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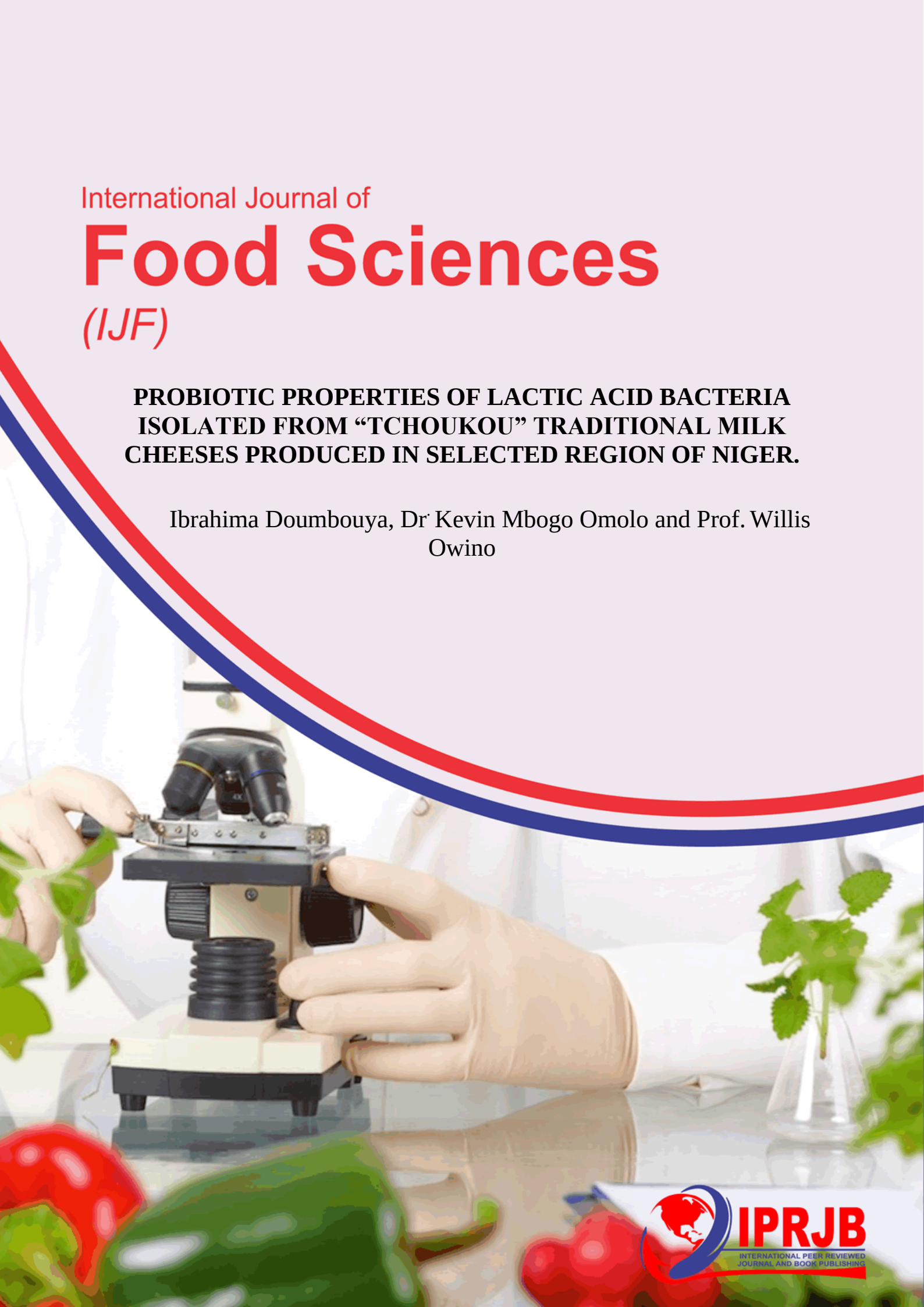

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# PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM "TCHOUKOU" TRADITIONAL MILK CHEESES PRODUCED IN SELECTED REGION OF NIGER.

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## Abstract

**Purpose:** The current study's aim is to evaluate the probiotic potential of lactic acid bacteria strains isolated from traditional "Tchoukou" milk cheeses produced in a selected region of Niger.

**Methodology:** Nine Samples were collected in selected regions of Niger (Tahoua, Maradi, and Zinder).Probiotic properties of isolated LAB were identified based on their acid tolerance, bile salt tolerance, auto-aggregation ability, simulated stomach and duodenum passage, simulated gastric juice survivability and their antimicrobial activities.

**Findings:** A total of eighteen strains were analysed *in vitro* for acid tolerance, bile tolerance, survival under simulated gastro-intestinal tract conditions and antimicrobial activity against index organisms. The results indicated that all seventeen strains exhibited a high viability after twenty-four hours of incubation at pH 2.5 and pH 3, but a decreased viability at pH 2.0 in which only eight strains were able to survive, strain C13 failed to grow at the three different pH. In this study, the isolates generally survived better after exposure to 0.3% bile salt. Also were able to survive exposure to simulated stomach and duodenum passage (SSDP) for three hours ranging from (89%-100%).All strains were able to survive under simulated gastric juice at different pH (2, 2.5 and 3). for auto-aggregation Only fifteen isolates showed the best auto-aggregation abilities ranging from (15-83%) and the other two had less auto-aggregation ability (2-11%). The isolates showed diverse antimicrobial activity against the index organisms. Among the isolates, only three (C1, C2 and C9) could not inhibit any of the selected pathogens.

**Unique contribution to theory, practice and policy:** This study was conducted to characterize the probiotic properties of LAB isolate which could serve as a potential source for industries and commercial applications.

**Keywords:** *Tchoukou, Probiotics, Lactic acid bacteria, Antimicrobial*

## INTRODUCTION

Lactic acid bacteria (LAB) are an important microbial group in the food sector. They have been consumed in dairy products for a long time by people all over the world and most of them are considered as "generally recognized as safe" (GRAS) microorganisms since they are non-pathogenic, suitable for technological and industrial processes, resistant to acids and bile and able to produce antimicrobial substances(Reuben *et al.*, 2020). Over the past decade, LAB has gained significant interest and is used widely as a probiotic (Reuben *et al*., 2020).Probiotics are an upcoming area for food companies, mainly in the dairy industry, with considerable potential for growth(Yadav *et al*., 2016).Probiotics are viable microorganisms that provide health effects when consumed. The minimum recommended amount of probiotic bacteria is to be at least $10^6$ cfu/g in the food product being fermented at the time of ingestion to provide their health benefits(Mahmoud *et al.,* 2020). The growing acceptance of probiotic use has been due to their diverse effects, which include antimicrobial activities, anti-cancer, anti-oxidant and anti-allergic effects, lowering of blood lipids, and enhanced immune response, all of which depend on the strain.(Lee *et al.*, 2014).

Probiotics have now many uses in human and animal health, especially in the management and prevention of diseases (Anandharaj *et al,* 2014). In the present day, probiotics are not only used as an enhancer of growth but also as a booster of the immune system and prevention of many illnesses (Del Piano *et al.*,2004). Probiotic food products due to their nutritive value and health sector in earlier times along with the therapeutic benefits are considered, and they have become the subject of extensive studies and commercial development(Smid *et al.*, 2005).

Probiotics release a number of compounds, namely exopolysaccharides, organic acids and bacteriocins. The bacteriocins produced by probiotics have been demonstrated to antagonize pathogens (Smid *et al.*, 2005).There is evidence of their role in the treatment of ulcerative colitis, necrotizing enterocolitis, pouchitis, severe infectious diarrhea, antibiotic-associated diarrhea, irritable bowel syndrome and Crohn's disease (Chong *et al*.,2014). An effective probiotic has to be viable, safe, tolerant to bile and gastric juices, capable of surviving in the gastrointestinal tract, and able to colonize and attach to epithelial cells in the gut (Lee *et al*.,2021). Phenol sensitivity is also an important selection characteristic, as gut bacteria can disrupt diet-derived amino acids, resulting in the generation of phenolic compounds (Lee *et al.*, 2014). While researchers in other countries have characterized LAB probiotic strains from a variety of dairy products and food or animal sources. Disease breakouts are becoming increasingly recognized as a critical challenge to agriculture, human health, and economic development in many countries. Intestinal infections due to *Escherichia coli*, *Campylobacter fetus subsp. jejuni*, *Clostridium perfringens* and *C. botulinum* are common, however, antibiotics used to manage these diseases may induce and propagate antimicrobial resistant bacteria and resistance genes, (Aly *et al.,* 2009). In addition, there is increasing interest in the use and, particularly, the overuse of antimicrobial drugs in human medicine, which may pose risks related to the spread of cross-resistance to antimicrobials in human medicine, (FAO/WHO/OIE 2006).

Artisanal “tchoukou” cheese has important organoleptic features that make it popular in Niger though limited data exist on the composition of this cheese microbiota. This study was build up on the study carried out by Mahamadou Rabiou *et.al.* (2021) who characterized

lactic acid bacteria isolated from Tchoukou cheese sampled from three geographical locations in Southern Niger. Despite the growing interest in probiotics, there is not much scientific information on the functional properties of LAB isolated from traditional cheese (tchoukou), therefore, extensive studies are needed to assess probiotic properties of these LAB isolates for their possible application as probiotics in fermented products, our objective is to characterize the probiotic properties of lactic acid bacteria isolated from traditional cow's milk cheese (tchoukou) from a selected region of Niger and to profile the LAB bacteriocin gene.

## MATERIALS AND METHODS

### Sample collection

Nine (9) samples of cow's milk cheese were collected from three different regions of Niger (Tahoua, Maradi, and Zinder). In each location, three (3) samples were collected. The samples were aseptically packaged in ZIP lock bags and then coated in aluminium foil after drying. This form of packaging allowed the collected samples to be transported safely and in good shape for the intended purpose. LAB were isolated from the samples and characterized using phenotypic and biochemical characteristics as well as genotypic methods to determine the taxonomic placement of LAB isolates (Mamadou *et al.*, 2021),

### In vitro determination of functional probiotic properties of the isolated LAB strains

### Tolerance to low pH

The low pH tolerance was assessed according to Mulaw *et al (*,2019), with modifications. Isolates were grown individually in MRS broth overnight at 37°C under anaerobic conditions. 1ml of the overnight isolate culture was inoculated into sterile MRS broth which was adjusted to pH values of 2.0, 2.5, and 3.0 using 1N HCI. At 37°C, each test tube was incubated for 24 hours. After an appropriate incubation period, 1 ml of the culture was diluted in 9 ml sterile ringer solution, prepared according to the manufacturer's instructions, and plated on MRS agar medium. Each inoculated plate was incubated at 37°C for 24 hours under anaerobic conditions. Each cultured colony was reported in colony forming units per milliliter (CFU/ml). A positive control consisting of regular MRS broth inoculated with the isolates was used (Grosu-Tudor *et al.*,2012). The survival rate was calculated as the percentage of LAB colonies grown on MRS agar relative to the initial bacterial concentration:

$$survival\ rate\ (\%) = \frac{logCFUN1}{logCFUN0} \times 100$$

Where $N_1$ is the viable count of isolates after incubation and $N_0$ is the initial viable count(Mulaw *et al.*, 2019).

### Bile salt tolerance

To assess the bile tolerance of acid-tolerant LAB (those grown only at pH 2.0, 2.5, and/or 3.0), the isolates were grown overnight in MRS broth sufficient cell suspension to give $10^6$ CFU/mL concentration of each isolate was added into 10 mL of sterile MRS broth containing 0.3% of bile salts (Oxoid, UK). The broths were incubated for 24 hours and cell viability was determined by serial dilution and plating onto MRS agar after 24, and 48hours incubation and the number of LAB was estimated using colony forming units per milliliter (CFU/ml).

The rate of survival was calculated as the percentage of LAB colonies grown on MRS agar relative to the initial bacterial concentration:

$$survival\ rate\ (\%) = \frac{logCFUN1}{logCFUN0} \times 100$$

Where $N_1$ is the viable count of isolates after incubation and $N_0$ is the initial viable count(Jose *et al.*, 2015).

**Simulated gastric juice survivability test**

The simulated gastric juice was prepared according to (Corcoran *et al.*, 2005), with modifications. Simulated gastric juice was formulated using pepsin (3g/l), and 1ml of each isolate was re-suspended in 5ml of simulated gastric juice at pH 2, pH 2.5 and pH 3. This was followed by incubation at 37°C for 90 min with constant shaking at different time intervals (0, 30, 60 and 90 min), after incubation the samples was taken and serially diluted, then plated on MRS agar plates and incubated at $37^0$C for 24 hours. The rate of survival was calculated as the percentage of LAB colonies grown on MRS agar relative to the initial bacterial concentration:

$$survival\ rate\ (\%) = \frac{logCFUN1}{logCFUN0} \times 100$$

Where $N_1$ is the viable count of isolates after incubation and $N_0$ is the initial viable count(Corcoran *et al.*, 2005).

**Response to simulated stomach duodenum passage**

Response to simulated stomach duodenum passage was assess according to (Mathara *et al.*, 2008), with modifications. Synthetic duodenum juice was prepared by completely dissolving $NaHCO_3$ (6.4 g $L^{-1}$), KCl (0.239 g $L^{-1}$), and NaCl (1.28 g $L^{-1}$) in distillate water. The pH was adjusted to 7.4 with 5M HCl before sterilizing at $121^0$C for 15 min. The bile salt solution was prepared by reconstituting 10g of bile salt in 100 mL distillate water and sterilizing at $121^0$C for 15 min. 4ml of bile salt solution was added to the culture in the flasks, followed by 17 mL of duodenum juice. After mixing, the initial count was determined by spread plating. The flasks were incubated at $37^0$C. Samples were withdrawn after 1h and viable counts were determined by spread plating. The flasks were further incubated at $37^0$C. Samples were withdrawn after 2 hours to 3 hours, and counts were determined. The rate of survival was calculated as the percentage of LAB colonies grown on MRS agar relative to the initial bacterial concentration:

$$survival\ rate\ (\%) = \frac{logCFUN1}{logCFUN0} \times 100$$

Where $N_1$ is the viable count of isolates after incubation and $N_0$ is the initial viable count(Mathara *et al.*, 2008).

**Auto aggregation assay**

The auto-aggregation was carried out according to (Balakrishna *et al.*, 2013), with modifications. LAB isolates were cultured for 18 hours at $37^0$C in MRS broth. Cells were collected by centrifugation (15000rpm, 5 min) and pellet was washed three times with sterile PBS. Next, the pellet was incubated at 37 $^0$C for 5 hours. The optical density of the top layer suspension was analysed at 0 hours and 5 hours using UV spectrophotometer at 600nm ($OD_{600}$). The auto-aggregation ability was calculated as follows:

$$\text{Auto-aggregation ability (\%)} = (1\text{-}A_t/A_0) \times 100\%$$

Where $A_t$ is $OD_{600}$ 5 hours, and $A_0$ is $OD_{600}$ at 0 hours (Balakrishna *et al.,* 2013).

**Antimicrobial activities of isolated probiotic against selected pathogenic bacteria.**

Antimicrobial activities analysis

Antimicrobial activity of isolates against pathogenic strains was assessed using agar well diffusion method according to (Ridwan *et al.,* 2008), with modification. Test microorganisms were *Escherichia coli* ATCC25922, *Staphylococcus aureus*, ATCC25923, *Pseudomonas* ATCC27853, and *Candida albicans*, ATCC 90028.100µl of the pathogen was spread over the nutrient agar plates. A 100µl of overnight grown LAB isolate was poured into a well on plates. Plates were allowed to dry and incubated at $37^0$C for 24 to 48 hours (Ridwan *et al.,* 2008).

**Screening for bacteriocin genes in the isolates**

Specific primers were used to screen the selected bacteriocin genes in the isolates. Cultures grown overnight in 9ml MRS broth at $37^0$C were used for DNA extraction. About 1ml of overnight culture was centrifuged at (15,000× g, 5 minutes, and the supernatant was discarded. Total gDNA was extracted using Quick-$DNA_{TM}$ Fungal/Bacterial Miniprep Kit (Zymo Research, USA) according to manufacturer's protocols. The concentration and purity of each DNA sample were determined using a Nano Drop spectrophotometer (PCR max Lambda) at 260/280 and 260/230. The DNA integrity was analyzed by gel electrophoresis on a 1% agarose gel. Samples were stored at -20°C for further analysis.

Screening for known bacteriocin genes, including those encoding Nisin, Pediocin and plantaricin was conducted using the specific primer, (table 1).

**Table 1:** Designed primers for bacteriocin genes

| Genes | Primers | Amplicon size (bp) |
|---|---|---|
| Plantaricin | F: 5' ACCTGAAATAGCATTTAATTCACG 3'<br>R: 5' AGTGTTCGACATGTTGTTGATG 3' | 147 |
| Nisin | F: 5' ACTTGGATTTGGTATCTGTTTCGA 3'<br>R: 5' TTTGATTTGGTTATTTGCTTACGTG 3' | 165 |
| Pediocin | F: 5′-GGTAAGGCTACCACTTGCAT-3′<br>R: 5′-CTACTAACGCTTGGCTGGCA-3′ | 287 |

**PCR Amplification of target genes:**

The PCR were conducted according to (Anandharaj *et al.*, 2014) . using a total volume of 25 µL, containing 12.5 µL of Master Mix (GoTaq Green Master Mix, Promega, Madison, WI), 1µL each of forward and reverse primers, 8.5µL of nuclease-free water, and 2 µL of DNA template using a thermal cycler (SimpliAmp Thermal Cycler, Thermo Fisher Scientific, Waltham, MA). PCR conditions, the initial denaturation was at 94°C for 60 seconds followed by annealing temperature of $56^0$C for 60seconds, 34 cycles of elongation at $72^0$C for 1 minute and final hold at 4°C indefinitely. Agarose gel was prepared to visualise PCR amplicon products using a 100bp ladder to estimate the products size.

**Statistical Analysis**

All data are revealed as mean value ± standard error from triplicate experiments, SPSS (25 version) was used .The results of each analyse were compared with an appropriate control

and statistical analysis was performed using the unpaired two-tailed *t*-test at a significance level of $P < 0.05$.

## RESULTS AND DISCUSSION

### Tolerance to low pH

Acid tolerance is an essential selection factor for potential probiotics. Probiotic strain has to be able to survive the acidic conditions of the stomach. (Yadav *et al.*, 2016).The ability of 18 LAB strains (C1 to C18) from traditional cow's milk cheese produce in Niger to tolerate low pH values of 2.0, 2.5 and 3 for 24 hours is shown in (Table 2). Seventeen of these strains except C13, showed survival rates of at least from 75% to 88% at pH 2.5, whereas at pH 2.0 only 8 strains(C3,C4,C5,C6,C7,C8,C11 and C17) showed survival rate from 51% to 65% and no viable cells were detected for 9 strains (C1,C2,C9,C10,C12,C14,C15,C16, and C18) after exposure for 24 hours at pH 2 (table 2). seventeen LAB isolates (C1,C2,C3,C4,C5,C6,C7,C8, C9,C10,C11,C12,C14,C15,C16, and C18) has showed survival rate from 76% to 94% at pH 3,.( table 2)

**Table 2:** The Growth of the LAB under pH 2, 2.5, and 3. Data expressed as Mean±SE of the Mean of triplicate experiments. Values in bracket represent the percentage survival rate of each isolate.

| **Strain** | **pH=2** | | **pH=2.5** | | **pH=3** | |
|---|---|---|---|---|---|---|
| **Code** | 0hour | 24hours | 0hour | 24hours | 0hour | 24hours |
| C1 | 7.50±0.01 | 0.00±0.00 (0) | 8.79±0.03 | 7.26±0.04 (83) | 9.56±0.06 | 8.36±0.01 (87) |
| C2 | 7.33±0.01 | 0.00±0.00 (0) | 8.72±0.05 | 7.51±0.06 (86) | 9.77±0.03 | 8.50±0.06 (87) |
| C3 | 8.86±0.02 | 5.04±0.01 (57) | 9.39±0.13 | 7.06±0.02 (75) | 10.62±0.04 | 8.06±0.02 (76) |
| C4 | 8.36±0.12 | 5.39±0.08 (64) | 9.80±0.03 | 7.55±0.06 (77) | 10.69±0.01 | 8.78±0.02 (82) |
| C5 | 8.59±0.04 | 5.59±0.04 (65) | 9.73±0.01 | 7.35±0.03 (76) | 10.59±0.04 | 8.22±0.07 (78) |
| C6 | 8.68±0.01 | 5.23±0.05 (60) | 9.60±0.02 | 7.47±0.02 (78) | 10.68±0.01 | 8.40±0.04 (79) |
| C7 | 7.37±0.12 | 4.24±0.08 (58) | 8.73±0.02 | 7.25±0.02 (83) | 9.67±0.04 | 8.22±0.03 (85) |
| C8 | 8.68±0.04 | 4.40±0.07 (51) | 9.78±0.02 | 7.40±0.04 (76) | 10.86±0.01 | 8.46±0.02 (78) |
| C9 | 0.00±0.00 | 0.00±0.00 (0) | 8.66±0.06 | 7.24±0.05 (84) | 9.55±0.05 | 8.31±0.03 (87) |
| C10 | 7.090.03 | 0.00±0.00 (0) | 8.73±0.01 | 7.66±0.02 (88) | 9.11±0.55 | 8.55±0.02 (94) |
| C11 | 8.55±0.04 | 5.02±0.01 (59) | 9.60±0.03 | 7.30±0.03 (76) | 10.64±0.02 | 8.28±0.04 (78) |
| C12 | 0.00±0.00 | 0.00±0.00 (0) | 8.91±0.01 | 7.43±0.02 (83) | 9.97±0.01 | 8.29±0.06 (83) |
| C13 | 0.00±0.00 | 0.00±0.00 (0) | 0.00±0.00 | 0.00±0.00 (0) | 0.00±0.00 | 0.00±0.00 (0) |
| C14 | 7.83±0.02 | 0.00±0.00 (0) | 8.48±0.04 | 7.37±0.04 (87) | 10.46±0.04 | 8.43±0.02 (81) |
| C15 | 7.45±0.11 | 0.00±0.00 (0) | 8.49±0.06 | 7.48±0.05 (88) | 10.50±0.05 | 8.57±0.02 (82) |
| C16 | 0.00±0.00 | 0.00±0.00 (0) | 8.21±0.07 | 7.02±0.01 (86) | 10.31±0.04 | 8.02±0.01 (78) |
| C17 | 7.10±0.03 | 4.15±0.05 (58) | 8.84±0.02 | 7.12±0.04 (81) | 10.15±0.05 | 8.17±0.02 (80) |
| C18 | 7.49±0.06 | 0.00±0.00 (0) | 8.23±0.08 | 7.15±0.05 (87) | 10.35±0.04 | 8.22±0.03 (79) |

### Bile salt tolerance

Seventeen (17) LAB isolates (C1,C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15,

C16, C17) were able to survive above 80% in the presence of 0.3% of bile salt. Isolate C12 was the most tolerant with 93% survival rate followed by isolates C15 with 92% and C14 with 91% survival rates, after 24hours incubation. After 48 hours incubation all the isolate were able to survive above 95% in the presence of 0.3% of bile salt. Isolate C17 was the most

tolerant with 105% survival rate followed by isolates C11, C12 with 103% and C2, C5, C6, C10, 15 with 102% and C9, C14 with 100% survival rates in presence of bile salt are present in (Table 3)

**Table 3:** Growth of the LAB under 0.3% of bile salt. Data expressed as Mean±SE of the Mean of triplicate experiments. Values in bracket represent the percentage survival rate of each isolate.

| Strain Code | 0.3 % bile salt | | |
|---|---|---|---|
| | 0 hours | 24 hours | 48 hours |
| C1 | 8.81 ±0.06 | 7.54 ±0.10 (86) | 8.37 ±0.13 (95) |
| C2 | 8.90 ±0.04 | 7.49 ±0.11 (84) | 8.95 ±0.02 (102) |
| C3 | 9.05 ±0.02 | 7.25 ±0.08 (80) | 8.62 ±0.02 (98) |
| C4 | 9.04 ±0.01 | 7.42 ±0.10 (82) | 8.73 ±0.02 (99) |
| C5 | 9.09 ±0.03 | 8.02 ±0.01 (88) | 9.03 ±0.01 (102) |
| C6 | 9.20 ±0.07 | 7.89 ±0.04 (86) | 8.97 ±0.03 (102) |
| C7 | 9.14 ±0.05 | 7.64 ±0.07 (84) | 8.49 ±0.14 (96) |
| C8 | 8.45 ±0.15 | 7.47 ±0.10 (88) | 8.46 ±0.12 (96) |
| C9 | 9.13 ±0.04 | 7.39 ±0.13 (81) | 8.80 ±0.01 (100) |
| C10 | 9.06 ±0.02 | 7.77 ±0.07 (86) | 8.97 ±0.02 (102) |
| C11 | 9.10 ±0.03 | 8.08 ±0.03 (89) | 9.09 ±0.03 (103) |
| C12 | 8.64 ±0.11 | 8.03 ±0.01 (93) | 9.05 ±0.02 (103) |
| C13 | 8.97 ±0.02 | 7.29 ±0.10 (81) | 8.52 ±0.05 (97) |
| C14 | 8.52 ±0.18 | 7.75 ±0.03 (91) | 8.84 ±0.02 (100) |
| C15 | 8.68 ±0.09 | 7.97 ±0.01 (92) | 8.98 ±0.01 (102) |
| C16 | 8.90 ±0.04 | 7.34 ±0.11 (80) | 8.70 ±0.01 (99) |
| C17 | 8.97 ±0.03 | 7.19 ±0.07 (80) | 9.26 ±0.09 (105) |

**Simulated gastric juice survivability**

The effect of simulated gastric juice at pH 2.0, 2.5 and 3 on LAB isolates is presented in (Table 4).only isolates C12 failed to survive in simulated gastric juice at pH 2.0 after 60minutes and 90 minutes of incubation, and the rest of 16 isolate were able to survive above 50%. Isolate C1 was the most tolerant at pH 2 with 69%, following by C17 with 65%, and C10 with 63% (table 4). At pH 2.5 all the 17 isolate were able to survive above 81%, isolate C1 is most tolerant with 89%, followed by C15 with 87%, C14, C13, C12, and C7 with 86%, C4 with 85%, C16, C11, C5, C2 with 84% and C8, C10 with 82% (table 4) .whereas all the isolates were able to survive at pH 3, the survival rate were above 94%.but the isolate C13 was the most tolerant at pH 3 with 105% after 60 and 90min of incubation, followed by C14, C10, C7, C2, with 104%, C4 with 103%, C8, C16 with 102%, C17 with 101% and C12 with 100% (table 4)

**Table 4:** The Growth of the LAB under pH 2, 2.5, and 3 of simulated gastric juice. Data expressed as Mean±SE of the Mean of triplicate experiments. Values in bracket represent the percentage survival rate of each isolate.

| **Strain** | pH=2 | | pH=2.5 | | pH=3 | |
|---|---|---|---|---|---|---|
| **Code** | 0hour | 24hours | 0hour | 24hours | 0hour | 24hours |
| C1 | 7.50±0.17 | 5.14±0.12 (69) | 8.94±0.02 | 7.96±0.02 (89) | 10±0.00 | 9.90±0.03 (99) |
| C2 | 7.94±0.05 | 4.24±0.08 (53) | 9.04±0.01 | 7.55±0.06 (84) | 9.54±0.18 | 9.95±0.02 (104) |
| C3 | 8.05±0.02 | 4.74±0.09 (59) | 9.05±0.02 | 7.34±0.12 (81) | 10.05±0.02 | 9.70±0.09 (97) |
| C4 | 8.03±0.02 | 4.10±0.03 (51) | 9.04±0.01 | 7.67±0.08 (85) | 9.55±0.18 | 9.82±0.03 (103) |
| C5 | 8.09±0.03 | 4.40±0.03 (54) | 9.13±0.02 | 7.66±0.02 (84) | 10.09±0.03 | 9.90±0.03 (98) |
| C6 | 8.20±0.07 | 4.15±0.05 (51) | 9.55±0.05 | 7.96±0.01 (83) | 10.20±0.07 | 9.97±0.01 (98) |
| C7 | 8.08±0.03 | 4.50±0.03 (56) | 9.04±0.01 | 7.76±0.03 (86) | 9.54±0.19 | 9.93±0.03 (104) |
| C8 | 7.95±0.02 | 4.65±0.02 (58) | 8.95±0.02 | 7.38±0.13 (82) | 9.64±0.09 | 9.88±0.04 (102) |
| C9 | 8.13±0.04 | 4.67±0.01 (57) | 9.33±0.02 | 7.53±0.09 (81) | 10.13±0.04 | 9.97±0.01 (98) |
| C10 | 7.99±0.03 | 5.02±0.01 (63) | 9.04±0.01 | 7.37±0.11 (82) | 9.55±0.18 | 9.92±0.02 (104) |
| C11 | 8.10±0.03 | 4.15±0.05 (51) | 9.15±0.02 | 7.73±0.01 (84) | 10.11±0.03 | 10.00±0.00 (99) |
| C12 | 7.82±0.05 | 0.00±0.00 (0) | 8.96±0.01 | 7.75±0.01 (86) | 9.99±0.01 | 10.00±0.00 (100) |
| C13 | 8.02±0.01 | 4.53±0.02 (56) | 9.02±0.01 | 7.76±0.03 (86) | 9.47±0.20 | 9.99±0.01 (105) |
| C14 | 8.02±0.01 | 4.64±0.05 (58) | 9.02±0.01 | 7.77±0.01 (86) | 9.52±0.18 | 9.92±0.03 (104) |
| C15 | 7.91±0.01 | 3.94±o.02 (50) | 9.14±0.05 | 7.95±0.02 (87) | 9.98±0.01 | 9.43±0.02 (94) |
| C16 | 7.91±0.04 | 4.43±0.02 (56) | 9.07±0.02 | 7.60±0.05 (84) | 9.76±0.09 | 9.93±0.01 (102) |
| C17 | 8.02±0.02 | 5.18±0.06 (65) | 9.12±0.02 | 7.60±0.03 (83) | 9.98±0.03 | 10.12±0.06 (101) |

**Response to simulated stomach duodenum passage**

The LAB isolates were tested under conditions of simulated stomach duodenum passage to determine their survival. All the isolates showed survival rates of 95 to 103% after 1hour incubation under simulated stomach duodenum passage,C1 had a high survival rate after 1hour with 103%, followed by C12 with 102% ,C4,C6,C8,C11,C13,C14,C16 with 100% (table 5). And they also showed survival rate ranging from 89 to 100% after 3 hours incubation (Table 5). The strains C7 had significantly higher survival rate with 100% while the other isolates namely C1, C2, C3, C4, C5, C6, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17 had survival rates of less than 100% and after 2hours incubation . This suggests that they can tolerate the conditions of the stomach and can therefore be potent probiotics.

**Table 5:** The Growth (percentage) of the LAB under simulated stomach duodenum condition. Data expressed as Mean±SE of the Mean of duplicate experiments. Values in bracket represent the percentage survival rate of each isolate.

| Strain Code | 0hour | 1hour | 2hours | 3hours |
|---|---|---|---|---|
| C1 | 6.73±0.01 | 6.98±0.00 (103) | 6.29±0.00 (93) | 6.43±0.02 (96) |
| C2 | 6.70±0.07 | 6.73±0.01 (98) | 6.62±0.01 (97) | 6.59±0.03 (97) |
| C3 | 6.94±0.01 | 6.61±0.00 (95) | 6.19±0.00 (89) | 6.47±0.01 (93) |
| C4 | 6.64±0.08 | 6.83±0.00 (100) | 6.25±0.01 (91) | 6.39±0.01 (93) |
| C5 | 7.15±0.05 | 6.49±0.00 (93) | 6.82±0.01 (98) | 6.44±0.01 (93) |
| C6 | 6.91±0.00 | 6.88±0.00 (100) | 6.75±0.03 (99) | 6.71±0.00 (97) |
| C7 | 6.90±0.01 | 6.43±0.01 (93) | 6.67±0.01 (97) | 6.89±0.01 (100) |
| C8 | 6.97±0.01 | 6.91±0.00 (100) | 6.89±0.00 (99) | 6.23±0.01 (89) |
| C9 | 6.91±0.01 | 6.57±0.01 (95) | 6.38±0.00 (92) | 6.22±0.01 (90) |
| C10 | 7.07±0.02 | 6.91±0.01 (97) | 6.65±0.02 (94) | 6.42±0.01(90) |
| C11 | 7.03±0.01 | 7.02±0.01 (100) | 6.64±0.00 (94) | 6.72±0.01 (95) |
| C12 | 6.43±0.15 | 6.98±0.01 (102) | 6.69±0.01 (98) | 6.20±0.01(90) |
| C13 | 6.90±0.02 | 6.94±0.01 (100) | 6.76±0.00 (97) | 6.56±0.01 (95) |
| C14 | 7.03±0.01 | 7.01±0.00 (100) | 6.69±0.01 (95) | 6.42±0.01 (91) |
| C15 | 6.93±0.01 | 6.72±0.01 (97) | 6.25±0.00 (90) | 6.43±0.01 (93) |
| C16 | 6.86±0.02 | 6.95±0.01 (100) | 6.10±0.00 (88) | 6.41±0.01 (93) |
| C17 | 7.00±0.00 | 6.78±0.00 (97) | 6.39±0.00 (91) | 6.70±0.01(96) |

**Auto aggregation assay**

The auto-aggregation abilities of all the LAB strains studied are shown in Table 6. The results indicated that each strain can auto aggregate. Among these probiotic strains, C9 showed the highest auto aggregation percentage of 83%, followed by C1 with 73%, C8 with 61%, C17 with 53%, C16 with 42%, C6 with 36%, C7 with 34%, C4 with 29%, C2, C10, C11 with 28% (table 6). Five (5) strains had less auto-aggregation abilities; C3, C12, C13, C14 and C15 ranging between 12%, and 19%. Isolate, C5 had auto-aggregation ability of 2% (auto-aggregation percentage <10%).

**Table 6:** Auto-aggregation ability (percentage) of the isolated LAB. Data expressed as Mean±SE of the Mean of triplicate experiments. Values in bracket represent the percentage of auto-aggregation abilities of each isolate

| Strain Code | Ohour | 5hours |
|---|---|---|
| C1 | 0.68±0.08 | 0.17 ±0.02 (73) |
| C2 | 2.02±0.13 | 1.46 ±0.13 (28) |
| C3 | 1.13±0.15 | 1.00 ±0.14 (12) |
| C4 | 2.31±0.06 | 1.63 ±0.01 (29) |
| C5 | 1.66±0.11 | 1.63 ±0.22 (2) |
| C6 | 2.36±0.09 | 1.54 ±0.17 (36) |
| C7 | 2.00±0.03 | 1.33 ±0.27 (34) |
| C8 | 2.08±0.05 | 0.82 ±0.04 (61) |
| C9 | 0.48±0.13 | 0.08 ±0.01 (83) |
| C10 | 2.27±0.02 | 1.63 ±0.12 (28) |
| C11 | 2.38±0.05 | 1.72 ±0.16 (28) |
| C12 | 2.20±0.06 | 1.79 ±0.10 (19) |
| C13 | 1.60±0.16 | 1.35 ±0.01 (16) |
| C14 | 2.28±0.03 | 1.93 ±0.11 (15) |
| C15 | 2.04±0.12 | 1.68 ±0.03 (180 |
| C16 | 2.29±0.11 | 1.32 ±0.09 (42) |
| C17 | 2.37±0.05 | 1.11 ±0.08 (53) |

**Antimicrobial activities analysis**

The LAB isolates were also tested for antimicrobial activity against indicator organisms (*Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, and *Pseudomonas* ATCC27853 and *Candida albicans* ATCC 90028,). The cell-free supernatants from different *Lactobacillus* strains inhibited the growth of indicator organisms as shown by inhibition zone results (Table 7). Among the test strains isolate C17 showed the highest antibacterial activity against *E. coli with* (21±0.34mm), strains C12,(19±0.34mm), followed by C16(18±0.34mm),C7(15±0.34mm),C5,C10,C13,C14 (14.5±0.17mm),C11 (13.5±0.17mm) and C17 (24.5±0.17mm) has showed a higher antibacterial activity against staphylococcus aureus. Followed by strains C14 (23.5±0.51mm), C15 (22±0.34mm), C13 (21±0.34mm), C12, C16 (20.5±0.17mm).The strains showed the highest antibacterial activity against *pseudomonas* ranging from 18.5±0.51 to 26±0.34mm with C8 which has showed highest antagonistic activity (26±0.34mm). They also showed the antibacterial activity against candida albicans from 11.5±0.17 to 24.5±0.17mm. (Table 7)

**Table 7:** Antimicrobial effects of the strains against selected pathogens, 7.5 represent the diameter of halo in milimeter.

| Strain code | *E.coli* | *Staphylo coccus aureus* | *Pseudo monas* | *Candida albicans* |
|---|---|---|---|---|
| C1 | 7.5 ± 0.00 | 7.5±0.00 | 7.5±0.00 | 7.5±0.00 |
| C2 | 7.5 ±0.00 | 7.5±0.00 | 7.5±0.00 | 7.5±0.00 |
| C3 | 12±0.34 | 16±0.34 | 22±1.03 | 18±0.34 |
| C4 | 13±0.34 | 7.5±0.00 | 20.5±0.51 | 14.5±0.86 |
| C5 | 14.5±0.17 | 14.5±0.17 | 18.5±0.51 | 19±0.34 |
| C6 | 12±0.34 | 12.5±0.86 | 21±0.34 | 17±0.34 |
| C7 | 15±0.34 | 12±0.34 | 25.5±0.17 | 17.5±0.17 |
| C8 | 11±0.34 | 19.5±0.17 | 26±0.34 | 14±0.34 |
| C9 | 7.5±0.00 | 7.5±0.00 | 7.5±0.00 | 7.5±0.00 |
| C10 | 14±0.69 | 16.5±0.51 | 25.5±0.17 | 11±0.34 |
| C11 | 13.5±0.17 | 19±0.34 | 24±0.34 | 17.5±0.17 |
| C12 | 19±0.34 | 20.5±0.17 | 23.5±0.51 | 11.5±0.17 |
| C13 | 14.5±0.17 | 21±0.34 | 23.5±0.86 | 11±0.34 |
| C14 | 14.5±0.17 | 23.5±0.51 | 22±0.69 | 11.5±0.51 |
| C15 | 11.5±0.17 | 22±0.34 | 22.5±0.17 | 22.5±0.17 |
| C16 | 18±0.34 | 20.5±0.17 | 21±0.34 | 24.5±0.17 |
| C17 | 21±0.34 | 24.5±0.17 | 23±0.34 | 22.5±0.17 |

## Screening for bacteriocin genes

The isolates were screened for selected bacteriocin genes (figure 1). All the isolates were negative for Nisin gene and Pediocin gene. However, all the LAB strains were positive for the bacteriocins, plantaricin gene (147 bp) (figure 1). Furthermore, the BLAST search of the plantaricin sequence for the strain confirmed the similarity of the detected plantaricin gene.

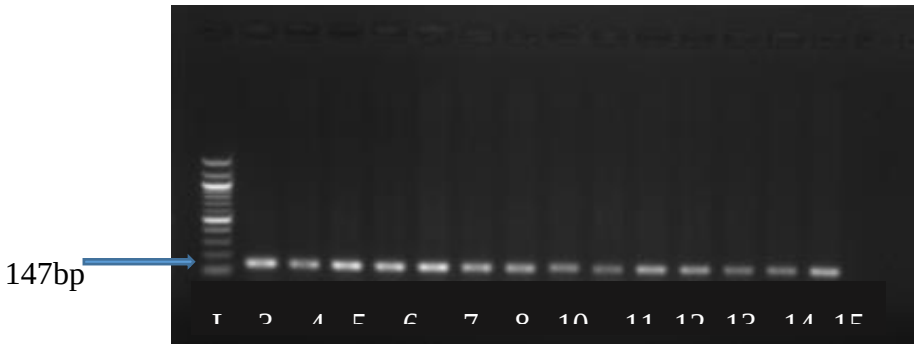


Figure 1: PCR bands for plantaricin

## Discussion

Lactic acid bacterial (LAB) strains are bacteria that are advantageous to the body. They're common in the healthy gut microbiota and can be found in a variety of fermented dairy products including cheese and fermented milk (Tan *et al*., 2013). Low pH, bile salts, and digesting conditions are the key elements determining probiotic bacteria's capacity to survive in the GI tract, which is one of the most sought features.(Tan *et al*., 2013). The probiotic characteristics of seventeen LAB strains isolated from cheese samples produced in Niger were studied. The most frequent way for determining the viability and activity of probiotic bacteria in the small intestine and stomach is to assess their tolerance to acidic conditions. A prior study (Azat *et al*., 2016) found that the growth rate at pH 3.0 is regarded as an excellent acid resistance for the chosen probiotic strains. All the isolates in this study were capable of tolerating pH 3.0 with a survival percentage of more than 70%, qualifying the isolates to be classified as acid-tolerant LAB strains. Only 8 isolates showed tolerance to pH 2 for 24 h (Table 2). Suggesting that the 8 LAB strains grew under pH 2 are the presumed acid tolerant LAB strains. However, all the 17 strains can be considered for probiotic strains, as the stomach acidic pH range from 1.5 to 3. Tolerance to bile salts is commonly thought to be a prerequisite for LAB strains to survive in the small intestine (Azat *et al*.,2016) The average bile concentration in the human gastrointestinal tract is about 0.3%, which is regarded critical and high enough to screen resistant strains (Azat *et al*.,2016). All the LAB isolates under the current study grew at a rate of more than 80% on 0.3% bile salt for 24 and 48 hours incubation (table 3). This finding is identical to (Damayanti *et al.*,2014), which had a viability percentage of greater than 100%. Biliary salts, which are compounds capable of damaging bacterial cell membranes and DNA, are key antibacterial agents in the digestive system (Machado *et al.*, 2014). In the gastric juice (pH 2, 2.5, and 3) tolerant assay all the LAB isolates demonstrated cell viability percentages (Table 4) ranging from 50% to 69% after a 24-hours incubation at pH 2, with the exception of strain C12. However, after 24 hours of incubation, all the strains were able to show high survival rates of 81-89% at pH 2.5 and 94-105% at pH 3. In a previous study, Bile Salt and Acid Tolerant of Lactic Acid Bacteria isolated from Proventriculus of broiler chicken had the highest viability at pH 2 (40.84-76.76%) (Damayanti *et al.*,2014). However, the strains in this investigation had a lower survival rate (50% to 69%) at pH 2 after 24 hours.

The purpose of the simulated stomach duodenum passage test was to see how all of the components (bile, low pH, and duodenal juice) worked together in a combined system. The majority of the isolates were able to survive this setting, indicating that they are able to reach the intestines. All of the isolates under investigation were still viable after 3 hours of exposure (table 5). Auto-aggregation was observed in all strains examined in this investigation (Table 6). After 24 hours, highest percentage of auto-aggregation was recorded, ranging from 12% to 83%. Auto-aggregation was found to be somewhat lower in strains C15, C14, C13, C12 and C3. This result was comparable to that of (Krausova *et al.*,2019). In which all tested strains showed high auto-aggregation capacity after 24 hours of incubation, ranging from 21.7 to 69.7%. The 17 selected potential probiotic lactic acid bacteria exhibited varying degrees of antagonism against *Staphylococcus aureus*, *pseudomonas*, *Candida albicans* and *Escherichia coli* (Table 7). According to Mulaw *et al*., (2019), isolates with clearance zones ≤9 mm and ≥12 mm diameter had low and significant antibacterial efficacy against the test pathogens, respectively. As a result, only 15 of the putative probiotic LAB strains tested showed high antibacterial activity against food-borne pathogens, with diameters

ranging from 12±0.34 to 26±0.34 mm. C12 demonstrated low antimicrobial activity against *Candida albicans*, with an inhibition zone of 11.5±0.17mm, whereas C1, C2 and C9 had no activity against any of the pathogenic microbes tested. In agreement with the present study, (Mulaw *et al.*, 2019).demonstrated that all *Lactobacillus* isolates obtained from Egyptian dairy products had a high level of antibacterial effect against *E. coli* and other pathogens with a zone of inhibition ranging from 19.33 to 21 mm in diameter.. LAB are capable of producing many types of bacteriocins such as Nisin, plantaricin and other bacteriocins. In this study, among all 17 LAB isolates, 15 were found to be plantaricin gene-producing LAB with high zone of inhibition, which affirmed its potential as a strong antibacterial agent.

## 6. Conclusion and recommendation

The present study has shown that the seventeen strains of lactic acid bacteria isolated from "Tchoukou" traditional milk cheeses produced in selected region of Niger had desirable probiotic properties as they were tolerant to acid, bile, able to survive simulated stomach duodenum passage as well as inhibit test pathogenic microorganisms. Further in vivo studies should be carried out using cell lines and animal models with a view of developing a consumer product that can benefit society.

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