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Inhibitory Activity of *Lactobacillus plantarum* Strains from *Akamu* - A Nigerian Fermented Maize Food against *Escherichia coli*

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Abstract *Lactobacillus plantarum* strains: NGL5 and NGL7 previously identified from *akamu*-a lactic acid bacteria fermented maize food using PCR sequencing analysis were evaluated for inhibitory activity against *Eschericia coli* NCTC 11560 in fermenting ground maize slurries at 22 and 30°C, and in porridges prepared from 24 h fermented slurries. The *L. plantarum* strains fermented ground maize slurries and produced significant levels of titratable acidity ≥5 g L^{-1} and low pH ≤3.63, displaying inhibitory activity against *E. coli* NCTC 11560. Inhibition was significantly ($p \leq 0.05$) greater at 30°C than at 22°C. At 30°C, *E. coli* was inhibited after 24 h in the co-inoculation assay ($\mu < -0.17\ h^{-1}$; D value <10 h) and after 180 min in the already fermented slurries ($\mu < -0.13\ min^{-1}$; D value <14 min). In the porridges, *E. coli* decreased below detection limit after 20 mins. This study showed that *E. coli* NCTC 11560 introduced during or after fermentation and into freshly prepared porridges from the *L. plantarum* strains fermented slurries would be unable to survive and proliferate at pH <4.2 and acidity >5 g L^{-1}. For safety of the product however fermentation has to be for at least 24 h at 30°C or more at 22°C.

Keywords: *Lactobacillus plantarum, maize slurry, fermentation, acidity, inhibition, Escherichia coli*

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1. Introduction

In most West African countries, lactic acid fermented cereal-based foods play important role in the nutrition of infants and young children as a major infant complementary food and forms the most integral part of adult main meals [1]. *Akamu* is a traditional Nigerian lactic acid fermented food, made from maize (*Zea maize*), sorghum or millet [2,3,4]. Its traditional processing technique has been described by Obinna-Echem *et al.*, [5] and the uncontrolled fermentation process increases the variability in quality and safety of *akamu*. Contaminations may occur through different channels before, during, and after preparation. The raw materials may themselves harbour some contaminants. Enteropathogenic microorganisms from polluted water, dirty utensils, insects, pests, domesticated animals, excreta from the environment could be introduced during and after preparation. Storage at ambient temperature due to economic constrains in turn favours the growth of the pathogens and/or formation of their toxins and insufficient cooking or reheating of the probably uncovered foods prior to consumption results in food borne illnesses [6], [7]. Diarrhoea etiological agents are broad but infections due to pathogenic *E. coli* are probably the commonest in developing countries with the transmission of about 25% of diarrhoeal episodes attributed to *E. coli* [8,9].

Fermentation of cereal-based foods has been reported to provide antagonistic environment against pathogenic microorganisms. This was attributed to the production of organic acids that lower pH to levels inhibitory to pathogenic organisms. Other reasons include the overcrowding of the food with desirable lactic acid bacteria that makes competition difficult for unwanted microorganisms and the production of antimicrobial agents. Some studies have utilized an accelerated fermentation (back slopping) method to demonstrate the beneficial effect of fermentation on quality and safety of cereal-based gruels [7,10-16].

The increased growth of mixed endogenous cultures of the substrate is inevitable with the back slop fermentation. Antimicrobial studies using this accelerated methods may not distinguish clearly between pathogens and the natural fermenting microorganisms present in the raw materials and those that are been introduced during the inhibitory test. Another alternative would be the use of pure starter cultures endogenous to the substrate. Recently, Obinna-Echem *et al.*, [5] isolated and identified two strains of *L. plantarum* (NGL5 and NGL7) from a Nigerian traditional *akamu* sample using both conventional microbiological methods and direct polymerase chain reaction and sequencing analysis.

This study was therefore aimed at determining the inhibitory ability of these *L. plantarum* strains (NGL5 and NGL7) and a commercial probiotic strain (LpTx) isolated from a probiotic food supplement capsule against *E. coli* NCTC 11560 in fermenting ground maize slurries at 22 and 30°C and in porridges prepared from 24 h *L. plantarum* fermented slurries.

2. Materials and Methods

2.1. Ground Maize

Ground organic maize (L1530) was obtained from Health Food Shop, Rickard Lanes', Plymouth City Centre, UK. About 50±0.01 g of the flour were weighed into cellophane bags, sealed and irradiated with ^{60}Co at 25.88 ± 0.79 kGy (Becton Dickinson and Company, Plymouth, UK).

2.2. Microorganisms and Inocula Preparation

The *L. plantarum* strains used in this study were: two strains (NGL5 and NGL7) previously isolated from Nigerian traditional *akamu* sample by Obinna-Echem *et al.*, [5] and a commercial probiotic strain (LpTx) isolated from a probiotic food supplement obtained from Health Food Shop, Rickard Lanes', Plymouth City Centre, UK. The *L. plantarum* strain (LpTx) was isolated and identified using the same conventional and molecular methods described by Obinna-Echem *et al.*, [5]. *E. coli* NCTC 11560 a category two pathogen was obtained from stock cultures in the microbiological laboratory of Plymouth University, UK. *E. coli* is an indicator organism most commonly chosen in temperate climates and one of the pathogens of concern in African fermented cereal foods [8].

The *L. plantarum* strains were cultivated on de Man, Rogosa and Sharpe (MRS) agar and *E. coli* NCTC 11560 on Nutrient agar incubated at 37°C for 24 h. MRS and Nutrient broths were used for broth cultures of the *L. plantarum* strains and the *E. coli* respectively. A distinct colony of the respective microorganism from the agar plate culture was inoculated into 10 mL of the appropriate broth media and incubated at 37°C without agitation for 18 – 20 h. The cultures were harvested by centrifugation (Hettich Zentrifugen Rotina 46 S, Tuttlingen, Germany) at 4000 *x g* for 10 min and washed twice in phosphate buffered saline (PBS) (pH 7.3±0.2) and re-suspended in PBS such that 1 mL of inoculum produced 10^9 and 10^8 CFU mL^{-1} for the *L. plantarum* strains and the *E. coli* respectively. The media and the diluent used were obtained from Oxoid Limited (Basingstoke, Hampshire, UK).

2.3. Antimicrobial Activity

The inhibitory ability of the *L. plantarum* strains against *E. coli* NCTC 11560 was determined firstly by co-inoculating the *L. plantarum* with *E. coli* into the ground maize slurries and secondly by inoculating the *E. coli* NCTC 11560 into 24 h *L. plantarum* fermented slurries and its porridges.

2.3.1. Co-Inoculation Test

Equal volumes (1 mL) of one of the *L. plantarum* strains and *E. coli* NCTC 11560 at a time were inoculated into 100 mL of sterile distilled water that was thoroughly mixed with 50±0.02 g irradiated ground maize. The inoculated slurries were distributed in 18 mL quantity into sterile transparent 50 mL plastic pots with lids and incubated at 22 and 30°C. The transparent pot was to enable the visualization of any changes like air bubbles from the metabolic activities of some of the microorganisms in the fermentation. Samples were aseptically withdrawn for analysis immediately after inoculation and every 3 h for the first 9 h and after 24 and 48 h at 30°C, and after 72 h at 22°C. Samples inoculated with only *E. coli* NCTC 11560 served as control.

2.3.2. Challenge Test in 24 h *L. plantarum* Strains Fermented Samples

One millilitre of the *L. plantarum* strain cell suspension was inoculated into 100 mL of sterile distilled water that was thoroughly mixed with 50±0.02 g irradiated ground maize. The inoculated slurries were fermented at 22 and 30°C. After 24 h, the samples were distributed in 18 mL quantity into sterile transparent 50 mL plastic pots with lids before the inoculation of 1 mL of *E. coli* NCTC 11560 followed by incubation at the same temperatures. Sampling was carried out after the initial inoculation and every 10 min for 40 min and at 60, 120 and 180 min. The control sample was without *L. plantarum* strain inoculation during the 24 h fermentation but had *E. coli* NCTC 11560 inoculation thereafter.

2.3.3. Challenge Test in Porridges from 24 h *L. plantarum* Strains Fermented Samples

Porridges were prepared from 150 mL of 24 h *L. plantarum* fermented samples by adding equal volume of boiling water and then microwaving for 2 minutes with vigorous stirring after each minute to obtain a lump free porridge. The porridges were distributed in 18 mL amount into well labelled transparent plastic mini pots with lids and allowed to cool in a water bath maintained at 45°C. Thereafter, *E. coli* NCTC 11560 was inoculated and enumerated after every 10 min for 40 min.

2.4. Determination of pH and Total Titratable Acidity (TA) of the Fermentation

The pH of 1 mL of sample in 10 mL of sterile distilled water was determined with a pH meter (Accumet^R AB10, Fisher Scientific, Loughborough, UK). Thereafter, the samples were titrated against 0.1 mol L^{-1} NaOH with phenolphthalein as indicator to determine the amount of acid as titratable acidity (TA) produced in the fermentation.

2.5. Microbial Enumeration

Ten-fold dilutions of 1 mL of sample slurries in 9 mL of PBS were prepared and plated out using the drop method by [17] on MRS agar plates for the enumeration of the *L. plantarum* strains in the fermentation without *E. coli* NCTC 11560 and on MRS agar supplemented with cyclohexamide at a final concentration of 1 x 10^{-3} g L^{-1} in the fermentation co-inoculated with *E. coli*. The enumeration of *E. coli* was on MacConkey agar plates. All the plates were incubated aerobically at 37°C for 24 – 48h.

2.6. Statistical Analysis

Data obtained were statistically analysed using Minitab (Release 16.0) Statistical Software English (Minitab Inc.

Coventry, UK). Statistical differences were evaluated by analysis of variance (ANOVA) under general linear model and Tukey's pairwise comparisons at 95 % confidence level. Relationship between *E. coli* NCTC 11560 viable count and pH, titratable acidity and *L. plantarum* viable counts utilised multiple regression analysis and correlations. For variables pairs (e.g. pH and *E. coli*) values of other variables were held constant to eliminate interaction effect of the variable held constant [18]. P-values lower than or equal to 0.01 signified that there was no correlation. The growth rates of the microorganisms and the fitted sigmoid curves were obtained using the microbial model (DMFit version 2.0) by [19]. The decimal reduction times or D values and the doubling times (*T*) were computed from the potential growth rates in Microsoft Excel 2010 using the formula: D = 1/*k* and *T* = 0.693/μ; where *k* and μ represent the death and specific growth rates respectively [20].

3. Results and Discussion

3.1. pH and Total Titratable Acidity (TA)

The pH and TA of the co-inoculation fermentations are shown in Figure 1 and Figure 2 respectively. All the *L. plantarum* strains co-inoculated with *E. coli* NCTC 11560 fermented the sterile ground maize slurry with significant reduction in pH from ≤6.23±0.08 to ≤3.72±0.01 and ≤5.50±0.04 to ≤3.35±0.01 after 48 h at 22 and 30°C respectively. While TA increased from ≥0.9 to ≥7.2 and to ≥8.1 g L^{-1} after 48 h at 22 and 30°C respectively. Presented in Table 1 are the pH and TA of the fermentations before the inoculation of *E. coli* NCTC 11560. At 22°C, the pH and TA was ≤4.17 and ≥5.11 g L^{-1} respectively while at 30°C the pH was ≤3.48 with TA ≥6.01. The pH (3.41) and TA (≥ 7.21 g L^{-1}) of the porridges are shown in Table 2.

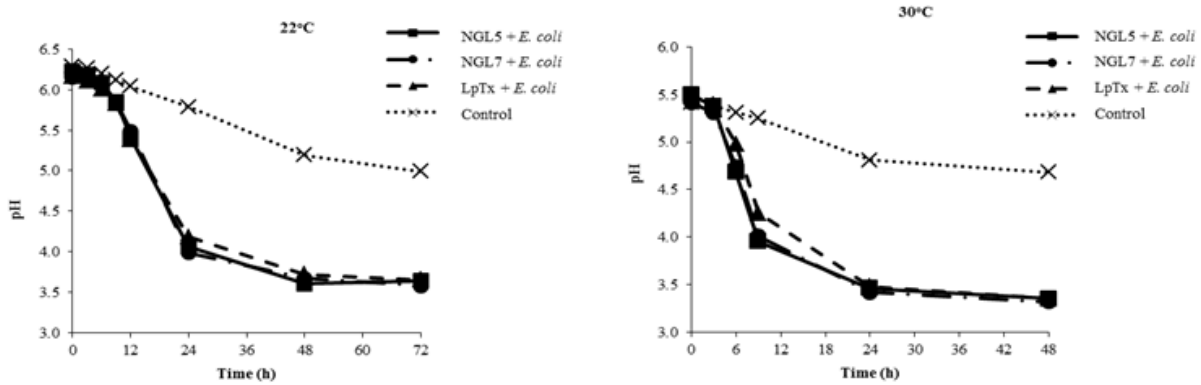

Figure 1. pH of sterile ground maize slurries fermented by co-inoculation of *L. plantarum* strains (NGL5, NGL7 and LpTx) and *E. coli* NCTC 11560 at 22 and 30°C

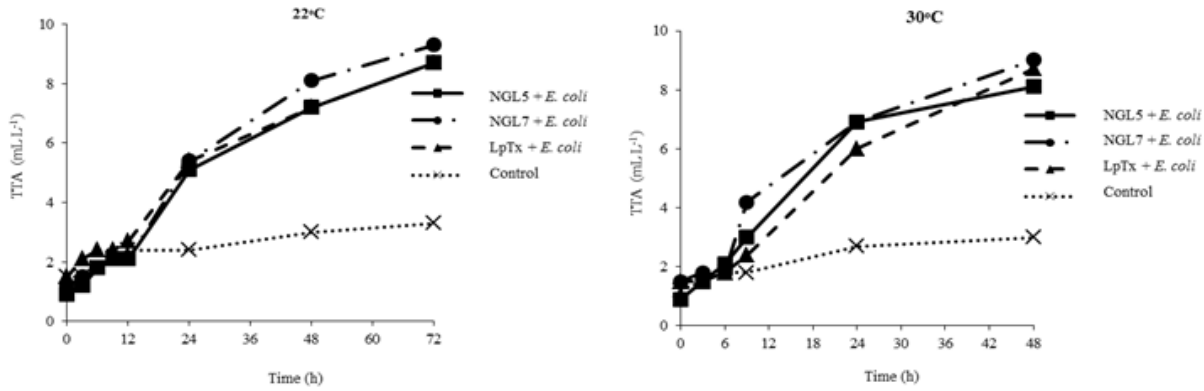

Figure 2. Titratable acidity (TA) (g L^{-1}) of sterile ground maize slurries fermented by co-inoculation of *L. plantarum* strains (NGL5, NGL7 and LpTx) and *E. coli* NCTC 11560 at 22 and 30°C

Table 1. pH, titratable acidity (TA) and *L. plantarum* strains (NGL5, NGL7 and LpTx) viable count in the fermentation before the inoculation of *E. coli* NCTC 11560

Samples	Temp (°C)	pH		TA (g L^{-1})		*L. plantarum* viable count (Log_{10} CFU mL^{-1})	
		0 h	24 h	0 h	24 h	0 h	24 h
NGL5	22	6.19±0.02^{bc}	4.01±0.03^{b}	1.80±0.00^{a}	5.71±0.52^{ab}	7.44±0.02^{a}	9.15±0.03^{ab}
	30	5.37±0.01^{a}	3.48±0.01^{a}	0.90±0.00^{a}	6.31±0.00^{ab}	7.19±0.05^{b}	9.20±0.01^{a}
NGL7	22	6.14±0.07^{bc}	4.04±0.01^{b}	1.20±0.52^{a}	5.71±0.52^{ab}	7.11±0.08^{bc}	9.03±0.09^{b}
	30	5.36±0.03^{a}	3.42±0.00^{a}	0.90±0.00^{a}	6.61±0.52^{ab}	7.22±0.05^{a}	9.03±0.01^{b}
LpTx	22	6.08±0.08^{b}	4.17±0.02^{c}	1.50±0.52^{a}	5.11±0.52^{b}	6.97±0.08^{d}	9.06±0.05^{ab}
	30	5.38±0.00^{a}	3.42±0.01^{a}	1.20±0.52^{a}	6.01±0.52^{ab}	6.99±0.03^{cd}	8.76±0.12^{c}
Control	22	6.20±0.02^{c}	6.14±0.01^{e}	1.20±0.52^{a}	2.40±0.52^{c}	-	-
	30	5.40±0.03^{a}	5.22±0.01^{d}	0.90±0.00^{a}	1.80±0.00^{c}	-	-

Values with same superscript in the same column do not differ significantly (p≤0.05).

Table 2. pH, titratable acidity (TA) and counts of *E. coli* NCTC 11560 in porridges from 24 h *L. plantarum* strains fermented maize slurries at 30°C

Samples	*pH	*TA (g L^{-1})	*E. coli* NCTC 11560 counts (Log_{10} CFU mL^{-1})		
			Time (min)		
			0	10	20
NGL5	3.41	7.21	7.21±0.03	2.90±0.57	<2
NGL7	3.41	8.11	7.18±0.05	3.29±0.57	<2
LpTx	3.41	9.01	7.16±0.08	3.26±0.57	<2

*pH and TA of the porridges before *E. coli* NCTC 11560 inoculation. N=3±SD.

Decrease in pH has been established as an important parameter for assessing how fast a process will reach conditions (pH <4.5) which can inhibit the growth of pathogenic microorganisms [21]. The fermentation ability of the *L. plantarum* strains characterised with significant reduction in pH and increase in TA provided a condition that had significant inhibitory influence on the survival of *E. coli* NCTC 11560. Similar fermentation abilities of *L. plantarum* strains were reported by Teniola and Odunfa [16] and Mugula *et al.*, [22]. The pH and TA of the control sample did not differ with temperature and varied significantly ($p \leq 0.05$) from the *L. plantarum* fermentation. This was expected as the maize flour samples were irradiated and sterile distilled water.

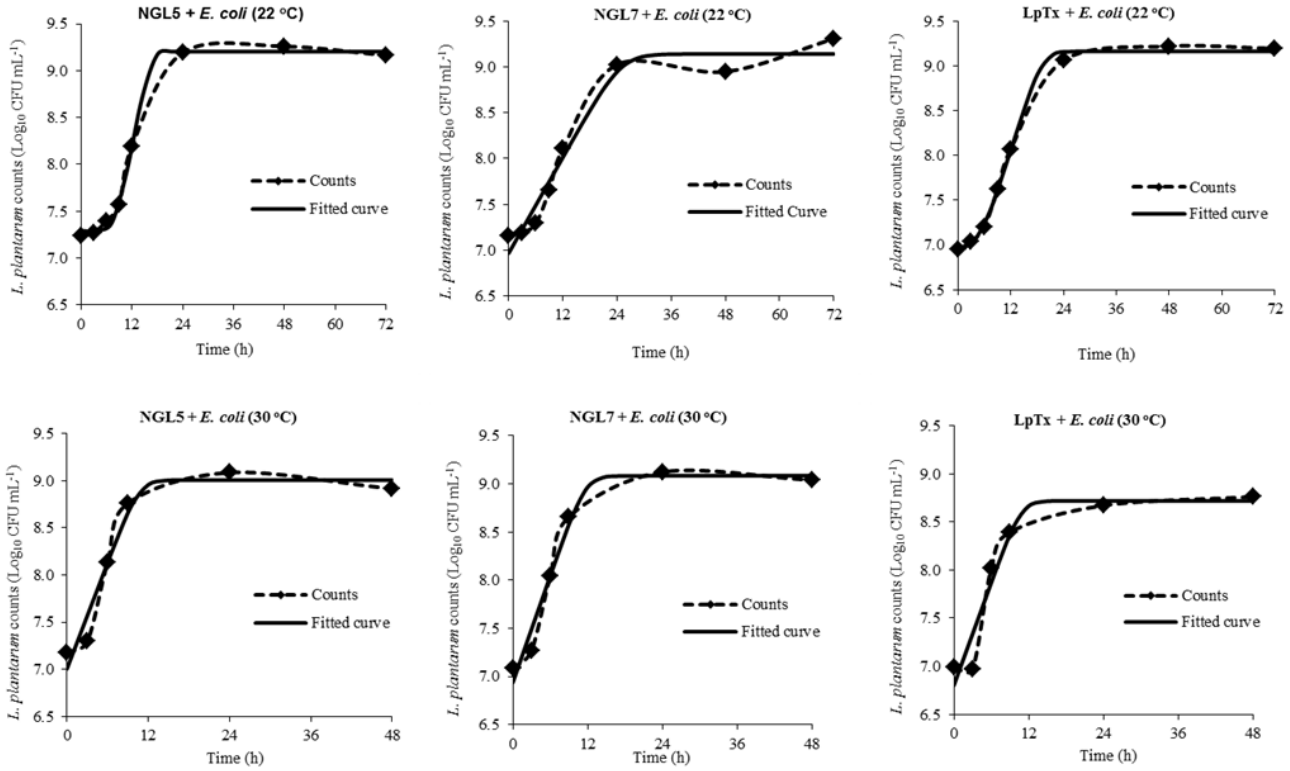

Figure 3. Viable counts with the fitted sigmoid curve for the *L. plantarum* strains (NGL5, NGL7 and LpTx) in the co-inoculation fermentation with *E. coli* NCTC 11560 at 22 and 30°C

Table 3. Modelling parameters for the growth of *L. plantarum* strains (NGL5, NGL7 and LpTx) in sterile ground maize slurries co-inoculated with *E. coli* NCTC 11560

curve	Temp (°C)	[a]Experimental data		Modelling parameters: [b]Curvature parameters		[c]Primary parameters				[d]Statistics		*T* (h)
		yDatMin	yDatMax	mCurv	nCurv	Rate	lag	y0	yEnd	se(fit)	R^2	
NGL5 + *E. coli*	22	7.20±0.02^{a}	9.28±0.05^{ab}	10	1	0.22±0.06^{a}	7.85±1.04^{a}	7.28±0.04^{a}	9.21±0.03^{a}	0.08	0.99	3.04
	30	7.18±0.02^{a}	9.09±0.07^{c}	10	0	0.22±0.05^{a}		7.07±0.11^{ab}	9.01±0.00^{c}	0.17	0.95	3.13
NGL7 + *E. coli*	22	7.15±0.02^{ab}	9.31±0.07^{a}	10	0	0.14±0.06^{a}	4.38±3.81^{b}	7.11±0.08^{ab}	9.12±0.06^{ab}	0.13	0.97	4.71
	30	7.08±0.08^{abc}	9.15±0.04^{bc}	10	0	0.20±0.03^{a}		6.98±0.07^{b}	9.08±0.05^{bc}	0.13	0.97	3.36
LpTx + *E. coli*	22	6.93±0.08^{c}	9.24±0.05^{ab}	10	1	0.14±0.06^{a}	3.38±3.17^{c}	6.91±0.10^{b}	9.19±0.04^{ab}	0.09	0.99	4.71
	30	6.96±0.13^{bc}	8.78±0.00^{d}	10	0	0.20±0.05^{a}		6.84±0.17^{b}	8.72±0.04^{d}	0.18	0.93	3.44

[a]yDatMin and yDatMax: Initial and maximum viable counts (Log_{10} CFU mL^{-1})
[b]mCurv and nCurv: Curvature parameters at the beginning and end of the linear phase respectively
[c]Rate: The potential maximum growth rate of the *L. plantarum* strains (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper asymptote of the sigmoid curve; Lag: The lag phase duration (h)
[d]Se(fit) Standard error of fitting (estimated standard deviation of the observed independent values); R^2: Adjusted R-square statistics of the fitting
T: Doubling time
Means that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD.

3.2. Microbial Growth Analysis

3.2.1. Co-inoculation Test

The viable counts with the fitted sigmoid curve for the *L. plantarum* strains in the co-inoculation fermentation are presented in Figure 3, while Table 3 shows the model parameters. The growth of the *L. plantarum* strains in the fermentation followed a typical microbial growth pattern. The main phases of growth observed were the log and the stationary phases. The period of lag observed at 22°C was probably for the adaptation of the *L. plantarum* strains to

the low temperature. Although at both temperatures, there was a difference of more than 1 h between the doubling times in the NGL7 + *E. coli* and LpTx + *E. coli* samples, there was no significant difference between their growth rates. Maximum viable counts of 9 Log_{10} CF mL^{-1} were also obtained after 24 h at both temperatures. This implied that the growth and metabolism of the *L. plantarum* strains were not limited by the temperatures. The *L. plantarum* strains however were unable to continue to grow exponentially at 22°C after 24 h and at 30°C after 12 h. This could be attributed to the accumulation of organic acid, depletion of utilisable nutrients and carbon dioxide in the closed fermentation system [23].

Figure 4 shows the viable counts of *E. coli* NCTC 11560 in the co-inoculation fermentation. The ability of *E. coli* NCTC 11560 to carry out its metabolic activities at near neutral pH in the co-inoculation with the *L. plantarum* strains must have accounted for the growth at the early stages of the fermentation.

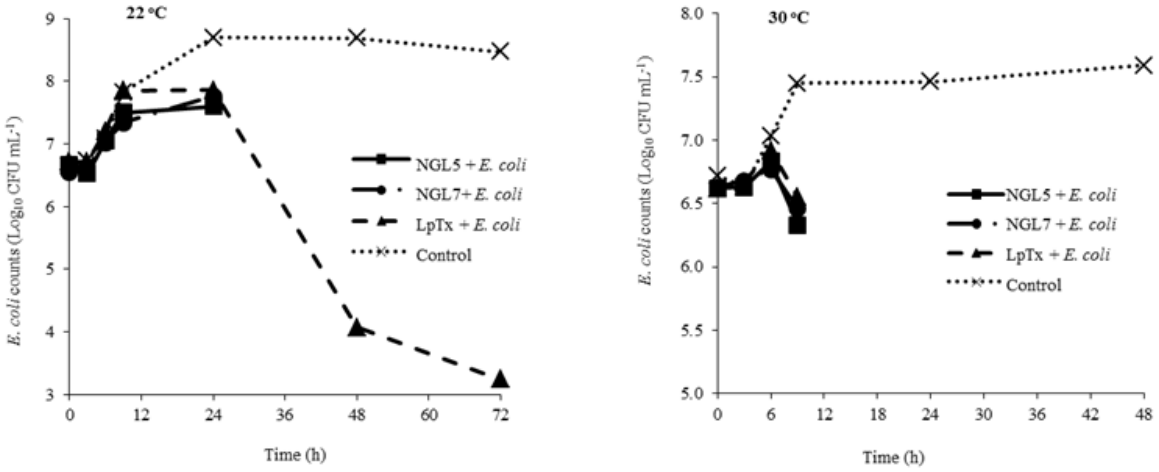

Figure 4. *E. coli* NCTC 11560 counts in the co-inoculation fermentation with *L. plantarum* strains (NGL5, NGL7 and LpTx) at 22 and 30°C

Table 4. Modelling parameters for the growth of *E. coli* NCTC 11560 in sterile ground maize slurries co-inoculated with *L. plantarum* strains

Curve	Temp (°C)	[a]Experimental data		Modelling parameters				
				[b]Primary parameters		[c]Statistics		*T* (h)
		yDatMin	yDatMax	Rate	y0	se(fit)	R^2	
NGL5 + *E. coli*	22	6.52±0.02^{b}	7.88±0.17^{bc}	0.11±0.00^{a}	6.44±0.04^{b}	0.21	0.86	6.09^{b}
	30	6.60±0.06^{ab}	6.83±0.06^{d}	0.04±0.01^{b}	6.59±0.06^{ab}	0.08	0.76	20.08^{a}
NGL7 + *E. coli*	22	6.51±0.05^{b}	7.98±0.06^{b}	0.13±0.03^{a}	6.44±0.04^{b}	0.15	0.90	5.30^{b}
	30	6.60±0.03^{ab}	6.77±0.06^{d}	0.03±0.02^{b}	6.60±0.04^{ab}	0.01	0.97	25.12^{a}
LpTxE + *E. coli*	22	6.71±0.10^{a}	8.01±0.07^{b}	0.12±0.00^{a}	6.56±0.10^{ab}	0.20	0.90	5.59^{b}
	30	6.58±0.02^{ab}	6.92±0.05^{d}	0.05±0.01^{b}	6.59±0.03^{ab}	0.10	0.86	13.84^{a}
Control	22	6.62±0.06^{ab}	8.70±0.02^{a}	0.09±0.00^{a}	6.69±0.07^{a}	0.28	0.88	7.49^{b}
	30	6.63±0.09^{ab}	7.66±0.22^{c}	0.10±0.02^{a}	6.60±0.07^{ab}	0.13	0.90	6.64^{b}

[a]yDatMin and yDatMax: Estimated initial and maximum viable counts (Log_{10} CFU mL^{-1})
[b]Rate: The potential maximum growth rate of *E. coli* NCTC 11560 (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper asymptote of the sigmoid curve had no values; There was no lag duration
[c]Se(fit) Standard error of fitting (the estimated standard deviation of the observed independent values); R^2: Adjusted R-square statistics of the fitting
T: Doubling time
The curvature parameters: mCurv and nCurv at the beginning and end of the linear phase were 10 and 0 respectively
Means that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD.

Table 5. Modelling parameters of the death of *E. coli* NCTC 11560 in sterile ground maize slurries co-inoculated with *L. plantarum* strains

Curve	Temp (°C)	[a]Experimental data		Modelling parameters				D value (h)
				[b]Primary parameters		[c]Statistics		
		yDatMin	yDatMax	rate	y0	se(fit)	R^2	
NGL5+ *E. coli*	22	7.60±0.08^{a}	7.88±0.17^{a}	-0.02±0.01^{a}	7.88±0.17^{a}	0	1	41.10^{a}
	30	6.33±0.06^{b}	6.83±0.06^{b}	-0.17±0.04^{b}	6.83±0.06^{b}	0	1	6.00^{b}
NGL7+ *E. coli*	22	7.76±0.21^{a}	7.98±0.06^{a}	-0.02±0.02^{a}	7.98±0.06^{a}	0	1	56.18^{a}
	30	6.46±0.07^{b}	6.77±0.06^{b}	-0.10±0.01^{c}	6.77±0.06^{b}	0	1	9.85^{b}
LpTx + *E. coli*	22	7.86±0.05^{a}	8.01±0.07^{a}	-0.01±0.01^{a}	8.01±0.07^{a}	0	1	77.82^{a}
	30	6.55±0.48^{b}	6.92±0.05^{b}	-0.12±0.17^{bc}	6.92±0.05^{b}	0	1	8.17^{b}

[a]yDatMin and yDatMax: Estimated initial and maximum viable counts (Log_{10} CFU mL^{-1})
[b]Rate: The potential maximum death rate of *E. coli* NCTC 11560 (h^{-1}); y0: initial point of the sigmoid curve; yEnd: lower asymptote of the sigmoid curve had no values; There was no lag duration
[c]Se(fit) Standard error of fitting (estimated standard deviation of the observed independent values); R^2: Adjusted R-square statistics of the fitting
The curvature parameters: mCurv and nCurv at the beginning and end of the linear phase were 10 and 0 respectively
Means that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD.

In addition to the growth phases observed, *E. coli* in the test samples exhibited decelerating and death phases with some inhibitions. The model parameters for the growth and death of *E. coli* are presented in Table 4 and Table 5

respectively. *E. coli* persisted longer at 22°C (doubling time < 6 h) than at 30°C (doubling time > 14 h). *E. coli* was set on decline after 6 h at 30°C with D-value < 10 h, while at 22°C decline in *E. coli* count was observed after 24 h with D-value > 41 h. After 12 h *E. coli* was therefore below detection limit and inhibited after 24 h at 30°C but viable counts of over 7.0 Log_{10} CFU mL^{-1} where obtained after 24 h at 22°C. This suggested the role of the fermentation temperature on the viability of the pathogen. According to the report by Adams and Moss [20] at higher temperatures, pH decrease to levels lower than the strength of the predominant acid (lactic acid; p*K*a 3.86) could result in greater concentration and higher diffusion of the un-dissociated acids into the cytoplasm of the pathogen. It may have been the case that sudden cytoplasmic acidification at 30°C compared to the likely effect at 22°C would have necessitated the complete inhibition of *E. coli* NCTC 11560 after 24 h at 30°C. With the absence of the fermenting *L. plantarum* strains in the control samples there was no significant change in the fermentation for the inhibition of the growth of *E. coli* NCTC 11560 at both temperatures.

The correlation coefficients between the variables: pH, titratable acidity (TA), *E. coli* NCTC 11560 and *L. plantarum* viable counts in the co-inoculation fermentation are shown in Table 6. There was correlation between all the variables after 24 h at both temperatures. Progression in microbial fermentation of cereal and cereal-legume mixture has often been linked with increase in microbial numbers concomitant with decrease in pH and increase in titratable acidity [24]. This was evidenced in the co-inoculation test where after 6 h the correlation between *L. plantarum* strains counts and pH was negative and that between TA after 24 h was positive. There was decrease in pH of the fermentations as TA increased. Acid production particularly lactic acid in the case of *L. plantarum* fermentation is responsible for the lowering of pH in the fermentation.

Table 6. Correlation coefficient between pH, titratable acidity (TA), *L. plantarum* and *E. coli* NCTC 11560 counts in the co-inoculation fermentation

Time (h)	Temp (°C)	TA & pH	*L. plantarum* & pH	*L. plantarum* & TA	*E. coli* & pH	*E. coli* & TA	*E. coli* & *L. planatrum*
0	22	NS	-0.62(0.03)	NS	NS	NS	NS
	30	NS	NS	NS	NS	NS	-0.61(0.04)
3	22	NS	-0.77(0.00)	NS	NS	NS	NS
	30	-0.63(0.03)	NS	NS	NS	-0.78(0.00)	NS
6	22	NS	-0.70(0.01)	NS	NS	NS	NS
	30	NS	-0.89(0.00)	NS	0.77(0.00)	NS	-0.65(0.02)
9	22	NS	-0.93(0.00)	NS	NS	NS	NS
	30	-0.58(0.05)	-0.99(0.00)	0.52(0.09)	0.88(0.00)	NS	-0.89(0.00)
12	22	NS	-0.98(0.00)	NS	0.55(0.06)	NS	-0.54(0.07)
	30	ND	ND	ND	ND	ND	ND
24	22	-0.93(0.00)	-1.00(0.00)	0.93(0.00)	0.96(0.00)	-0.85(0.00)	-0.95(0.00)
	30	-0.94(0.00)	-1.00(0.00)	0.95(0.00)	1.00(0.00)	-0.94(0.00)	-1.00(0.00)
48	22	-0.97(0.00)	-0.99(0.00)	0.97(0.00)	0.90(0.00)	-0.90(0.00)	-0.87(0.00)
	30	-0.98(0.00)	-1.00(0.00)	0.97(0.00)	1.00(0.00)	-0.97(0.00)	-1.00(0.00)

The values in parenthesis are the p-values. NS – Not significant with p-values higher than 0.10. ND – No data was observed for the variables.

Suppression of the growth of *E. coli* NCTC 11560 in the co-inoculation fermentation when decline in viable count had no relationship with pH or acidity may be an attribute of competition for nutrient. However, the onset of the decline in the counts of *E. coli* at 30°C after 6 h when correlation between *E. coli* count and pH was first observed suggested the effect of pH on the viability of the *E. coli*. The mechanism of microbial growth suppression and inhibition at low pH has been associated with the rate of diffusion of un-dissociated acid into the Gram negative cell cytoplasm where they dissociate producing protons which decreases the cell pH. The cell therefore shifts its energy from growth to that of proton expulsion [20]. With progression in fermentation and subsequent decrease in pH, increase TA and the *L. plantarum* counts, *E. coli* NCTC 11560 fell below detection limit (2 Log_{10} CFU mL^{-1}) and was completely inhibited. At lower pH, the rate of diffusion of the un-dissociated acids into the pathogen cytoplasm may have exceed the rate of proton expulsion and in the presence of competition for substrate with the fermentative *L. plantarum* strains, the survival of *E. coli* NCTC 11560 were adversely affected as compared with the control at a pH of 4.68.

3.2.2. Challenge Test in 24 h *L. plantarum* Strains Fermentation and the Porridge Samples

The viable count of the *L. plantarum* strains in the fermentation before the inoculation of *E. coli* NCTC 11560 was presented in Table 1. The *L. plantarum* strains had an increase of 2 log cycles. The adaptive nature of the *L. plantarum* strains shown by the quick onset of the log phase and their fermentative ability is important for efficient production of the desired metabolic end product and for product safety.

The effect of the already fermented samples on the viable counts with the fitted sigmoid curve for the death of *E. coli* NCTC 11560 at 22 and 30°C are shown in Figure 5 and Figure 6 respectively, while the model parameters in the test samples are presented in Table 7. There were no significant changes in the *E. coli* counts in the control samples at both temperatures within the 120 min, hence there was no curve to fit. *E. coli* counts at 30°C were significantly ($p \leq 0.05$) reduced below detection limit after 60 min and completely inhibited in LpTx sample after 180 min. Although at 22°C, *E. coli* counts of 4.62±0.08 and 5.53±0.25 Log_{10} CFU mL^{-1} were obtained after 120 min in NGL5 and NGL7 samples respectively, inhibition was observed after 24 h. At 30°C, the death rate of the *E. coli* in LpTx (0.10 min^{-1}) and NGL7 (0.13 min^{-1}) fermented samples were significantly ($p \leq 0.05$) greater than the death rate at 22°C (0.02 h^{-1}).

Table 7. Modelling parameters of the death of *E. coli* NCTC 11560 in the 24 h *L. plantarum* strains fermented ground maize slurries

Curve	Temp (°C)	[a]Experimental data		Modelling parameters [b]Curvature parameters		[c]Primary parameters			[d]Statistics		D value (min)
		yDatMin	yDatMax	mCurv	nCurv	rate	y0	yEnd	se(fit)	R^2	
NGL5+ *E. coli*	22	4.62±0.08[b]	7.70±0.10[a]	10	0	-0.06±0.02[ab]	7.48±0.17[a]		0.32	0.89	16.81[b]
	30	4.07±0.06[c]	7.08±0.15[b]	0	0	-0.07±0.00[abc]	7.15±0.10[a]		0.22	0.96	13.90[bc]
NGL7+ *E. coli*	22	5.53±0.25[a]	7.74±0.04[a]	10	0	-0.02±0.01[a]	7.39±0.20[a]		0.24	0.86	41.96[a]
	30	3.78±0.18[cd]	7.31±0.47[ab]	10	0	-0.13±0.05[c]	7.17±0.47[a]	3.98±0.17	0.21	0.97	7.67[d]
LpTx+ *E. coli*	22	5.30±0.15[a]	7.74±0.03[a]	0	0	-0.02±0.00[a]	7.51±0.21[a]		0.21	0.92	47.33[a]
	30	3.33±0.27[d]	7.25±0.09[ab]	0	0	-0.10±0.01[bc]	7.08±0.10[a]		0.28	0.96	10.10[cd]

[a]yDatMin and yDatMax: Initial and maximum viable counts (Log_{10} CFU mL^{-1})
[b]mCurv and nCurv: Curvature parameters at the beginning and end of the linear phase respectively
[c]Rate: The potential maximum death rate of *E. coli* NCTC 11560 (min^{-1}); y0: initial point of the sigmoid curve; yEnd: lower asymptote of the sigmoid curve had no value
[d]Se(fit) Standard error of fitting (estimated standard deviation of the observed independent values); R^2: Adjusted R-square statistics of the fitting
Means that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD.

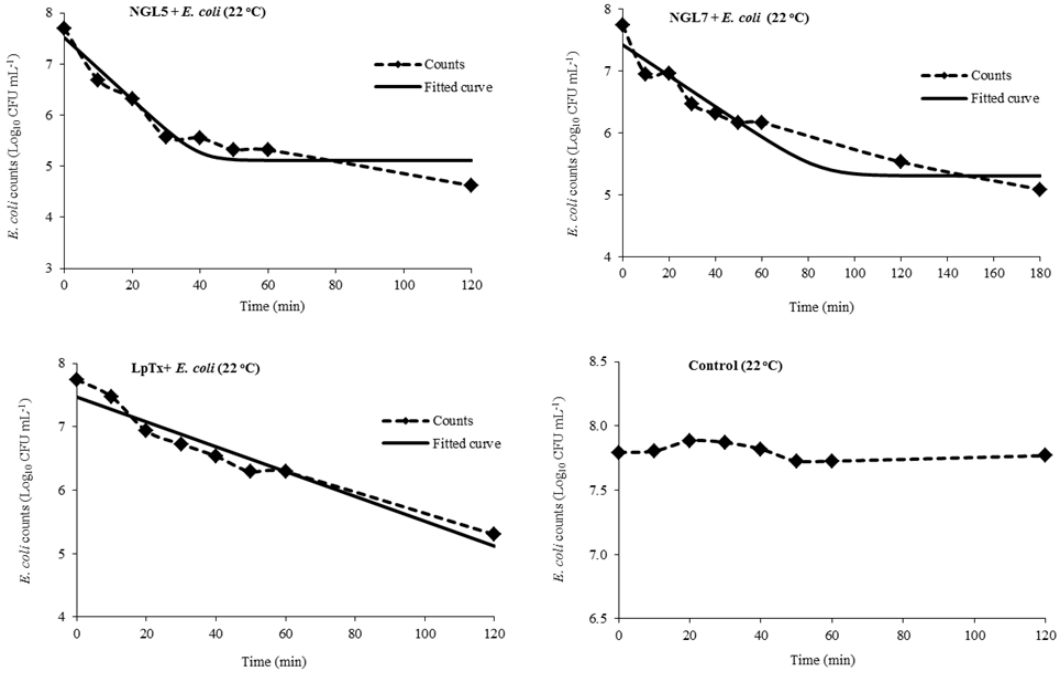

Figure 5. Viable counts with the fitted sigmoid curve for the death of *E. coli* NCTC 11560 at 22°C in the 24 h *L. plantarum* strains (NGL5, NGL7 and LpTx) fermented maize slurries (No significant ($p \leq 0.05$) change in the control to fit a sigmoid curve)

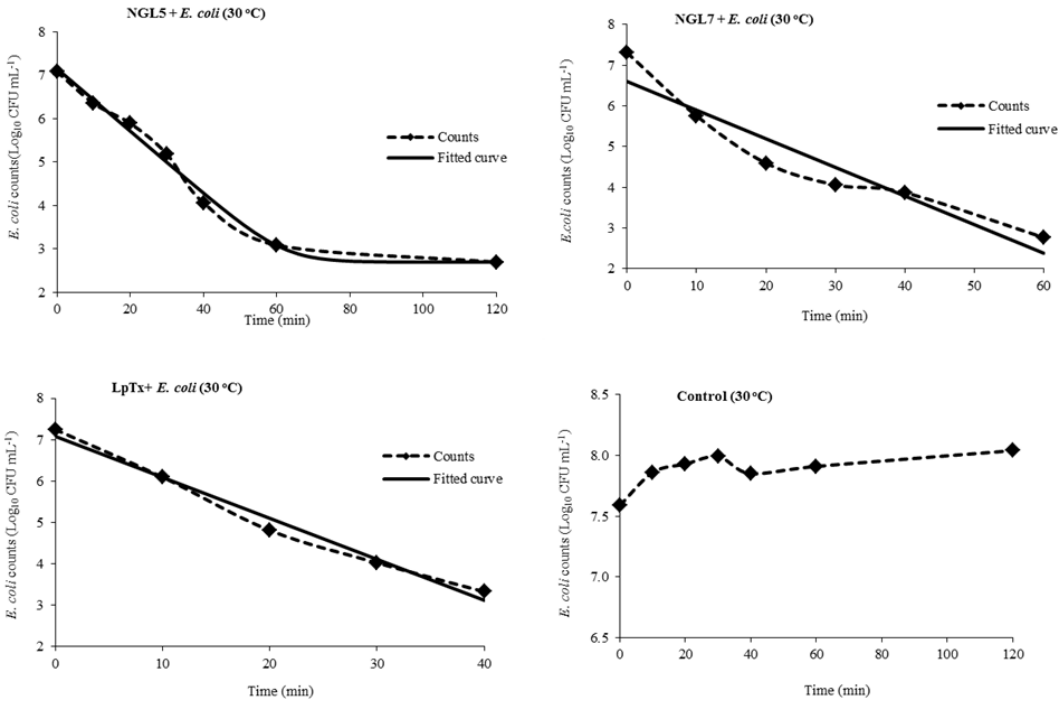

Figure 6. Viable counts with the fitted sigmoid curve for the death of *E. coli* NCTC 11560 at 30°C in the 24 h *L. plantarum* strains (NGL5, NGL7 and LpTx) fermented maize slurries (No significant ($p \leq 0.05$) change in the control to fit a sigmoid curve)

The absence of growth of *E. coli* in the control samples may imply that while the cells in the control sample were adapting for substrate utilization, the adverse condition of low pH and acidity in the test sample made *E. coli* NCTC 11560 to enter into the decelerating phase after inoculation with subsequent inhibitions. Despite the known influence of temperature on acid dissociation and microbial growth, it may be interesting to know whether *E. coli* NCTC 11560 expressed genes that enabled its survival in the acid condition at 22°C.

The *E. coli* NCTC 11560 counts in the porridges are shown in Table 2. Although the *L. plantarum* strains used in the fermentation of the ground maize slurries may have been killed by heating during the porridge preparation, the inoculated pathogen was unable to survive in the porridges having the same range of pH as their fermented slurries. *E. coli* counts decreased below detection limit after 20 mins. This finding is different from the report by [25] where *E. coli* survived for hours in weaning porridges fermented using inoculum recycling.

4. Conclusion

This study has shown that the *L. plantarum* strains (NGL5, NGL7 and LpTx) were able to ferment the ground maize slurries with significant acid production and low pH displaying inhibitory activity against *E. coli* NCTC 11560. The inhibition of *E. coli* NCTC 11560 was concomitant with the decrease in pH and increase in TA and *L. plantarum* counts. The fermentation temperature also showed significant influence on the survival of *E. coli* NCTC 11560 with death rates significantly ($p \leq 0.05$) greater at 30°C. It could be suggested that the low pH and high acidity of *akamu* fermentation with *L. plantarum* strains at temperatures higher than or equal to 30°C would assure product safety.

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