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Growth Studies of Potential Probiotic Lactic Acid Bacteria in Cereal – Based Substrates

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Abstract. In this study, the growth of three lactic acid bacteria in cereal substrates of maize, sorghum and malt were evaluated. Molecular analysis was used to characterize the organisms and their probiotic abilities ascertained. After 48 h of fermentation, the viable counts revealed that *L. fermentum* had a count of 6.21 log Cfu/mL, *L. plantarum* had a count of 6.62 Cfu/mL and *L. nantensis* had a count of 7.51 Cfu/mL in maize substrate. The counts for *L. fermentum*, *L. plantarum* and *L. nantensis* in the sorghum substrate were 4.66, 8.77 and 9.36 log Cfu/mL respectively, whereas the viable counts for malt fermentation were 8.05, 8.92 and 9.42 log Cfu/mL for *L. fermentum*, *L. plantarum* and *L. nantensis*, respectively. This research therefore suggests that cereals (maize, sorghum and malt) are suitable for the growth of the three probiotic strains being *L. fermentum, L. plantarum* and *L. nantensis*.

Keywords: cereal-based substrates, probiotics potential, lactic acid bacteria, fermentation

Introduction

Cereals are food materials basically known to have a special place as far as African continental dishes are involved. They remain the most paramount food crop with an overwhelming global production of 2500 metric tonnes in 2015 alone (FAO, 2016). Cereals could be applied in the design of cereal-based fermented formulations with probiotic capabilities if such formulations fulfil probiotic requirements as well as possess acceptable physico-chemical characteristics and organoleptic properties (Salmeron *et al*., 2015).

Pandey *et al*. (2015) and Salmeron (2017) did define prebiotics as food materials comprehended by fibres of natural origin that are not digested in the upper gastrointestinal tract and such do improve the health of the host by selectively supporting the development and activity of particular genera of micro-organisms in the colon mostly lactobacilli and bifidobacteria. In 2013, International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host, while in 2016, defined prebiotic as a substrate that is selectively utilized by host organisms conferring a health benefit (Swanson *et al*., 2020). Probiotic foods are typically dairy formulations made of milk and fermented milk products, such as beverages and cheese that contain live organisms of the lactic acid bacteria group. However, issues with vegetarian populations in third-world countries and lactose intolerance, as well as the cholesterol content of dairy products, have caused a shift in attention to non-dairy beverages (Enujiugha and Badejo, 2017).

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Kandylis *et al*. (2016) did acknowledge that beverages made from fruit and vegetable juices are a next generation class of food supplements that serve to convey probiotic bacteria.

Similarly, cereals are also potential substances that serve as carriers of probiotics as they contain nutrients that are easily accessible by probiotics organisms (Martins *et al*., 2013). Cereals are efficient in carrying lactobacilli through the harsh conditions of the gastro system and are also known to enhance both single and mixed fermentations (Rathore *et al*., 2012). Hassan *et al*. (2012) stated that cereal products often do ferment spontaneously resulting in elevated shelf-life and better nutritional properties compared to the raw materials in that single as well as mixed cereals are used as substrates in the production of fermented foods and the final products do vary going by the microbial population involved and the fermentation conditions applied. Fermented formulations have evolved from traditional

naturally fermented products to beverage supplemented with functional ingredients which enhance cardio-vascular functions followed by fermented drinks that improve the composition of gastrointestinal tract, they could be further improved with specific bioactive nano-particles (Onyimba *et al*., 2022; Salmeron, 2017).

Omemu and Faniran (2011) evaluated the antibacterial activity of lactic acid bacteria isolated from two fermented maize products, Ogi and Kunnun-zaki and found that they were sources of probiotic lactobacilli and also able to be isolated from fermented sorghum by Sifeeldein *et al*. (2019) which is study on the phylo-genetic identification of lactic acid bacteria isolates and their impact on the fermentation quality of sweet sorghum (*Sorghum bicolor*) silage.

This work seeks to under study of the use of single fermentations of maize, sorghum and malted sorghum as carriers of probiotic lactobacilli and could further be improved upon by future research endeavour. At consumption the level of probiotic in the food material should be $\geq 10^6$ Cfu/mL.

Material and Methods

Micro-organisms used in this study are three lactic acid bacteria strains of the genus – lactobacillus. The strains were isolated from fermented Ogi, maintained at 4 °C and sub-cultured periodically on slants prepared from MRS agar. Colonies isolated from MRS agar plates were then pre-cultured twice in MRS both for approxi-mately 12 h at 37 °C. The 12 h pre-cultured cells were then centrifuged (5000 g, 10 min, 4 °C), washed twice with sterile quarter – strength Ringer's solution and re-suspended in Ringer's solution. The bacterial suspension was subsequently used to inoculate the fermentation media at varying percentages (v/v). In all cases, the initial microbial concentration was approximately 10^7Cfu/mL.

Molecular analysis. DNA Extraction was performed at the Anaerobe Laboratory, Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research Yaba Lagos. Methodology was based on PCR and metagenomics analysis, while sequencing analysis was done at Inqaba Biotechnology Pty south Africa.

DNA Extraction. DNA extraction was from growth of Algae broth harvested by centrifugation at 14, 000 × g for 10 min. The cells were washed three times in 1 mL of ultra-pure water by centrifuging at 12,000 rpm for 5 min. DNA extraction and purification was done using ZR soil DNA MiniPrep™50 Preps. Model D6001 (Zymo Research, California, USA). 50-100 mg of cells was resuspended in 200 µL of sterile water. This was transferred into a ZR Bashing Bead™ Lysis Tube. Exactly 750 µL Lysis solution was added to the tube. The bead containing the solution was secured in a bead beater fitted with a 2 mL tube holder assembly and process at maximum speed for 5 min. The ZR Bashing Bead™ Lysis Tube was centrifuged in a micro-centrifuge at 10,000 × g for 1 min. 400 µL of the supernatant was pipetted into a Zymo-Spin™ IV Spin filter in a collection tube and centrifuged at 7,000 × g for 1 min. This was followed by the addition of 1,200 µL of soil DNA binding buffer into the filtrate in the collection tube. After this 800 µL of the mixture was transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 × g for 1 min.

The flow through was discarded from the collection tube and the process was repeated to obtain the remaining products. The 200 µL DNA pre-wash buffer was added into the Zymo-Spin™ IIC column in a new collection tube and centrifuge at 10,000 × g for 1 min. This was followed by the addition of 500 µL soil DNA wash buffer into the Zymo-Spin™ IIC column and centrifuged at 10,000 × g for 1 min. The Zymo-Spin™ IIC column was transferred into a clean 1.5 mL micro-centrifuge tube and 100 µL of DNA elution buffer was then added directly to the column matrix. This was centrifuged at 10,000 × g for 30 s to elute the DNA. The ultra-pure resulting filtrate (DNA) obtained was used as a template during the assay. This was transported in ice the labouratory for sequencing.

DNA Sequencing. DNA sequencing was performed by Sanger (dideoxy) sequencing technique to determine the nucleotide sequence of the specific micro-organism isolated using automated PCR cycle-sanger sequencer™ 3730/3730XL DNA analyzers from applied biosystems (Metzenberg, 2003; Russell, 2002). This result was obtained as nucleotides in Fasta format. Identification of the specie present was done using the resultant nucleotides base pairs. This was performed by blast analysis by direct blasting. For every set of isolates, a read was blasted and the resultant top hits with minimum E-score for every blast result showing species name was used to name the specific organism.

Bile tolerance test. The method described by Jin *et al*. (1998) and Gilliland *et al*. (1984) was applied in this

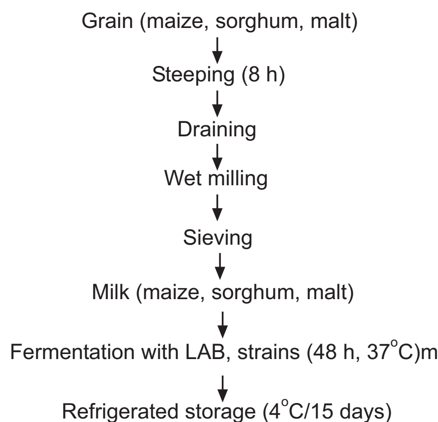
experiment. The lactic acid bacteria strains were sustained overnight in MRS broth, then 0.1 mL of the suspension was inoculated into 10 mL of MRS broth containing 0.2% cow bile and incubated at 37 °C for 24 h. At the end of incubation, 0.1 mL of the culture was plated in MRS media and viable counts taken.

Acid tolerance test. The method of Conway *et al*. (1987) was used. The cultures were grown in MRS broth overnight at 37 °C and subcultured into MRS broth and incubated for another 24 h. The cultures were subsequently centrifuged at 2000 g for 10 min, 4 °C. The pellets were washed in sterile phosphate buffered saline and re-suspended in 1 mL of the saline. Hydrochloric acid was used to adjust the pH to 2.0 and incubated. 0.1 mL of the sample was cultured an MRS media after 2 and 4 h respectively and viable colonies counted.

Haemolytic activity. Haemolytic activity of LAB strains was determined by the method described by Maragkoudakis *et al.* (2006) with slight modification. The isolates were grown in MRS broth and streaked on cow blood agar plates. The plates were incubated at 37 °C for 24 h and the non-haemolytic activity of strains were determined.

Cereal-based fermented media. The cereal based fermentation process and refrigerated storage were carried out using the procedure stated by Hassan *et al*. (2012) with slight modification show in (Flowchart).

Analytical methods. Fermentation samples were decimally diluted at 12 h intervals in sterile peptone water and plated on MRS agar using a re-calibrated pipette and incubated at 37 °C for 48 h. Colony-forming units were counted (Cfu/mL) and then the results expressed in log value. Cell values were given as mean values of nine to replicate measurements and the standard error (S.E) of the mean was calculated at 95% confidence level (Charalampopulos *et al*., 2002).



Flowchart. Flow chart for fermentation and refrigeration procedure.

Chemical analysis. The buffering capacity of each cereal medium was determined by titrating 100 mL of the medium with HCL (1 mol/L). The values then expressed as the amount of HCL (mM) required to drop 1 pH unit/unit volume (Pai *et al*., 2001).

The free amino nitrogen was determined by the ninhydrin-method (Magne and larher, 1992), while changes in pH were determined by using a pH meter.

The lactic acid concentration was determined using the method of titratable acidity (Nwachukwu *et al*., 2010; Halm *et al*., 1996).

Shelf-life determination. The shell-life of the fermented beverages of maize, sorghum and malt substrates were determined under refrigerated condition *i.e*. 4 °C for 15 day. Viable counts pH changes and acidity ere measured at 3 days intervals (Hassan *et al*., 2012).

Results and Discussion

The purpose of this research is to ascertain the probiotic abilities of three lactic acid bacteria strains using various tests such as the bile salt test, haemolysis test as well as acid tolerance test. The lactic acid bacteria strains being *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus nantansis* were isolated from fermented ogi and subsequently subjected to molecular analysis involving DNA (deoxyribonucleic acid) extraction and sequencing as demonstrated in Table 1. This research findings supports the work of Dawlal *et al*. (2019) on the visualisation and quantification of fumonisins bound by lactic acid bacteria isolates from traditional African maize-based fermented cereals, Ogi and Mahewu.

Points of great importance in the design and evaluation of food fermentation of this sort include the contents of the raw materials, specific growth rate of starter culture, final cell population, acidification rate and distribution of primary metabolites – (Charalampopolous and Pandiella, 2010). Aside these other tests involved aided in the confirmation of the probiotic potentials of the organisms.

Table 1. Molecular analysis result

Lab no.	Sample no.	Sequence	E-Score/ID similarities	Accession number/ version	Id of organism
CZ1	NKA	>CZ1_907-R_B09_06 AGGCGGGTGTTAGCGTTAGCTGCGCACTGAAGGGC GGAAACCCTCCAACACTTACACTCATCGTTTACGGC GACTACCGGGTATCTAACCTGTTTGCACCCATGCTTT CGAATCTCAGCGTCAGTTACAGACCAGAAGCCCCT TC CCCTGGTGTCTTCCAATATCTACCTTCCACCGCTA CACAGGATTCCCTTTCCTCTTCTGCACTCAAGTTACC ATTTTCAAGCACTTCCCGGTTGAGCCGAGGGTTTCA CTTCAAATTAAAAACCGCCACATTCTCTTTACCCCAA AAATCCGAAACGCTGCCCCTACGTATTACCGCGGCT GCGGCACGATTAGCCGTGGTTTCTGGTTGAATACCG TCATACTGAACATTACTCTCACCATGTTCTTCTTCAA CAACAGAGTTTTACGACCAAACTTCTTCACTCACGC GGCATTGCTCCATCAGGTTTCCCCTTGTGAAGATTCC CTACTGCTGCCTCCCGTAGGAGTTGGGCCGTGTCTC ATCCCATGTGGCGATTACCCTCTCAGTCGGCTACGTA TCATTGCTTGGTGAGCCGTTACCTCACCAACTAGCTA ATACGCCGCGGGTCCATCCAAAAGCGATAGCAGAGC ATCTTTCAAGTACATCATGTGAAAGTAGTTGTTATGC GGTATTACACCTGTTTCCAGTTATCCCCCACTTTTGG GCAGGTTACCCACTGTTACTCACCCGTCCGCCACTC ATCAAATGTGATCATGAAGCAAGCTTCATCATACCGA GTTCGTTCGACTTGCATGATTAGGCATGCCGCACAG CGATCGTCCTGAGACATGATCAAACTCTAGTG	0.0/90%	NR_043114.1 GI:343198632	*Lactobacillus nantensis*
	NKB	>CZ2_907-R_C09_09 TAGGCSGGGRATGCTTAATGCGTTAGCTCCGGCACTG AAGGGCGGAAACCCTCCAACACCTAGCACYCWTCG TTTACGGCATGGACTACCAGGGTATCTAATCCTGTTC GCTACCCATGCTTTCGAGTCTCAGCGTCAGTTGCAG ACCAGGTAGCCGCCTTCGCCACTGGTGTTCTTCCAT ATATCTACGCATTCCACCGCTACACATGGAGTTCCAC TACCCTCTTCTGCACTCAAGTTATCCAGTTTCCGATG CACTTCTCCGGTTAAGCCGAAGGCTTTCACATCAGA CTTAGAAAACCGCCTGCACTCTCTTTACGCCCAATAA ATCCGGATAACGCTTGCCACCTACGTATTACCGCGGC TGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTAAA TACCGTCAACGTATGAACAGTTACTCTCATACGTGTT CTTCTTTAACAACAGAGCTTTACGAGCCGAAACCCT TCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGC CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGG AGTATGGGCCGTGTCTCAGTCCCATTGTGGCCGATCA GTCTCTCAACTCGGCTATGCATCATCGCCTTGGTAGG CCATTACCCCACCAACAAGCTAATGCACCGCAGGTC CATCCAGAAGTGATAGCGAGAAGCCATCTTTTAACG TTGTTCATGCGAACAACGTTGTTATGCGGTATTAGCA TCTGTTTCCAAATGTTGTCCCCCGCTTCTGGGCAGGT TACCTACGTGTTACTCACCCGTCCGCCACTCGTTGGC ACAAAATCAAATCAGGTGCAAGCACCATCAATCAAT	0.0/99%	KT159935.1 GI:953562636	*Lactobacillus fermentum*

continued ....

	TGGGCCAACGCGTTCGACTTGCATGTATTAGGCACA CCGCCGGCCGTTCATCCTGAGCAGAAACTGG			
NKE	>CZ5_16S 27-F_F09_16 TACGAMSTGMGTGATCTGTCTCAGGAACTCGTACAA RGWASCCGTAGCCKTTGATTTTTTTTCMSCCACTCAA AAMAAMRGAMCAGSAACCCGTTTTTTTTTTTTTTK AWYCGRAARAAAAAACCAGRAAACWTATTTTTTTT TTTTTTTTTCTMAMMAAAMATGGCCCGAGCTTGAAA GATGGTTTCTGTTATCACTTTKGGATGGWCCCGCGG MGTATTAKCTAKATGGTGWGGTAACGGSTCACCATG GMAATGATACGTARCCGACCTGAKAGGGYAATCSSC CACMTTGRGACTGARACACGGMCCAWACTCCTACG GGAGGCAGCAKKAGGGAATCTTCCACAATGGACGA AAGTCTGATGGAGCWACGCCGCGTGAGTGAAGAAG GGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAA CATATCTGAGAGTAACTGTTCAGGTATTGACGGTATT TAACCASAAAGCCACGGCTAACTACGTGCCAGCAGC CGCGGTAATACGTAGGKGGCAAGCGTTGYCCGGATT TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTARG TCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTG CATCGGAAACTGGGAAACTTGAGKGCAGAAGAGGA CAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGA TATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCT GGTCTGTAACTGACGCTGAGGMTCGAAAGTATGGGT AGCAAACAGGATTAGATACCCTGGTAGTCCCATACC GTAAACGATGAATGCTAAGTGTTGRAGGGTTTCCGC CCTTCAGTGCTGCRGCTAACGCATTAAGCATTCCGCC TGGGGAKTACGGCCGCAAGGCTSAAACTCAAAGGAA TTGACGGGGGCCCGMACAAGCGGWGGAGCATGTGG TTAATTCGAAKCTACGCGAASAACCYTACCAGGTCTT GACATACTWTGCAATCWAARAAAATAGACGYTCCCT TCGGGACATSGAWWMMGGTGGAKGMWTGGATGWC GTCAGCTYCGCGCCRMGC	0.0/96%	KM374733.1 GI:723266321	*Lactobacillus plantarum* strain S4

The bile salt test had *L. Fermentum* producing a cell population of 3.87 log Cfu/mL, while the organisms *L. Plantarum* and *L. Nantensis* produced 3.58 log Cfu/mL and 3.50 log Cfu/mL respectively at 0.4% bile as shown in Fig. 1.

In a test, involving the determination of the effect of an acidic condition on the organisms at pH of 2.0, *L. fermentum* produced the highest survival rate of 3.96 log Cfu/mL at the 20 min of the experiment and 3.82 log Cfu/mL as well as 3.80 log Cfu/mL at 60 min and 120 min (Fig. 2).

In Table 2, we also observed that the three organisms when grown in blood agar media produced an (α) haemolysis test.

There were slight variations between those of *L. plantarum* and *L. nantensis* as cell populations of 3.94 log Cfu/mL, 3.87 log Cfu/mL and 3.84 log Cfu/mL were observed for *L. plantarum* and 3.93, 3.85 and 3.80 log Cfu/mL were for *L. nantensis* at 20, 60 and 120 min (Fig. 2).

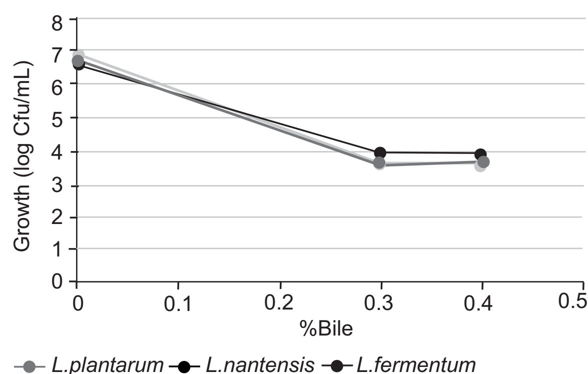


Fig. 1. Response of LAB to concentration of bile salt.

Table 2. Haemolysis test

Strain	Type of haemolysis
L. fermentum	-(α)
L. plantarum	-(α)
L. nantensis	-(α)

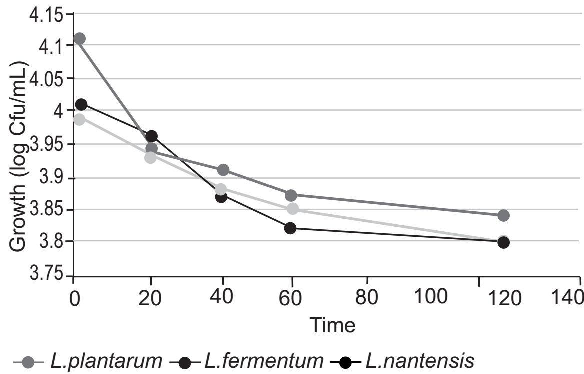


Fig. 2. The effect of low pH (2.0) on three lactic acid bacteria stains.

Slight pH changes were also observed for each of the organisms in the different cereal substrates (Fig. 3-5) with *L. fermentum* producing a significant drop in pH from 5.8 to 5.62 in the malt medium (Fig. 6). Charalampopolous and Pandiella (2010) reported that such pH changes compared with moderate concentration of lactic acid detected at the same time could be linked to the low buffering capacity of the malt (18.43) mmol/pH1 as shown in Table 3. This confirms the importance of substrate composition in the total fermentation process. Hence, the need arises for an augmentation of the malt with additives to improve it buffering capacity thereby boasting the fermentation process.

The free amino nitrogen (FAN) content of the maize, sorghum and malt media which measured 15.6 mg/L, maize), (27.3 mg/L, sorghum) and (62.3 mg/L, malt) was also important growth factor that aided the fermentation process (Table 4).

Table 3. Buffering capacity

Substrate	Buffering capacity (mmol/pH1)
Maize	9.64
Sorghum	6.21
Malt	18.43

Lactic acid production in the maize substrate showed 1.21 mold/m^3 for *L. fermentum,* 1.10 for *L. plantarum* and 0.98 for *L. nantensis* (Fig. 6), while the sorghum substrate had 1.22, 1.46 and 1.12 for each of the strains respectively after 48 h (Fig. 7). Higher acid contents were observed in the malt media*, L. fermentum* 2.73 mold/m^3, *L. plantarum* and *L. nantensis* were 2.66 mold/m^3 and 2.26 mold/m^3 at the end of the fermentation process (Fig. 8). Due to the nature of the experiment

Table 4. Free amino nitrogen (Fan)

Substrate	Fan (mg/L)
Maize	15.6
Sorghum	27.3
Malt	62.3

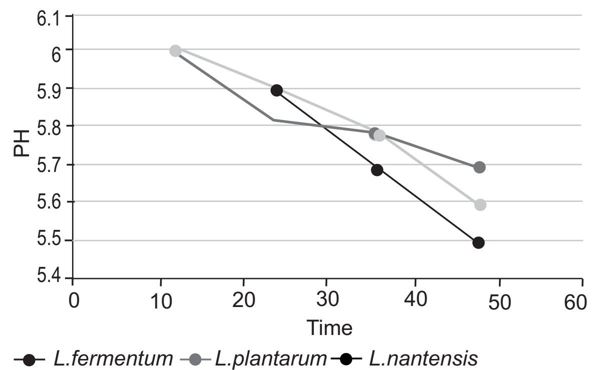


Fig. 3. pH determination for Maize during fermentation.

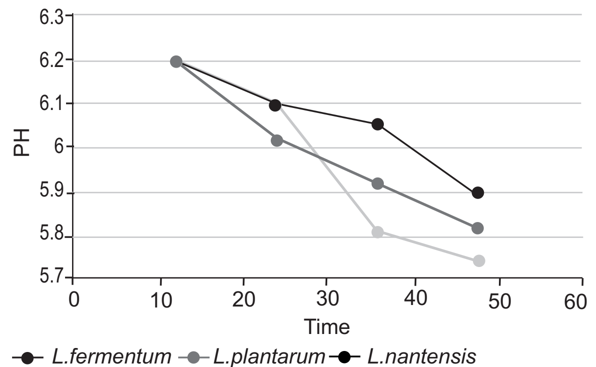


Fig. 4. pH determination for sorghum during fermentation.

i.e. absence of regulation of pH and synthesis of metabolic products, there was a consequent reduction in the pH of the fermentation media. The reduction stems from the synthesis and accumulation of lactic acid in that both the dissociated and undissociated forms or by the indirect release of protons into the medium (Anyasi *et al*., 2017). Low concentrations of this acid could inhibit the growth of cells after the exponential phase (Narendranath *et al*., 2001).

Bacterial cell counts of the probiotic strains had shown an increase in cell population from the start of the experiment up to 12 h, while counts did depreciate towards the end of the fermentation process as shown in Fig. 9-11. In the maize medium, *L. fermentum* had a cell count of 6.30 log Cfu/mL at 12 h, while a count of 6.21 log Cfu/mL was observed at the end, the case wasn't any different in the other two substrates of sorghum and malt as shown in Fig. 10 and 11.

Growth rates were also observed with *L. plantarum* and *L. nantensis,* the slight drop in cell population towards the end of the fermentation process (the decline phase – 48 h) could have been as a result of decrease in the growth – limiting factor being the free amino nitrogen (FAN) as well as acidification leading to changes in pH of the growth media (Peyer *et al*., 2017).

Available data (Fig. 12-14) showed that storage time brought about a significant change in acid level of the beverage, the maize beverage produced using *L. fermentum* had variations in acidity during the period of storage (15days) at 4 °C, *L. fermentum* produced acidity of 1.22 and 126 mold/m^3 during the period of storage (15 days) at 4 °C those of *L. plantarum* and *L. nantensis* ranged between 1.13 and 1.30 mold/m^3 as well as 1.10 and 1.17 respectively (Fig. 12). Readings were also taken for the other beverages of sorghum and malt and results recorded (Fig. 13 and 14), these results

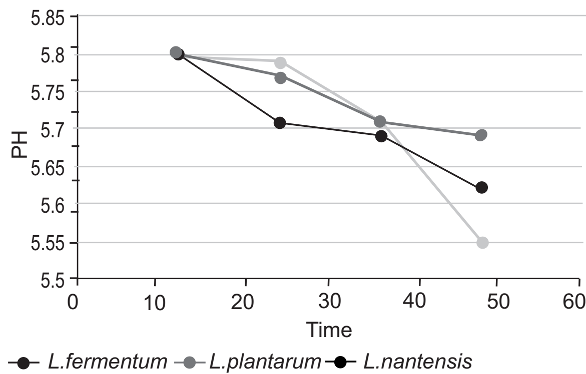


Fig. 5. pH determination for malt during fermentation.

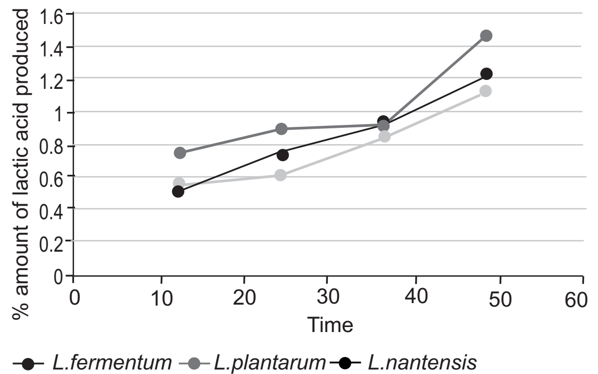


Fig. 7. Lactic acid production for sorghum during fermentation.

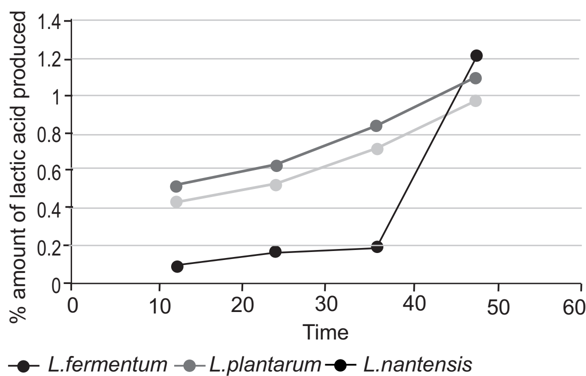


Fig. 6. Lactic acid production for maize during fermentation.

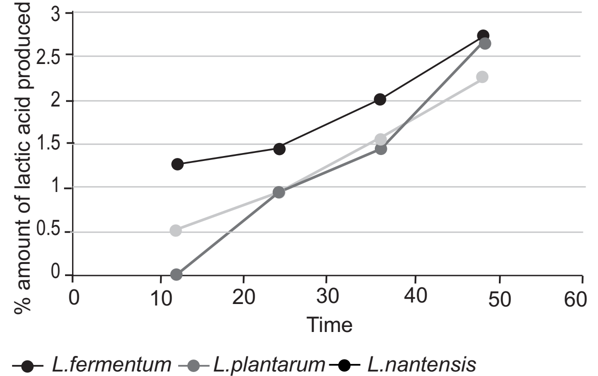


Fig. 8. Lactic acid production for malt during fermentation.

were in agreement with the observation of Hassan *et al*. (2012). The titratable acidity (Fig. 15-17) showed a very sharp increase as the in maize during refrigerated

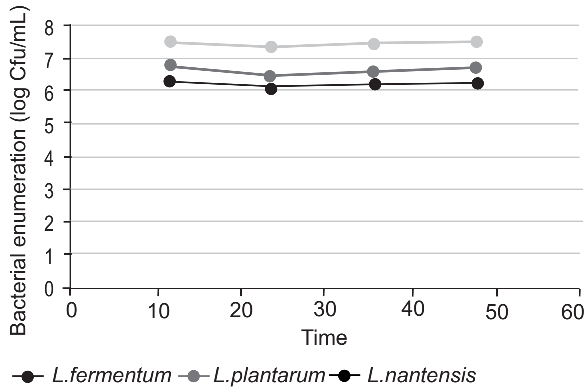


Fig. 9. Bacterial enumeration in maize during fermentation.

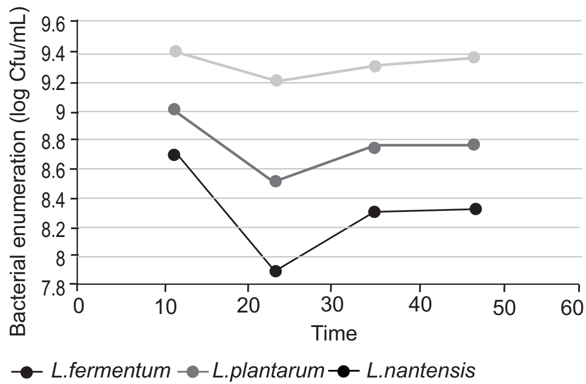


Fig. 10. Bacterial enumeration in sorghum during fermentation.

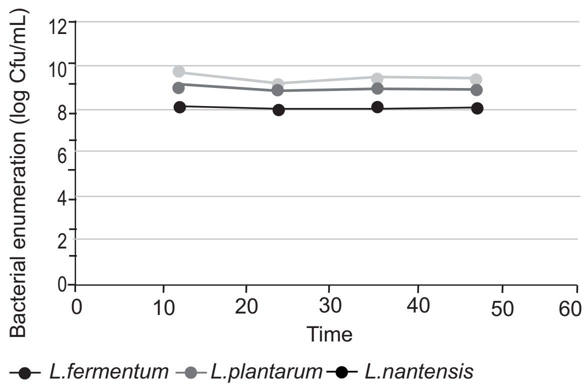


Fig. 11. Bacterial enumeration in malt during fermentation.

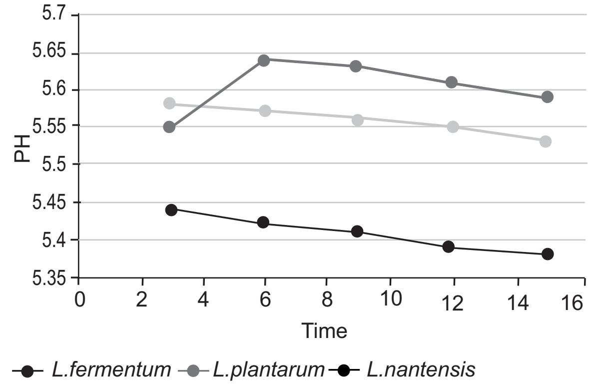


Fig. 12. pH during refrigerated storage in maize during refrigerated storage for 15days at 4 °C.

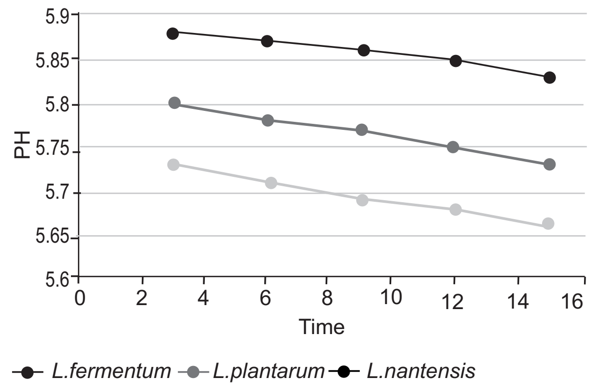


Fig. 13. pH during refrigerated storage in sorghum during refrigerated storage for 15 days at 4 °C.

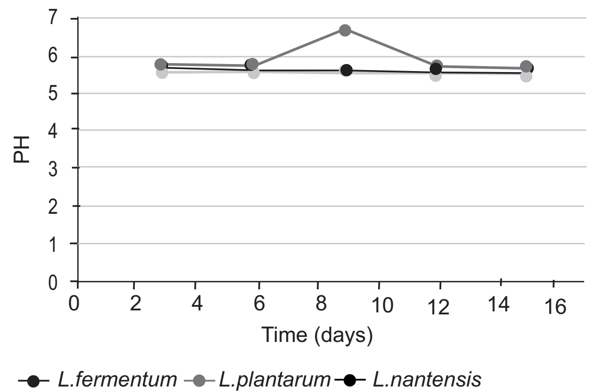


Fig. 14. pH during refrigerated storage in malt during refrigerated storage for 15 days at 4 °C.

storage for 15 days at 4 °C, while there is a slight increase for sorghum and malt. This supports the research work of Ohaegbu *et al*. (2022) on characterization and antimicrobial activities of lactic acid bacteria isolated from selected Nigerian traditional fermented foods, where there is an increase in titratable acidity after 72 h. Bacterial counts for the period (15 days) showed a constant decrease (Fig. 18-20). In the maize beverage,

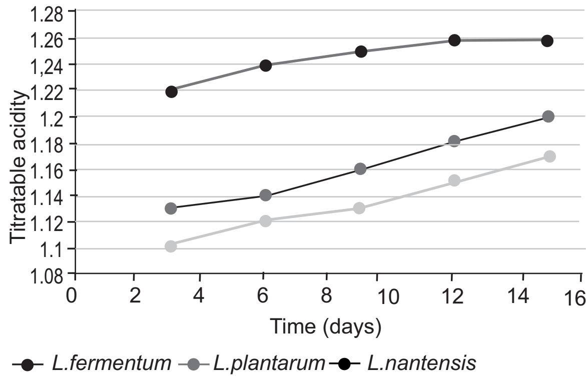


Fig. 15. Titratable acidity for maize during refrigerated storage for 15 days at 4 °C.

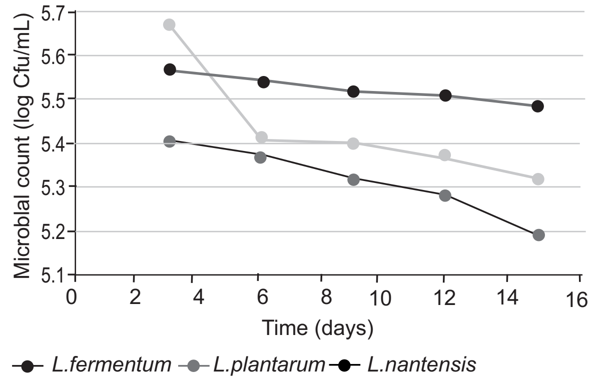


Fig. 18. Microbial count for maize during refrigerated storage for 15days at 4ºC.

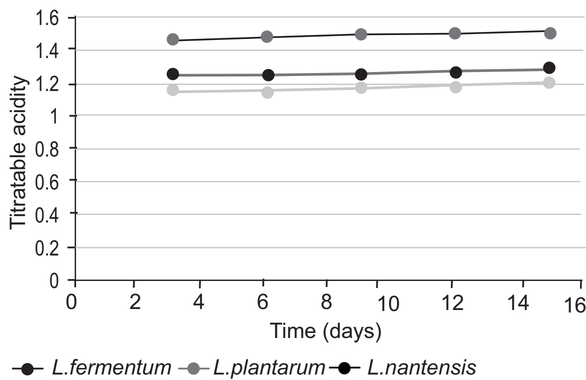


Fig. 16. Titratable acidity for sorghum during refrigerated storage for 15 days at 4 °C.

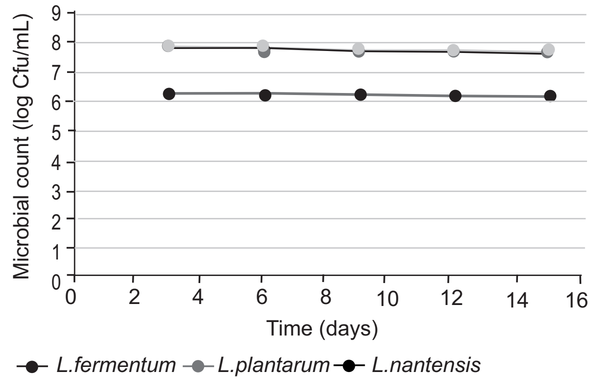


Fig. 19. Microbial count for sorghum during refrigerated storage for 15 days at 4 °C.

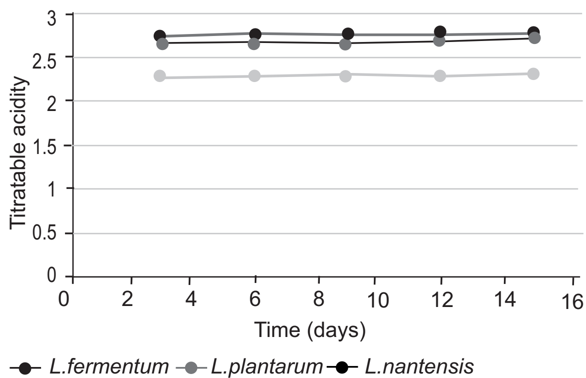


Fig. 17. Titratable acidity for malt during refrigerated storage for 15 days at 4 °C.

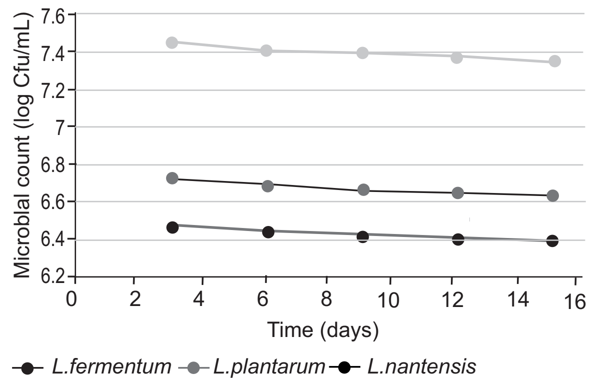


Fig. 20. Microbial count for malt during refrigerated storage for 15 days at 4 °C.

L. fermentum showed a decrease in count from 5.57 to 5.48 log Cfu/mL from day 3 to day 15, while those of *L. plantarum* and *L. nantensis* decreased from 5.41 to 5.19 log Cfu/mL and 5.67 to 5.32 log Cfu/mL respectively as shown in Fig. 18. Similar decrease were also observed for the other two beverages (Fig. 19 and 20), Mousavi *et al.* (2011) was reported a reduction for *L. plantarum* in probiotic pomegranate juice after 14 days of storage at 4 °C.

Conclusion and Recommendations

In the production of probiotic cereal beverages through fermentation using potential probiotic lactic acid bacteria, it is paramount to take into consideration the organoleptic quality of such beverage which to a great extent depends on the rate of acid production. Other factors that do play important roles in fermentation processes do include cell population and viability, nutrient content of substrate, addition of supplements such as vitamins and minerals as well as the synthesis of metabolic end products.

In fermentation processes the known inhibitory factors to microbial growth include pH and depletion of growth limiting factors which includes free amino nitrogen. Novel experimentation procedures could probably be designed such that the acids (lactic acid) produced could be channelled – out so as to curtail pH changes, other steps to take in ensuring continuity of the process do involve augmentation of the nutrient supply as well as microbial cell population be re-feeding. Addition of supplements could as well improve nutrient content of the fermentation substrate.

Conflict of Interest. The authors declare that they have no conflict of interest.

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