

## Adhesion Abilities of *Lactobacillus Plantarum* Strains Isolated from Nigerian Fermented Maize Food - *Akamu*

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### Abstract

Two strains of *Lactobacillus plantarum* isolated from *akamu* a Nigerian fermented maize food were investigated for probiotic potential based on: adhesion to hydrocarbons (hydrophobicity), porcine mucin and epithelial cell models. Gelatinase and haemolytic activities of the *L. plantarum* isolates were also studied. Adhesions to mono polar solvents (>22%) were significantly ( $p \leq 0.05$ ) higher than the *n*-alkanes (<13%) with significant maximal affinity (35%) for chloroform an acidic solvent. The general order of affinity was chloroform > ethyl acetate > hexadecane > hexane. NGL7 had significantly ( $p \leq 0.05$ ) the highest affinity for all the solvents. Both *L. plantarum* strains had significant adhesions to porcine mucin ( $\geq 6.51 \text{ Log}_{10} \text{ CFU mL}^{-1}$ ) after 2 h at 37°C. Viable counts on Caco-2 cells were 5.13 and 5.53  $\text{Log}_{10} \text{ CFU mL}^{-1}$  for NGL7 and NGL5 respectively. The *L. plantarum* strains possessed significant adhesion abilities: adhesion to hydrocarbons, porcine mucin and Caco-2 cells with no gelatinase and haemolytic activity. This suggested that the *L. plantarum* strains isolated from the Nigerian fermented maize food -*akamu* would be able to adhere to the intestinal mucosa and epithelial cells for beneficial health effects without posing any risk.

**Keywords:** *Lactobacillus plantarum*, hydrophobicity, adhesion, Caco-2 cells, gelatinasis; haemolysis

### 1. Introduction

Adhesion of probiotic bacteria to the intestinal mucosal surfaces and epithelial cells and cell lines are among the main selection criteria for probiotic microorganisms (FAO/WHO, 2002). Adhesion ability of probiotic bacteria offers competitive advantage important for bacterial persistence in the gut (Vinderola & Reinheimer, 2003). Bacterial cell surface hydrophobicity is an important factor that governs adhesion of bacteria to various surfaces (Liu et al., 2004; Zita & Hermansson, 1997). It represents an attractive force between cells and/or inert surfaces principally due to the role of electron-donor/electron-acceptor interactions between the two surfaces (Bellon-Fontaine, Rault, & van Oss, 1996). Bacterial cell surface hydrophobicity has often been assessed by adhesion to solvents or hydrocarbons (Del Re, Sgorbati, Miglioli, & Palenzona, 2000; Kos et al., 2003; Mathara et al., 2008; Schillinger, Guigas, & Heinrich Holzappel, 2005; Vinderola & Reinheimer, 2003), based on the comparison between bacterial cell affinity to a monopolar and a polar solvent. The monopolar solvent could be acidic (electron acceptor) or basic (electron donor) but must have similar van der Waals properties (Bellon-Fontaine et al., 1996). High cell surface hydrophobicity has been correlated to increased cell-to-cell adhesion (Del Re et al., 2000; Kos et al., 2003; Schillinger et al., 2005) and suggested to play significant role in interaction with organic mucin layer of the gut (Mathara et al., 2008) and adhesion to epithelial cells (Schillinger et al., 2005).

The epithelial cells of the human digestive tract is overlaid with mucus; a biochemically complex layer containing glycoproteins, antimicrobial peptides, immunoglobulins, lipids and electrolytes (Juge, 2012). The main component being high molecular weight surface active glycoproteins called mucins (Jonsson, Ström, & Roos, 2001; Svensson & Arnebrant, 2010). The gastrointestinal mucus is divided into two layers; an outer loose easily removable layer and an inner layer firmly attached to the epithelium (Juge, 2012). The outer mucus layer harbours glycan-rich domains that provide preferential binding sites and energy for bacterial proliferation while the inner mucus layer protects the underlying intestinal epithelia layer from intestinal contents such as corrosive

acid and pepsin of gastric juice and guards the host against infections by pathogens ( Zhou, Gopal, & Gill, 2001; Juge, 2012; Uchida et al., 2006). The outer mucus layer is continually being degraded and new components constantly secreted. For prolonged persistence that allows time for exertion of healthful benefits, bacterial adhesion to the mucus layer need to reach the epithelial cells to prevent quick dislodging and washing away by luminal content (Kirjavainen, Ouwehand, Isolauri, & Salminen, 1998). The assessment of adhesion properties of probiotic bacteria have been achieved through *in-vitro* adherence to mucus and epithelia layers using mucin extracts from human and animals, Caco-2 and HT-29 cells and human intestinal Int-407 (ATCC CCL 6) cells as intestinal models (Baccigalupi et al., 2005; Kalui, Mathara, Kutima, Kiiyukia, & Wongo, 2009; Lin et al., 2011; Montegudo-Mera et al., 2012; Wang et al., 2008).

Just as microbial adhesion to intestinal mucosa and epithelial cells is an important probiotic characteristic; their degradation is considered a potential virulence factor (Apostolou et al., 2001; FAO/WHO, 2002). Microorganisms with extensive mucin-degrading activity would have the potential to invade the host, facilitate mucosal penetration by potential pathogens and cause cessation of the underlying epithelial cell lines. Theoretically, mucinolysis can be defined by changes in the carbohydrate and/or protein content of ethanol-precipitated mucin portions (Zhou, Gopal, & Gill, 2001). The *in-vitro* ability of bacteria to hydrolyse gelatine would imply their ability to derange the protective mucus lining of the GIT, while the ability to breakdown red blood cells would cause cessation of the underlying epithelial layer. Although most studies had been with intestinal and dairy isolates, there is increasing interest not just in the development of food products containing beneficial *Lactobacillus* strains but the characterisation of non-intestinal isolates for possible health-enhancing effects.

*Akamu* is a lactic acid fermented cereal-based food that constitutes a major infant complementary food, serves as a component of adult main meals and also an important food for convalescence in most Africa countries. *Lactobacillus plantarum* is one of the main Lactic acid bacteria associated with spontaneous fermentation of *akamu* (Obinna-Echem, Kuri, & Beal, 2014). To be able to develop *akamu* for more than its nutritional value to that of health enhancing benefits would require the characterization of the fermenting microorganism for probiotic potentials. This study was therefore aimed at the characterisation of two strains of *L. plantarum* isolated from *akamu* a Nigerian fermented maize food for possible probiotic potential based on: adhesion to hydrocarbons (hydrophobicity), adherence to mucin and epithelial cell models and, gelatinase and haemolytic activities.

## 2. Method

### 2.1 Microorganism and Inoculum Preparation

The two *L. plantarum* strains: NGL5 and NGL7 characterised in this study were previously isolated from *akamu* a Nigerian traditionally fermented maize food and identified using both conventional and molecular methods by (Obinna-Echem et al., 2014). The commercial probiotic strain (LpTx) was isolated from a probiotic food supplement obtained from Health Food Shop, Rickard Lanes', Plymouth City Centre, UK, using the same method described by Obinna-Echem et al., (2014). *L. reuteri* NCIB 11951 was obtained from stock culture in the Microbiology laboratory of Plymouth University.

The lactic acid bacteria (LAB) were cultivated on de Man, Rogosa and Sharpe (MRS) agar with incubated at 37°C for 24 h. MRS broth was used for the LAB broth cultures. A distinct colony from the agar plate culture was inoculated into 10 mL of broth 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  $\times$  g for 10 min and washed twice in phosphate buffered saline (PBS) (pH 7.3 $\pm$ 0.2) and re-suspended in PBS such that 1 mL of inoculum produced 9 Log<sub>10</sub> CFU/mL. The media and the diluent used were obtained from Oxoid Limited (Basingstoke, Hampshire, UK) (Obinna-Echem et al., 2014).

### 2.2 Hydrophobicity

Hydrophobicity was demonstrated as described Bellon-Fontaine *et al.*, (1996) with some modifications. The pairs of solvents used were Chloroform and hexadecane, and ethyl acetate and hexane. Equal volume (2 mL) of bacterial suspensions and the individual solvents were mixed by vortexing for 120 s. After 20 min of incubation at 22 - 24 °C for complete separation of the two phases, the absorbance of the aqueous phase (1 mL) at 600 nm was measured and the decrease in the absorbance of the aqueous phase was taken as a measure of cell surface hydrophobicity, expressed as follows:

$$(1 - A/A_0) \times 100 \quad (1)$$

where A and A<sub>0</sub> were absorbance values before and after addition of solvents.

### 2.3 Adhesion to Porcin Mucin

Adhesion to mucin was determined using the method of Roos, Karner, Axelsson, & Jonsson (2000) and Jonsson *et al.* (2001) with some modifications. Briefly, 100 mL of mucin (porcine stomach Type II, Sigma-Aldrich, Gillingham, Dorset, England) solution at a concentration of 100 µg mL<sup>-1</sup> in 50 mmol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> in Maxi Sorp surface Nunc-Immunomicrotitre wells (Life Technology, Paisely, UK) were incubated at 4°C overnight. The wells were blocked with phosphate buffered saline supplemented with 0.05% Tween 20 (PBST) at room temperature (22±2°C) for 1 h and thereafter washed with the same buffer.

The bacteria were grown in MRS broth at 37°C for 16 h, washed once with PBST and re-suspended in the same buffer to an optical density of 0.5 at 600 nm. In one experiment, 100 µL of the bacterial cell suspensions for each test organism were added into the wells of one of the plates for absorbance measurement and to another set of plates for microbial enumeration. The plates were incubated on an orbital platform shaker (IKA vibrax-VXR S17, Staufen, Germany) at 40 rpm for 1 h at 37°C. Thereafter, the content of the wells were aspirated and the wells were washed thrice with PBST to remove unbound bacterial cells. The plates for absorbance measurement were left to dry in the incubator at 30°C for 30 minutes and the absorbance values (OD<sub>403 nm</sub>) were measured in a VersaMax ELISA microplate reader (Molecular Devices, Wokingham, Berkshire, UK). Bound bacterial cells for microscopic examination were estimated under phase contrast inverted microscope (Olympus CK30, Yaung-Guan St., Taipei, Taiwan) at a magnification of x 4000.

Each batch of experiment included control wells (mucin coated wells with PBST only) and *L. reuteri* NCIB 11951 was used as standard (Aleljung *et al.*, 1994). Wells that were not coated with mucin but inoculated with bacterial cell suspension were also examined, to ascertain whether the bacteria could bind to the walls of the wells. Adhesion was expressed as:

$$(OD_{ml} - OD_m) / OD_m \quad (2)$$

Where OD<sub>ml</sub> and OD<sub>m</sub> represented OD of mucin coated wells inoculated with the LAB and the mucin with only the buffer solution. To estimate the number of adhered bacterial cells, the bound cells after washing were lifted with 1 mL of buffer into 9 mL of PBST. 1 mL of appropriate dilutions was plated on MRS agar with incubated at 37 °C for 24 h.

### 2.4 Adhesion to Caco-2 cells

#### 2.4.1 Caco-2 Cell Culture

Caco-2 cells were grown according to the method of Xie, Zhou, & Li, (2012). Briefly, Caco-2 cells were grown at 37 °C in 5% CO<sub>2</sub>-95% air atmosphere (Thermo Scientific Forma Steri-Cult CO<sub>2</sub> incubator, Waltham, Massachusetts, USA) in Dulbecco modified Eagle's minimal essential medium (DMEM) containing L-glutamine (2 mmol L<sup>-1</sup>), supplemented with 10% (v/v) fetal calf serum, 1% (v/v) non-essential amino acids, 100 U mL<sup>-1</sup> penicillin and 0.1 mg mL<sup>-1</sup> streptomycin. The Caco-2 monolayers were prepared by seeding in 500 µL of cells (c. 5 Log<sub>10</sub> cells mL<sup>-1</sup>) into six-well tissue culture plates (Fisher Scientific, Loughborough, UK) that were incubated for 21 days, with daily changing of the cultured medium to replenish nutrients and maintain the correct pH.

#### 2.4.2 Caco-2 Adhesion Assay

Adhesion assay was performed following the method of Maragkoudakis *et al.* (2006). The growth medium in six-well tissue culture plates of Caco-2 monolayers was aspirated and the cells washed twice with PBS (pH 6.8). Subsequently, 100 µL of bacterial cell suspension OD<sub>600 nm</sub> (c. 8 Log<sub>10</sub> CFU mL<sup>-1</sup>) was added to the Caco-2 monolayers. After 1 h of incubation at 37°C in 5% CO<sub>2</sub>, the bacterial cells were aspirated and the monolayers washed 6 times with PBS to release unbound bacteria. Adhered bacterial cells for viable count were lifted with 1 mL of 1% Triton X-100 (BDH, England) into 9 mL of PBS and enumerated.

### 2.5 Gelatinase and Haemolytic Activities

Gelatinase activity of the *L. plantarum* strains was investigated using the method of Harrigan, (1998). Bacterial cell suspension (5 µL) were spotted on Nutrient agar (Oxoid, Basingstoke, England) supplemented with 0.4% gelatine (Merck, Germany) and incubated at 37°C for 2 - 7 days in anaerobic jars with anaerobic gas jackets (AnaeroGen, Oxoid, Basingstoke, England). Thereafter the plates were flooded with 8 mL of saturated NH<sub>3</sub>SO<sub>4</sub> and observed for clear zones as positive gelatinase activity. For haemolytic activity, a colony from 18 h plate culture of the *L. plantarum* strains, *E. coli* NCTC 11560 and *S. Enteritidis* NCTC 5188 was streak plated on blood agar composed of Columbia agar base (Oxoid, Basingstoke, England) supplemented with 5% defibrinated

sheep blood (TCS Biosciences, Buckingham, UK). The plates were incubated anaerobically at 37°C for 48 h. The pathogens served as control.

### 2.6 Statistical Analysis

Data obtained were statistically analysed using Minitab (Release 16.0) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences and relationship among variables were evaluated by analysis of variance (ANOVA) under general linear model and Tukey pairwise comparisons at 95% confidence level. Relationship between the variables utilised multiple regressions and correlations (Zar, 1999). Interpretations of the correlation results were made using  $\alpha$ -level of 0.01, in which p-values less than or equal to 0.01 signified that the correlation was not zero.

## 3. Results and Discussion

### 3.1 Hydrophobicity

Table 1. Adhesion (%) of *Lactobacillus plantarum* strains (NGL5, NGL7 & LpTx) to solvents

<i>L. plantarum</i>	Solvents				*Mean
	Chloroform	Hexadecane	Ethyl acetate	Hexane	
NGL5	18.09±0.34 <sup>c</sup>	5.86±0.43 <sup>h</sup>	9.71±0.90 <sup>g</sup>	5.59±0.19 <sup>h</sup>	9.8
NGL7	63.58±0.57 <sup>a</sup>	25.75±0.61 <sup>c</sup>	32.76±0.89 <sup>b</sup>	14.78±0.02 <sup>f</sup>	34.2
LpTx	23.48±0.30 <sup>d</sup>	6.47±0.48 <sup>h</sup>	23.88±0.81 <sup>d</sup>	10.07±0.46 <sup>g</sup>	16.0
<b>*Mean</b>	35.1	12.7	22.1	10.1	

Values with same superscript do not differ significantly ( $p \leq 0.05$ ). N=3±SD

NGL5 and NGL7 – *L. plantarum* strains isolated from *akamu*-a Nigerian fermented maize food

LPTX - Commercial probiotic strain isolated from a probiotic food supplement

In Table 1, there was significant variation in the ability of the *L. plantarum* strains to adhere to the solvent pairs chloroform/hexadecane and ethyl acetate/hexane. NGL7 had significantly ( $p \leq 0.05$ ) the highest affinity for all the solvents while NGL5 had the least affinity. The values were 63.58 – 18.09, 25.75 – 5.86, 32.76 – 9.71 and 14.78 – 5.59% for chloroform, hexadecane, ethyl acetate and hexane respectively. Adhesions to the mono polar solvents: chloroform (35.1%) and ethyl acetate (22.1%) were significantly ( $p \leq 0.05$ ) higher than the *n*-alkanes: hexadecane (12.7%) and hexane (10.1%) with significant maximal affinity for chloroform an acidic solvent. The general order of display of affinity was chloroform > ethyl acetate > hexadecane > hexane.

Hydrophobic cell surface properties of the *L. plantarum* strains were observed to be that of favourable acid-base interaction with increased affinity to the mono-polar solvents. The cells could be assumed to have more electron donating properties attributable to the presence of carboxylic groups on microbial cell surfaces (Bellon-Fontaine et al., 1996). Differences in the hydrophobicity of the strains further suggested variation in the structure and surface composition of each strain (Pan, Li, & Liu, 2006).

### 3.2 Adhesion to Porcine Mucin and Caco-2 Cells

Table 2. Adhesion of *Lactobacillus plantarum* strains (LpTx, NGL5 and NGL7) to porcine mucin

LAB	*Adhesion relative to Mucin OD <sub>403</sub>			Viable cell count at 37°C after 2 h (Log <sub>10</sub> CFU mL <sup>-1</sup> )	
	37°C (2 h)	37°C (20 h)	4°C (20 h)	Initial cells	Adhered cells
NGL5	0.72±0.03 <sup>b</sup>	1.07±0.22 <sup>a</sup>	0.11±0.13 <sup>b</sup>	8.43±0.07 <sup>a</sup>	6.52±0.02 <sup>a</sup>
NGL7	0.62±0.04 <sup>b</sup>	0.80±0.07 <sup>a</sup>	0.32±0.27 <sup>b</sup>	8.22±0.02 <sup>bc</sup>	6.60±0.11 <sup>a</sup>
LpTx	1.45±0.06 <sup>a</sup>	0.43±0.09 <sup>b</sup>	0.06±0.09 <sup>b</sup>	8.15±0.01 <sup>c</sup>	6.51±0.11 <sup>a</sup>
<i>L. reuteri</i> NCIB 11951	0.72±0.04 <sup>b</sup>	0.86±0.04 <sup>a</sup>	1.40±0.39 <sup>a</sup>	8.25±0.02 <sup>b</sup>	6.61±0.10 <sup>a</sup>

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

\*Adhesion =  $(OD_{mL} - OD_m) / OD_m$ . N=3 ±SD.

NGL5 and NGL7 – *L. plantarum* strains isolated from *akamu*-a Nigerian fermented maize food

LPTX - Commercial probiotic strain isolated from a probiotic food supplement

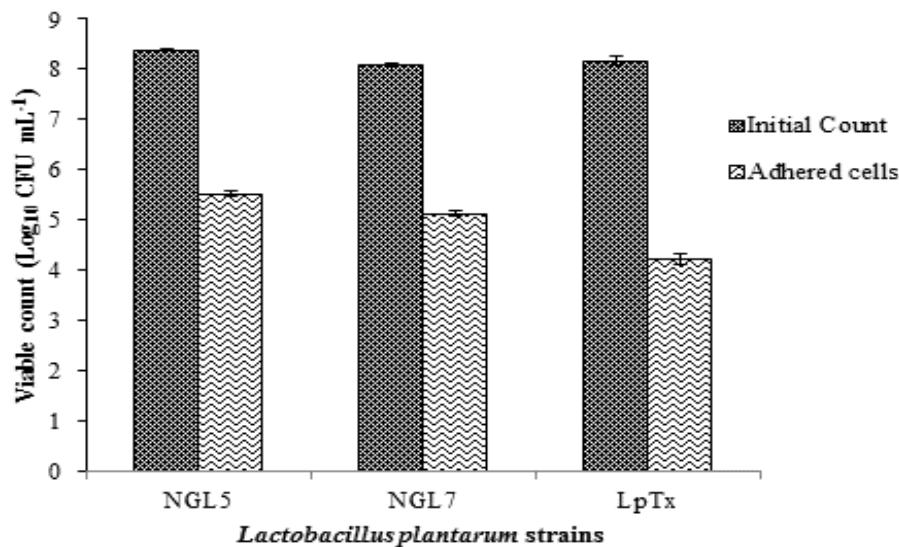


Figure 1. Adhesion of *L. plantarum* strains (LpTx, NGL5 and NGL7) to Caco-2 cells

NGL5 and NGL7 – *L. plantarum* strains isolated from akamu-a Nigerian fermented maize

LPTX - Commercial probiotic strain isolated from a probiotic food supplement

Table 2 presents the adhesion of the *L. plantarum* strains to porcine mucin. Adhesion of the *L. plantarum* strains was significantly ( $p \leq 0.05$ ) higher at 37°C ( $\geq 0.43$ ) than at 4°C ( $\leq 0.32$ ). The results obtained using the optical density method revealed that the adhesion for the test organisms at 37°C did not differ significantly ( $p \leq 0.05$ ) from that of the standard *L. reuteri* NCIB 11951 (0.72 after 2h and 0.86 after 20 h). The probiotic strain at 37°C had significantly ( $p \leq 0.05$ ) the highest adhesion after 2 h and significantly ( $p \leq 0.05$ ) the lowest after 20 h. Adhesion after 2 h at 37°C was taken as a reference condition and the adhered cells ranged between 6.51 - 6.61 Log<sub>10</sub> CFU mL<sup>-1</sup> for LpTx and *L. reuteri* NCIB 11951 respectively. There was no significant difference in the adhered cells for all the organisms. In Figure 1 the adherent viable counts on the Caco-2 cells ranged between 4.22 and 5.53 Log<sub>10</sub> CFU mL<sup>-1</sup> for LpTx and NGL5 respectively.

The *L. plantarum* strains were able to adhere to porcine mucin ( $\geq 0.62$  from the optical density assay and  $\geq 6.51$  Log<sub>10</sub> CFU mL<sup>-1</sup> for viable cell count). Some studies have revealed that effective binding of *L. plantarum* strains are mediated by the secretion of extracellular protein (Hevia et al., 2013; Sánchez, González-Tejedo, Ruas-Madiedo, Urdaci, & Margolles, 2011). It could be suggested that the *L. plantarum* strains in this present study may possess proteins with adherence capabilities in addition to the binding receptors of the mucin. The isolated *L. plantarum* strains were characterised with the ability to also adhere to Caco-2 cell linings. The adhesion of the *L. plantarum* strains to the epithelial cell culture (Caco-2) based on the enumeration of the bacterial colonies in this study indicated that 4 - 6 Log<sub>10</sub> CFU mL<sup>-1</sup> were adherent to the Caco-2 cells. This was higher than values reported in similar analysis for *L. plantarum* ACA-DC 112 (Maragkoudakis, Miaris, et al., 2006) and some other *Lactobacillus* spp. (Monteagudo-Mera et al., 2012). The adhesion of the isolated endogenous *L. plantarum* strains (NGL5 and NGL7) to Caco-2 cells was significantly ( $p \leq 0.05$ ) higher than the probiotic strain (LpTx). The type of strain and not its origin may be an influencing factor to adherent properties. Considering the adhesion potential of the isolated Nigerian akamu *L. plantarum* strains (NGL5 and NGL7) in comparison with the commercial probiotic strain (LpTx), it could be suggested that the *L. plantarum* strains (NGL5 and NGL7) would be able to utilize the binding sites and possibly proliferate and prevent colonization by pathogens in the GIT. There was an observed correlation ( $p \leq 0.01$ ) in Table 3 between hydrophobicity and adhesion to porcine mucin especially with Ethyl acetate ( $r = 0.88$ ). Increase in hydrophobicity would imply more ability to adhere to mucin. This confirmed the report that cell surface hydrophobicity offers that competitive advantage important for bacterial persistence in the gut (Vinderola & Reinheimer, 2003).

Table 3. Correlation between hydrophobicity, adhesion to mucin and Caco2 cells

		Adhesion		Hydrophobicity		
		Caco2	Mucin <sup>a</sup>	Hexadecane	Chloroform	Ethyl acetate
Adhesion	Mucin <sup>a</sup>	-0.42 (0.26)				
Hydrophobicity	Hexadecane	0.32 (0.41)	0.50 (0.17)			
	Chloroform	0.24 (0.54)	0.57 (0.11)	1.00 (0.00)*		
	Ethyl acetate	-0.30 (0.44)	0.80 (0.01)*	0.81 (0.01)*	0.85 (0.00)*	
	Hexane	-0.16 (0.68)	0.74 (0.02)	0.88 (0.00)*	0.92 (0.00)*	0.99 (0.00)*

a – Values used are the adhered viable cells

\*  $p < 0.05$  signified correlation between variables.

### 3.3 Gelatinase and Haemolytic Activity

The *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) were unable to hydrolyse gelatine. The haemolytic activities of the *L. plantarum* strains and the pathogens were shown in Figure 2. The strains of *L. plantarum* exhibited no haemolytic activity ( $\gamma$ -haemolysis), while greenish coloration ( $\alpha$ -haemolysis) was observed for *S. Enteritidis* NCTC 5188. *E. coli* NCTC 11560 had prolific growth with clear zones around its colonies ( $\beta$ -haemolysis).

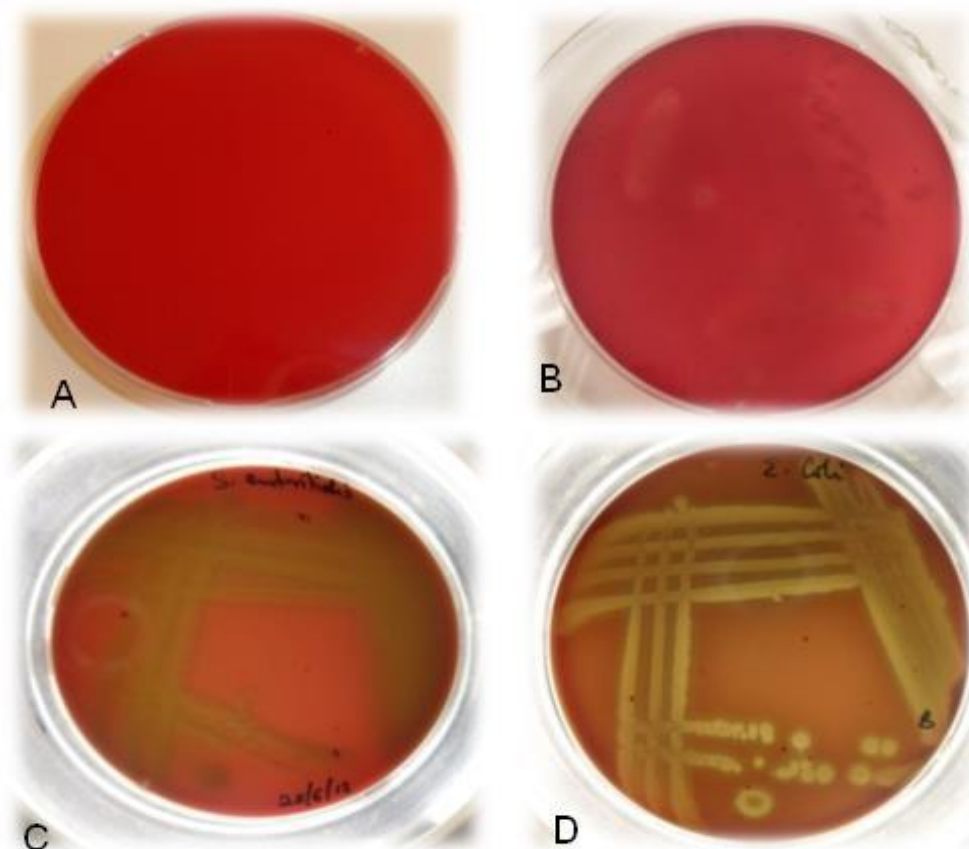


Figure 2. Demonstration of haemolytic activity: (A) Fresh blood agar, (B)  $\gamma$ -haemolysis of *Lactobacillus plantarum* strain, (C)  $\alpha$ -haemolysis of *Salmonella enteritidis* NCTC 5188 and (D)  $\beta$ -haemolysis of *Escherichia coli* NCTC 11560

In line with the FAO/WHO, (2002) recommended safety attributes, the *L. plantarum* strains did not exhibit gelatinase and haemolytic activity. This was in agreement with the report on non-haemolytic and non-gelatinase activities of strains of *L. plantarum* isolated from Kenyan fermented maize porridges (Kalui et al., 2009) and the non-haemolytic activity of other *Lactobacillus* species reported by Maragkoudakis et al., (2006) and Zhang, Li, & Li, (2012). This implies that the *L. plantarum* strains would neither derange mucus protective lining of the GIT nor cause cessation of the underlying epithelial layer. Other safety attributes that could further be investigated are resistance to antibiotics and the possession of transferable resistant genes.

The *L. plantarum* strains exhibited significant adhesion to hydrocarbons, porcine mucin and Caco-2 cells. With respect to safety issues, there was no gelatinase and haemolytic activity observed. This suggested that the *L. plantarum* strains isolated from the Nigerian fermented maize food -*akamu* would be able to adhere to the intestinal mucosa and epithelial cells for beneficial health effects without posing any risk.

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