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ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM FERMENTED *NONO*

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Abstract

Lactic acid bacteria refers to a large group of beneficial bacteria that have similar properties and all produces lactic acid as an end product of the fermentation process. A total of 128 LAB were isolated and 5 different species were identified using modern biochemical tools. These isolates were screened to determine their probiotic qualities as well as their capacities as yoghurt starter culture. Their acidification properties were determined using pH meter and titratable acidity. There was a significantly ($p<0.05$) high acidification rates by *L. acidophilus, L. bulgaricus* and *L. helveticus* when compared with the control and other isolates. L. *acidophilus* produced lactic acid ($2.74\pm0.22\%$) that was significantly higher ($p<0.05$) than other isolates after 36 h. A positively strong correlation (R-value = +0.833) was observed between acid production and change in pH of all the isolates. However, there was a significantly

Introduction

Fermented milk is the most common product from which other food products are derived (Akabanda *et al.*, 2014). Starter culture organisms used in fermentation belong to a group of organisms called Lactic acid bacteria (LAB). LAB has been used to ferment and culture foods for over 4000 years (Carr *et al.,* 2002). They are used in particular in fermented milk products from all over the world, including yoghurt, cheese, butter, buttermilk, kefir and koumiss (Jay, 2000). Lactic acid bacteria refer to a large group of beneficial bacteria that have similar properties and all produce lactic acid as an end

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($p<0.05$) high concentration of lactic acid (27.4±0.07% and (2.63±0.08%) and a corresponding change in pH (2.95±0.02 and 2.68±0.08) by *L. acidophilus* and *L. bulgaricus* respectively compared to other isolates. The fast- acidifying strains of LAB are good candidates for yoghurt starter cultures. The isolates from this study could be considered for further development through improvement strategies. However, LAB strains with slow acidification rates could be considered as adjuncts in dairy fermentation for some other beneficial qualities they may possess.

Key-words*:* Acid production Fermentation, nono, lactic acid bacteria.

Product of the fermentation process (Ali, 2011). They are widespread in nature and are also found in our digestive systems. Although they are best known for their role in the preparation of fermented dairy products, they are also used for pickling of vegetables, baking, winemaking, curing fish, meats and sausages (Bayas and Gayatri, 2014).

The traditional biotechnology of preservation of food through lactic acid fermentation has been known for thousands of years. Microorganisms had been tested in the 19th century to prevent and cure diseases and it is probably from the work of the Russian Nobel Laureate Elie Metchnikoff in 1908 that the first scientific assessments of probiotics were made. He firsthypothesized that a high concentration of lactobacilli in intestinal flora were important for health and longevity in humans (Ayman and Omer, 2009). Starter cultures, used for making thick sour cream in Copenhagen and Kiel in 1890 for the first time marked the beginning of modern industrial microbiology and food technology. The most commonly used probiotic microorganisms in foods for human consumption are *Lactobacillus* and *Bifidobacterium* species, which have given significant health benefits associated with ingestion of these micro-organisms (Dixit *et al.*, 2013).

The production of yoghurt largely depends on a symbiotic relationship between two bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* where each species of bacterium stimulates the growth of the

other. This interaction results in a shortened fermentation time and a product with different characteristics than one fermented with a single species (Salimen *et al*., 2004). With yoghurt and other fermented milk there are considerable opportunities for exploiting lactic acid bacteria as probiotic cultures. These supplement and help our normal gut bacteria to function more efficiently. The world-wide market for these products continues to increase in response to the demands of an increasingly health-conscious public (Ezeronye, 2004).Lactic acid bacteria are therefore excellent ambassadors for an often maligned microbial world (Ayman and Omer, 2009).

This study aims at isolating and characterizing lactic acid bacteria from fermented *nono*.

MATERIALS AND METHODS

Sample Collection:

A total of 20 *nono* samples were collected directly and aseptically from 10 different settlements within Abuja (FCT) and Minna (Niger State). The samples were labelled and kept in thermos coolers containing ice cubes and transported to National Biotechnology Development Agency (NABDA) laboratory as soon as possible where they were kept at 4°C for further use.

Isolation of Lactic Acid Bacteria

Culture Media and Growth Conditions

Nono samples were analyzed using the dilution pour plate method on MRS and M17 agar using the technique adopted by Ali (2011). One ml of each sample was taken and homogenized in 9ml of peptone water. Serial dilution of up to 10^{-8} dilutions was prepared and 1ml aliquots from 10^{-2}, 10^{-4} and 10^{-6}dilutions were plated in duplicates into the MRS agar (pH 6.2-6.8)(for the isolation and enumeration of lactobacilli) and M17 agar (pH 7.15) (for the isolation and enumeration of streptecocci and lactococci). Cycloheximide at a concentration of 0.01% (v/v) was added into the enumeration agar plate to prevent fungal growth. All plates were incubated for 72 h at 39ºC (this

temperature (39°C) was used in most parts of this research because it yielded the best growth) in microaerophilic conditions using anaerobic gas jar pack system to reduce oxygen level. After the incubation, the plates with colony forming units (CFU) ranging from 30 and 300 as described by Frederick and Luc (2013) were selected for enumeration.

Subculturing and Purification Protocols

After 72 h of incubation, discrete colonies were randomly picked and subsequently transferred to MRS and M17 agar plates accordingly using the streak plating technique(Holzapfel, 2001) and further incubated at 39°C for 24h. Purified colonies were screened and transferred to appropriate maintenance agar slant; For cocci-shaped isolates, M17 agar (pH 6.9) at 42°C was used while for rod-shaped organisms, MRS agar (pH 6.2) at 37°C. (Arokiyamary and Sivakumaar, 2011).

Screening and Identification of Isolates

Phenotypic Identification

The isolates were screened using the routine bacteriological methods which include; observing the cell colonial morphology, carrying out preliminary tests like Gram staining (Sneath and Holt, 2001).

Cell Colonial Morphology. For colony morphologies, the colour, shape, elevation, margin, size, texture and optical property of the colonies were observed and reported. **Gram's staining.** The Gram's staining was performed according to the procedure described by Christian Gram (1884). To carry out this procedure a colony of bacterial culture was smeared and fixed on a clean slide, the slide was flooded with crystal violet solution for up to one minute and then washed off briefly under a running tap. Gram's iodine solution was added and allowed to act for about one minute before washing it off with tap water. Excess water was then blotted off the slide and 95% alcohol was used as a decolorizing agent and allowed for 10 seconds before washing off with tap water and drained. The slide was subsequently flooded with Safranin

solution and allowed to counterstain for 30seconds before washing it off with tap water and drained. The Grams status of the isolates were determined by light microscopy under the oil immersion lens and recorded.

Total Bacterial Count (TBC)

Total Bacteria Count was done to enumerate the total number of viable organism that could grow under microaerophilic condition. This test was conducted by growing organism at appropriate incubation condition (usually 37^{o}C, 48 h). All plates counted were expressed as the number of colony forming units per milliliter (cfu/ml) of *nono* as described by Alexander and Strete (2001).

CFU/ML = Number of colonies (mean) x volume plated reciprocal of dilution factor

Biochemical Identification of Isolates

Catalase Test.

Overnight cultures of isolates grown on appropriate agar plate at 39^{o}C were used for this test. Catalase activity was determined using this procedure. A bacterial colony was pick using sterile wire loop and dropped on a clean slide, a drop of 3% hydrogen peroxide solution is added to the colony on the slide. A positive result was depicted by a rapid evolution of oxygen (within 5-10 sec) as evidenced by bubbling while the absence of bubbles or only a few scattered bubbles was reported as negative as described by Harrigan (1998).

Motility Test.

Isolates from overnight culture grown on appropriate agar plate at 39^{o}C was used for this test as described by Harrigan (1998). This was using the wet mount technique. A loopful of the pure culture was dropped on a clean slide and a cover slip was lowered over the drop at one side to avoid bubbles. The preparation was examined under microscope using 4x, 40x and 100x magnification. Migration away from line of inoculation depicted motility.

Oxidase Test.

Overnight culture of isolates grown on appropriate agar plate at 39°C was also used. A filter paper soaked in oxidase reagent (tetra-methyl-p-phenylenediamine dihydrochloride) and a well pure culture of the test organism was rubbed on the treated filter paper. Organisms were considered oxidase positive when the color changed to blue/purple within 5 to 10 sec and organisms which did not change colour were considered oxidase negative (Alexander and Strete, 2001).

Physiological Identification of Isolates

Each isolate was activated in 5 ml MRS broth for 24 h at 37ºC before use. Physiological identification of isolates was carried out in accordance with the methods described by Sneath and Holt (2001). The characteristics examined were growth at 10°C, 25°C, 37°C, 45°C, salt tolerance at 2%, 4%, 6.5% NaCl concentration and pH values of 3, 4, 6.5. Hydrolysis of arginine was also performed on the isolates as well as testing their ability to produce gas from glucose.

Growth at Different Temperatures

Aliquot of 0.5mlof overnight grown cultures were transferred into the tubes containing 5ml of the test media. This test media contains; per litre, peptone (10g), meat extract (10g), yeast extract (5g), D (-) Glucose (20g), Tween 80 (1ml), L2HP0_4(2g), Sodium acetate (5g), Triammonium citrate (2g), Mg$S0_4$.7$H_2$0 (0.2g), Mn$S0_4$. 4$H_2$0 (0.05g), Bromocresol purple (indicator)(0.04g), Deionized water (1000 ml). All ingredients were dissolved in deionized water and the pH was adjusted to 6.2. The medium was sterilized by autoclaving at 121°C for 15 minutes. After inoculation, they were incubated for 7 days at 10°C, 25°C, 37°C 45°C respectively. Cell growth at any of these temperatures was detected by the change in the color of the cultures, from purple to yellow.

Growth at Different pH

MRS and M17 broths were prepared and sterilized and bromocresol purple was added as an indicator. The pH of the media was adjusted to 3, 4 and 6.5 using hydrochloric acid. Aliquot of 0.5mlof overnight grown cultures were transferred into the tubes containing 5ml of each test medium and incubated for 5days at 39^{o}C. A change in colour of the growth medium from purple to yellow indicates growth of isolates. A change in the color of the basal medium from purple to yellow was considered as growth (Awan and Rahman, 2009).

Growth at Different NaCl Concentrations

Aliquot of 0.5ml of overnight grown cultures were transferred into the tubes containing 5ml modified test media as stated in 3.5.1. Isolates were tested for growth at 2%, 4% or 6.5% NaCl concentrations. They were incubated for 7 days at 39^{o}C. The change in colour from purple to yellow connotes that the cell could tolerate it, hence growth (Dagdemir and Ozdemir, 2008).

Arginine Hydrolysis

Aliquots of 0.5ml overnight cultures was inoculated into 5ml arginine test media and then incubated for 5 days at 37^{o}C. Nessler's reagent was used to detect production of ammonia at the end of incubation. About 0.5ml of culture broth was pipetted into each McCarthney bottles containing the test medium and 0.5ml of Nessler's reagent was added. Orange colour formation at the end of incubation period indicated ammonia production (Sneath and Holt, 2001)

Gas Production From Glucose

Liberation of carbon dioxide from glucose metabolism was performed to define the homo/heterofermentative status of the isolates. In order to carry out this test, a citrate lacking MRS broths and inverted Durham tubes were used. Aliquot of 0.5ml of overnight cultures was transferred into a 5.0ml test media. After incubation for 5 days at 37^{o}C, gas accumulation in Durham tubes

was taken as the evidence for CO_2 liberation from glucose (Yan and Polk, 2006).

Isolation of Lactic Acid Bacteria Based on Their Proteolytic Characteristics.

To determine their proteolytic activities, the selected organisms were subjected to grow in 2% skim milk medium agar and incubated at 37°C for 48 hand the zones of clearance around the colonies were measured (Dagdemir and Ozdemir,2008). Organisms that had areas of clear zone were stated positive while those without clear zone areas were noted and were presumed to have no proteolytic activity.

Sugar Fermentation Spectrum of Isolates

Isolates that yielded favourably to the tests above were selected and examined for their ability to ferment different sugars including monosaccharides and disaccharides such as;fructose, galactose, lactose, arabinose, cellobiose, mannose, melibiose, raffinose, ribose, sucrose, trehalose, maltose, rhamnose, xylose and glucose according the method of Bylund, (1995). This was done by inoculating a 0.1ml of overnight grown culture into tubes containing 5ml MRS and M17 broth containing each of the sugar and bromocresol purple was added as indicator. Durham tube was inserted into each tube and incubated for 48h at 37°C. Colour change from purple to yellow was taken as an evidence for acid production and growth while gas accumulation in the inverted tubes was taken for gas production.

RESULTS AND DISCUSSION

Isolation of LAB Isolates

Characteristics of Microorganisms Isolated From *Nono*

A total of 20 *nono* samples were screened. About 736 distinct colonies were picked and observed for colonial morphological characteristics out of which 128 isolates with LAB characteristics were identified and grouped into 5

based on their similarities (morphological and Gram stain). Table 4.1 shows the characteristics of the microorganisms isolated from the *Nono*.

Table 4.1: Colonial Morphology of Microorganisms Isolated from *Nono*on MRS and M17 AGAR

Isolate	Frequency %	Pigmentation	Form	Elevation	Margin form	Size	texture	Optical prop
TC11	46.1	Cream	Circular	Convex	Entire	Punctiform	Smooth	Opaque
TC18	10.2	Cream	Circular	Convex	Entire	Small	Smooth	Opaque
TC34	17.2	White	Circular	Umbonate	Entire	Small	Smooth	Opaque
TC58	7.0	Cream	Irregular	Raised	Undulate	Small	Smooth	Opaque
TC72	19.5	Cream	Circular	Raised	Entire	Moderate	Smooth	Translucent

Identification of Lactic Acid Bacteria

The morphological characteristics and some biochemical properties of the LAB isolates are represented in Table 4.2. Identification of colonies that were considered LAB according to Ali (2011) was conducted. A total of five (5) representative strains were picked out of the One hundred and twenty eight (128) isolates for further identification. All the isolates were gram positive, catalase negative, oxidase negative and non-motile (as shown in Table 4.2).

Table 4.2: Morphological and some biochemical profile of the isolates.

Isolates	Cell shape	Gram stain	Catalase test	Oxidase test	Motility test
TC11	Rod	+	-	-	-
TC18	Rod	+	-	-	-
TC34	Rod	+	-	-	-
TC58	Cocci	+	-	-	-
TC89	Rod	+	-	-	-

The Proteolytic Characteristics of Isolates.

The proteolytic activities of the isolates are presented in Table 4.3. Most of the isolates tested showed proteolytic activity to varying degree but isolates TC34 had the best proteolytic activity (about 20mm in 12 h) followed by TC72. Isolate TC58 showed the least proteolytic activity.

Table 4.3: Proteolytic activities of the selected LAB.

	Time (h)			
Isolates	12	24	36	48
TC11	+	+	++	++
TC18	+	+	++	++
TC34	+	++	+++	+++
TC58	_	_	+	+
TC72	+	+	++	+++

Key

-= No effect, + =Zone< 10mm, ++ =Zone =10-20mm, +++= Zone>20mm.

Physiological Tests.

The result of physiological tests carried out on the isolates is illustrated in Table4.4. The tests consist of gas production from glucose, arginine hydrolysis, the effects of temperature, pH, salt concentration (NaCl) on the growth of isolated LAB.

Table 4.4: Physiological Attributes of Isolates

	Isolates				
Treatments	TC11	TC18	TC34	TC58	TC72
Gas from glucose	_	_	_	_	+
NH_3 from arginine	_	_	+	_	_
Growth at Different Temperature					
10°C	_	_	_	_	_

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25°C	+	_	_	_	_
37°C	+	+	+	+	+
45°C	_	+	+	+	+
Growth at Different pH					
3.0	+	+	_	_	+
4.0	+	+	+	+	+
6.5	_	_	_	+	_
Salt Tolerance					
2%	+	+	+	+	+
4%	_	+	_	_	+
6.5%	_	_	_	_	+

Key

_ = no growth, + = growth.

Sugar Fermentation Spectra of the Isolates

The result of the isolates' capabilities to ferment sugars are presented in Table 4.5. Isolates TC58 and TC72 demonstrated weak fermentation capacities as they could not ferment majority of the sugars used. Two of the isolates TC11and TC34 showed high fermentation capacities and were able to ferment a larger range of sugars. Based on these fermentation capacity and other biochemical properties the isolates were identified as *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus fermentum, Streptococcus thermophilus* and *Lactobacillus helveticus* respectively.

Table 4.5: Sugar Fermentation Capacities of Isolates.

	Isolates				
Sugars	TC11	TC18	TC34	TC58	TC72
Sugar Fermentation					
Arabinose	+	_	+	_	_
Cellobiose	+	_	+	_	_

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Fructose	+	_	+	_	_
Mannose	+	_	+	_	_
Melibiose	_	_	+	_	_
Raffinose	_	_	+	_	_
Ribose	_	+	+	_	_
Sucrose	+	_	+	+	_
Galactose	_	_	_	_	+
Trehalose	+	+	+	_	_
Maltose	+	+	_	_	_
Lactose	+	+	+	+	+
Rhamnose	_	_	_	_	_
Xylose	+	_	+	_	_
Glucose	+	_	+	+	+
Identified Isolate	*Lactobacillus acidophilus*	*L. bulgaricus*	*L. fermentum*	*Streptococcus thermophilus*	*L. helveticus*

Key − = no growth, + = growth

Antibiotic Susceptibility Tests of Isolates

The result of antibiotic susceptibility pattern of isolates is presented in Table 4.6c. Most isolates were sensitive to most of the antibiotics used in this study. However, *L. fermentum* showed resistance to some of the antibiotics used with no zones of inhibition observed. The zones of inhibition for susceptible isolates ranged from less than 10mm to 22mm.

Table 4.6c Antibiotic Susceptibility Pattern (tests) of Isolates

	Antibiotics									
Isolates	CH	SXT	SP	CPX	AM	AU	CN	PEF	OFX	S
L. bulgaricus	++	+	+	++	++	+	++ +	++ +	++	++ +

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L. fermentum	++	++	+++	+	+++	+	++	+	+++	+
S. thermophilus	+	-	+	-	++	+	+	++	++	+
L. helveticus	++	+	+++	+	+++	+	+	+	++	+
L. bulgaricus	+++	++	++	++	++	++	+++	++	+++	++

KEY

CH	=	Chloranphenecol (30ug)	SXT	=	Septrin (30ug)
SP	=	Sparfloxacin (10ug)	CPX	=	Ciprofloxacin (10ug)
AM	=	Amoxacillin (30ug)	AU	=	Augumentin (30ug)
CN	=	Gentamycin (10ug)	PEF	=	Pefloxacin (10ug)
OFX	=	Tarivid (10ug)	S	=	Streptomycin (30ug)
-	=	No effect,	+	=	Zone< 10mm,
++	=	Zone =10-20mm,	+++	=	Zone>20mm,

Acid Tolerance Test of Isolates

The absorbance of the isolates grown in MRS broth atpH of 3.0, 3.5, 4.0, 4.5 and 5.0 for 8 hours is shown in Table 4.6d. All isolates grew at pH 4.0 as reflected in the absorbance. The culture turbidity value appreciated significantly. Their survival rate was reduced at pH 3.0 with *L. acidophilus* and *L. fermentum* being the most tolerant (0.349 and 0.280 respectively). At pH5.0 *L. helveticus* and*S. thermophilus* grew best. *L. acidophilus* was themost acid tolerant compared to other isolates, as evidenced by the increase in its culture turbidity value relative to the initial value. However, survival was best at pH 4 for *L. acidophilus, L. bulgaricus* and *L. fermentum* and pH 5 for *S.thermophilus* and *L. helveticus.*

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Table 4.6d Growth of Isolates on Different pH values (at 600nm).

	pH values				
Isolate	3.0	3.5	4.0	4.5	5.0
L. acidophilus	0.349	0.396	0.488	0.392	0.138
L. bulgaricus	0.280	0.295	0.394	0.288	0.143
L. fermentum	0.222	0.234	0.321	0.312	0.273
S. thermophilus	0.085	0.241	0.311	0.328	0.354
L. helveticus	0.064	0.232	0.317	0.219	0.322

Acidification Properties

pH Determination

The change in pH values of the fermenting medium by the LAB isolates tested is presented in Table 4.7a. Generally, the rate of acidification varied among the isolates. The acidification properties of the LAB strain tested showed that *L. acidophilus, L. bulgaricus* and *L. helveticus* demonstrated a significantly ($p<0.05$) high acidification rate compared to the control and other isolates from the second through the 14 hour. These LAB strains were able to achieve 0.4 pH change in two hours. *S. thermophilus* exhibited the slowest acidification rate (less than 0.4 change in pH) in 2 hours.

The acidification rate of yoghurt produced by using combined potential starter isolates is shown in Table 4.7b. Some of the combined isolates demonstrated a rapid decrease in pH with time, thereby increasing the acidity of the medium. After 2 hours, the combined isolates TC11+TC18 and TC58+TC72 showed a significantly ($p< 0.05$) high rate of acidification compared to others but did not measure up to the standard of 0.4 in 2hours. However, isolates TC11+TC18+TC58 recorded a consistent value greater than 0.4 in 10hours.

Discussion

Isolation and identification of lactic Acid Bacteria (LAB) found in *nono* was carried out. About 128 LAB strains were identified. Further characterization revealed that majority of the organisms found in *nono* belonged to the genera *Lactobacillus*and *Streptococcus*species.The different *nono* samples assayed showed the varying ratios of different organisms and this is likely to be caused by difference in the environment, the animals and the handlers.Most of the isolates tested demonstrated proteolytic activities to varying degree but *L. fermentum* had the best proteolytic activity (about 20mm in 12h). This was followed by *L. helveticus*. *Streptococcus thermophilus* showed the least proteolytic activity less than 10mm in 48h.This result was in agreement with Akabanda *et al.*(2014) who reported that *Latobacillusfermentum, L. plantarum*and *L. helveticus*isolated from *nono* in Ghana showed good proteolytic activities. Though these results were in contrast with those of Dagdemir and Ozdemir (2000) who noted high proteolytic activity for other strains of LAB isolated from cheese. The contrast in proteolytic activities may be due to the strain associated with the product. The production of high quality fermented dairy products is highly dependent on the proteolytic system of the starter culture. Since these have a direct impact on the organoleptic property (flavour) of the fermented product.

The probiotic properties of LAB strains isolated from various *nono* samples were evaluated. Majority of the LAB strains were able to grow in a broth medium containing 0.3% bile acid. *L. bulgaricus* was the most bile tolerant and was able to keep growing even after 8 hours followed by *L. acidophilus* while *L. helveticus* was most sensitive to bile. These findings are similar to the works of Begley *et al.,* (2005) and Hyronimus *etal.*, (2000) who also identified that most strains of *L. bulgaricus and L. acidophilus* demonstrate a high tolerance for bile. The ability of these organisms to grow well in the presence of bile indicates that such strain if used as an oral probiotic can survive the presence of bile and other gastric juice and acid encountered in the stomach (Bao*et al.,* 2010: Liong and Shah, 2000). The LAB strains isolated were also

able to inhibit the growth of some selected indicator organisms to varying degrees. Similar to these findings, Afolabi *et al.* (2008) and Udhayashree *et al*. (2012) also demonstrated that organisms that produce bacteriocin had the ability to inhibit the growth of other bacteria which included Gram positive and Gram negative bacteria. Akabanda *et al*. (2014) noted that the ability of some LAB strains to inhibit the growth of other organisms is due to their ability to produce substances that are deleterious to the indicator organisms which is dependent on the amount produced or the concentration. The works of Breukink *et al*. (2003) and Hawaz (2014) also shows that most strains of *Lactobacillus helveticus* produce high level of bacteriocin which inhibits the growth of other organisms. These also serve as a competitive advantage to the LAB strain when in consortium during fermentation (Cleveland *et al.,* 2001). Wakil and Osamwonyi (2012) also reported that LAB that produce lactic acid, hydrogen peroxide and diacetyl had a greater ability to inhibit the growth of other organisms.

The antibiotic susceptibility test revealed that most of the LAB strains tested were susceptible to the antibiotic used to varying degrees. The antibiotic susceptibility patterns were recognized by the presence of clear zones on the agar plates used. This study revealed that majority of the strains tested were highly susceptible to Amoxicillin and Taravid, this was also in agreement with the findings of Gamal, *et al.* (2014) who reported that most of the LAB isolated in their studies showed high susceptibility to Amoxicillin and ampicillin. This was also corroborated by the work of Ammor *et al*. (2007). The strain of *L. fermentum* tested was found to be resistant to Septrin and Ciprofloxacin. Such resistance are however not a favourable characteristic for starter culture development, owing to its health implications (Hummel *et al.,* 2014).

The acidification properties of the LAB strains tested are shown in Table 4.7a and 4.7b. Generally, the rate of acidification varied among the isolates tested. The acidification properties of the LAB strain tested were determined and the result shows that *L. acidophilus, L. bulgaricus and L. helveticus* demonstrated a very fast acidification rate compared to other isolates. These LAB strains

were able to achieve 0.4 pH change in two hours. Isolate TC58 exhibited the slowest acidification rate (less than 0.4 change in pH) in 2 hours this could be as a result of the isolates inability thrive in a high acidic medium (Azcarate-Peril *et al.,*2004).Fast acidification is a major preference for development of starter cultures for fermented dairy products. The fast acidifying strains are therefore good candidates for dairy fermentation process as primary starter culture, while poor acidification strains can be used as adjunct cultures depending on other properties (Ayad *et al.,* 2004).

Conclusion

Nono, a traditionally processed dairy product is a repository of lactic acid bacteria (LAB) which could also be harnessed for yoghurt production (Starter cultures). Also this product has been observed to be sources of several pathogenic organisms. The several LAB strains isolated in this work had high proteolytic potentials as well as good acidification activities as was observed throughout the experiment. This study reveals several strains of LAB isolated from *nono*have high resistance to acid as well as being able to produce lactic acid. Rapid decrease in pH which is strain-dependent is essential for coagulation and prevention or reduction of growth of most pathogenic or and spoilage microbes found in dairy products. Therefore fast acidifying strains of LAB are good candidates for yoghurt starter cultures. The isolates from this study could be considered for further development through improvement strategies.

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