	ND	*Bacillus* sp.
C_9	+	+	-	+	+	+	-	+	-	+	+	-	-	-	+	+	+	ND	ND	*Bacillus* sp.
C_{10}	-	+	-	-	+	-	-	-	-	+	-	+	+	+	-	+	-	ND	ND	*Klebsiella* sp.
C_{11}	-	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	+	ND	ND	*Pseudomonas* sp.
CL_{12}	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	-	+	ND	ND	*Lactobacillus* sp.
CL_{13}	+	-	-	-	+	-	-	-	+	-	+	+	+	+	+	-	+	ND	ND	*Lactococcus* sp.
CL_{14}	+	-	-	-	+	-	-	-	+	-	+	+	+	-	+	+	+	ND	ND	*Lactobacillus* sp.
CL_{15}	+	-	-	-	+	-	-	-	+	-	+	+	-	-	+	-	+	ND	ND	*Leuconostoc* sp.
CL_{16}	+	-	-	-	+	-	-	-	+	-	+	-	+	+	+	+	+	ND	ND	*Lactobacillus* sp.
CY_{17}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	-	+	+	+	-	+	-	-	*Saccharomyces cerevisiae*
CY_{18}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	-	+	+	+	+	+	+	-	*Geotrichum* sp.
CY_{19}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	-	-	-	+	-	-	-	-	*Candida* sp.

Key: += Growth; -= No growth

Table 3: Percentage occurrence of probable organisms during 72 hr of cassava spontaneous fermentation

Probable isolates	Number of occurrences	Percentage of occurrence (%)
S. aureus	4	1.90
Bacillus sp.	12	5.71
Corynebacterium sp.	8	3.81
Enterobacter sp.	6	2.86
Shigella sp.	4	1.91
Bacillus sp.	7	3.33
Lactococcus sp.	8	3.81
E. coli	11	5.24
Leuconostoc sp.	8	3.81
Bacillus sp.	13	6.19
Lactobacillus sp.	13	6.19
S. cerevisiae	14	6.67
Geotrichum sp.	8	3.81
Lactobacillus sp.1	11	5.24
Candida sp.	23	10.95
Lactobacillus sp.2	38	18.10
Klebsiella sp.	9	4.29
Pseudomonas sp.	8	3.81
Bacillus sp.	5	2.38

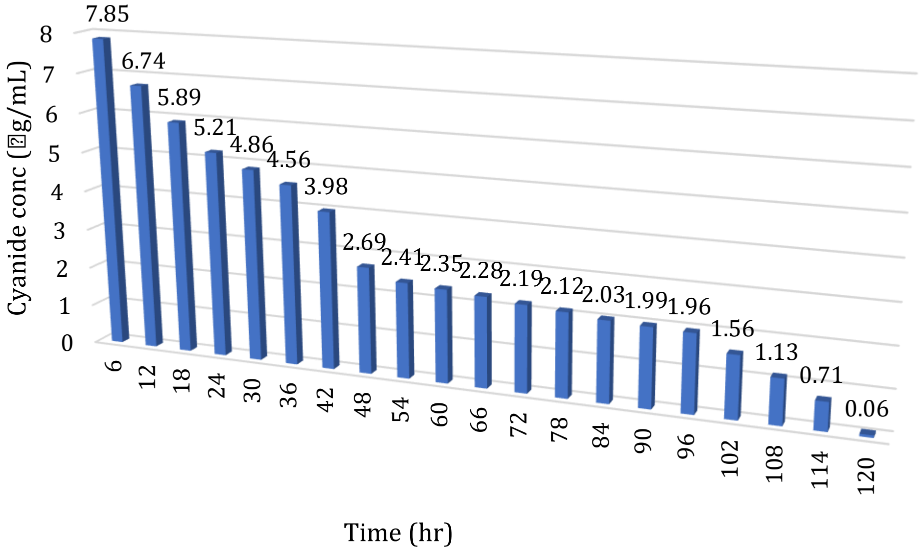

Figure 3. Cyanide degradation by *Lactobacillus* sp. 2 during a 5-day controlled fermentation process

Table 4: Microbial succession of probable organisms during 72 hr of cassava spontaneous fermentation

	Time (hr)											
Probable organisms	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60**	**66**	**72**
S. aureus	+	+	+	+	+	-	-	-	-	-	-	-
Bacillus sp.	+	+	+	+	+	+	+	+	+	+	+	+
Corynebacterium sp.	+	+	+	+	-	-	-	-	-	-	-	-
Enterobacter sp.	-	-	-	+	+	+	+	+	-	-	-	-
Shigella sp.	+	+	+	-	-	-	-	-	-	-	-	-
Bacillus sp.	+	+	+	+	+	+	+	+	+	+	-	-
Lactococcus sp.	-	-	+	+	+	+	+	-	-	-	-	-
E. coli	+	+	+	+	+	+	+	+	-	-	-	-
Leuconostoc sp.	-	-	-	-	-	-	-	+	+	+	+	+
Bacillus sp.	+	+	+	+	+	+	+	-	-	-	-	-
Lactobacillus sp.	-	-	+	+	+	+	+	+	+	-	-	-
S. cerevisiae	-	-	-	+	+	+	+	+	+	+	+	+
Geotrichum sp.	-	-	-	-	-	-	+	+	+	+	+	+
Lactobacillus sp.1	-	-	+	+	+	+	+	+	-	-	-	-
Candida sp.	-	-	-	+	+	+	+	+	+	+	+	+
Lactobacillus sp.2	-	-	+	+	+	+	+	+	+	+	+	+
Klebsiella sp.	-	-	-	+	+	+	-	-	-	-	-	-
Pseudomonas sp.	-	-	-	-	-	-	+	+	+	-	-	-
Bacillus sp.	-	-	+	+	+	+	+	+	+	+	-	-

Key:
+= Growth; -= No growth

Discussion

This study provides insights into the microbial succession and the cyanide degradation potentials of LAB. The isolation and identification of the recorded microorganisms have earlier been reported by Ezemba *et al*. (2021). It was observed that Enterobacteriaceae counts began to decline at the 42nd hr, and later, no counts were recorded between 60-72 hr. This occurrence might be due to the increasing acidity level of the fermenting substrate cassava, as the earlier reports of Olojede *et al.* (2020); Madilo *et al*. (2025) stated that LAB and yeast release organic acids into the fermentation matrix as time increases. The dominance of LAB and yeasts during the later stages of the fermentation process is an indication of low oxygen level due to the increase in TTA levels, resulting in the growth of facultative anaerobes (Tamang *et al*., 2016; Ozabor *et al* 2026b).

Additionally, the ability of LAB and yeast to dominate the fermentation matrix may be due to their better adaptation to acidic conditions, ability to utilize the fermenting substrate (cassava) for metabolic activities, as well as ability to produce microbial inhibitory compounds, which gives them a competitive advantage over the other organisms such as the Enterobacteriaceae (Dike, 2023). It has been reported that *Bacillus* sp. also contributes significantly to fermentation processes, one of the reasons being that they can form resistant spores with high tolerance levels to adverse environmental conditions (Obi and Ugwu, 2019; Ozabor *et al*., 2024). Earlier reports have documented that *B. subtilis* can produce amylases that are required for the breakdown of starch into sugars, used for the growth of fermenting organisms, and also have the capacity to use cyanohydric acid for their nutrition and metabolic activities. Thereby, it contributes to the reduction of cyanide degradation potentials (Safa *et al*., 2017).

Pathogens such as *S. aureus, Klebsiella*, *Shigella, Corynebacterium, Pseudomonas,* and some *Bacillus* occurred in the early stage of the spontaneous fermentation process but could not survive till the end as a result of the acidic conditions that developed as the fermentation time progressed, making the fermenting matrix difficult for them to adapt (Alvarado-Lopez *et al.,* 2023). The observed partial dominance of yeasts such as *S. cerevisiae* and *Candida* observed in this study not only contribute to the production of organic acids, but also contribute to the development of flavour compounds in the final products, as they work in synergy with LAB. Yeasts have been reported to break down starch with the use of amylolytic enzymes into simple sugars that can be utilized by LAB (Adegbehingbe *et al.,* 2019; Ozabor *et al*., 2025).

Furthermore, LAB and yeasts are also able to synthesize industrially important enzymes that have the potentials of degrading cyanide (Nwafor *et al*., 2015). The decrease in pH, with an increase in TTA favours microbial activities by converting carbohydrates into organic acids such as lactic acid, acetic acid, citric acid, etc which enhance product preservation, shelf life, and microbial stability (Obi and Ugwu, 2019; Ruiz-Rodriguez *et al*., 2019). However, the coexistence of LAB and yeasts as observed during the fermentation process leads to the conversion of starch (cassava) into simple sugars that can be utilized by these organisms for growth and metabolism (Bamidele *et al.,* 2015; Maiga *et al*., 2022; Ozabor *et al*., 2026b). Additionally, the organic acids and inhibitory compounds produced have antagonistic activities on foodborne spoilage organisms (Ozabor *et al*., 2025).

Lactobacillus sp. 2 tested negative for hemolytic, DNase, and gelatinase activities and persisted throughout the fermentation process. Hence, it was selected as a starter for the controlled fermentation, as earlier studies have mentioned that only food grade non-pathogenic organisms must be employed for food fermentation processes (Ozabor *et al*., 2024; Ozabor *et al*., 2025). In this study, increasing fermentation time was observed to enhance cyanide degradation and removal by *Lactobacillus* sp.2 during the controlled fermentation process.

Conclusions

Overall, this study concludes that reduction in pH values as a result of increased fermentation time led to an increase in TTA, which is a useful indicator of acidity and microbial activity in the fermented cassava tubers, in addition to improving the shelf-life. More so, *Lactobacillus* sp.2 can be suggested as a promising starter culture for cassava fermentation, as it was able to survive throughout the fermentation process, tested negative to the subjected pathogenicity tests (hemolysis, gelatinase and DNase), as well as significantly degraded cyanide during the controlled fermentation process.

Study limitation

Due to financial constraints, the isolated microbes were identified using the conventional morphological identification methods. Therefore, future studies are needed for molecular identification of the species present in the starter cultures.

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