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Isolation, Molecular Identification and Phylogenetic Analysis of Bacteriocinogenic Lactic Acid Bacteria from Traditionally Fermented Foods from four Major Cities in Cameroon against *Salmonella enterica* pathogens

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Abstract:- Traditionally fermented foods such as, 'Corn Pap' (fermented corn paste), "Kum-kum" (fermented cassava-derived powder), 'Water fufu' (Fermented cassava-derived paste), and Soya beans yogurt (processed from fermented soya beans), have an old history in Cameroon and are staple foods largely consumed on daily basis by majority of both young and elderly people in the main cosmopolitan cities such as Yaounde, Douala, Bamenda and Buea, where majority of the ethnic groups in Cameroon reside. Lactic acid bacteria (LAB) have been used in the production of fermented foods and bacteriocins produced by these bacteria are harmless and have the ability to inhibit the growth of pathogenic bacteria. The aim of this study was to screen and identify genotypically bacteriocin-producing lactic acid bacteria from locally fermented foods in Cameroon against two food born *Salmonella enterica* pathogens and to assess the phylogenetic relationship among the isolates.

A total of 112 fermented food samples were obtained randomly from different local foods markets in the four main cities in Cameroon. All the samples were cultured in the De man, Rogosa, and Sharpe (MRS) medium which supports the growth of lactic acid bacteria. A total of 70 isolates were found to be Gram-positive and catalase-negative and were further identified by using polymerase chain reaction (PCR) targeting the 16S rRNA gene. Genomic DNA of bacteria isolates were extracted using EZ-10 spin column genomic DNA Extraction kit and amplification of 16SrRNA gene of bacterial isolates was done using the bacteria universal MP096 forward and reverse primers and sequencing was carried out at Genewiz, NJ, USA. Selection for DNA sequencing was based on their bacteriocin-producing potentials which produced inhibition zones on culture plate. Sequence analysis was carried out using BLAST program and comparison with the resources at the NCBI database. Phylogenetic tree was constructed to determine the closest bacterial species and strains by Neighbour Joining method using MEGA X software, version 10.0.5.

Results showed that 70 PCR-amplified products were obtained which revealed clear single bands of presumed LAB isolates on 1.5% (w/v) agarose gel with average molecular weights in the range of 800bp-1300bp. The sequencing results identified 23 lactic acid bacteria strains (34.9%) which had similarity over 90% with those deposited in the NCBI GenBank. The isolates were identified as; *L. plantarum* (9 strains), Uncultured *L. spp* (4 strains), *L. paraplantarum* (3 strains), *L. pentosus* (2 strains), *L. salvarius* (2 strains), *Pediococcus acidilactici* (2 strains) and 1 strain belong to *Pediococcus cellicola*. There was a significant difference ($P<0.05$) in the number of isolates obtained from Corn pap compared to the other fermented food sources. The highest number of isolates were obtained from Corn pap sample (9), followed by 5 each from Kumkum and Soyabeans, and 4 from Water fufu samples. Results also showed genetic relationship of isolates from Yaounde, Douala and Bamenda to have ancestry from isolate obtained from Buea.

Therefore, this study has shown the presence of different LAB bacterial strains in the fermented foods and constitute potential sources of bacteriocinogenic LAB against *Salmonella* Typhimurium and *S.* Enteritidis. The distribution of the strains from Bamenda, Douala and Yaounde, were extremely likely to originate from the strain, *Lactiplantibacillus plantarum* strain 07B144, obtained from Buea.

Keywords:- *Bacteriocinogenic; Isolation; Lactic Acid Bacteria; Molecular Identification; Phylogenetic Analysis; Salmonella Enterica; Traditionally Fermented Foods.*

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I. INTRODUCTION

The four cities in Cameroon namely; Bamenda, Buea, Douala and Yaounde are cosmopolitant towns [1] located in the North West, South West, Littoral and Centre regions respectively, where majority of the ethnic groups in Cameroon and other nationals reside. Soy beans, Corn (maize) and cassava based products are the major sources of energy in the diets of sub-saharan Africans [2].

Corn is an important source of carbohydrate, protein, Vitamins B and Minerals. Corn pap is also a meal of choice for patients that are in need of soft and easily digestible foods. It has been reported that corn pap, produced from maize by lactic acid bacteria fermentation especially *Lactobacillus* [3] has sour taste and has become part of the staple diets for young adults, nursing mothers and for weaning infants between the ages of 1-2yrs [4].

Some cassava fermented food such as *Water fufu ,Kumkum, bobolo, Myondo* are largely spread in central and west Africa especially in Cameroon and diverse groups of microorganisms such as *Lactobacillus, Leuconostoc, Lactococcus, Enterococcus* have been reported to play active roles in the process [7, 8, 9]. *Lactobacillus fermentum* of different strains have been isolated from cassava-derived products such as water-fufu [10]**.** As a result, water fufu and kumkum, which are Cassava fermented foods constitute a significant proportion of the diet of the population of Africa and it provides over 50% of the average daily caloric intake in some countries [6].

Soy-based yogurts have emerged as a popular alternative to traditional dairy-based yogurt due to their reduced level of cholesterol, saturated fat and lactose, contain higher levels of essential fatty acids, soluble fiber, vitamins, and minerals. Thus soy based yogurts have gained significant consideration for their many nutritional health benefits including reducing cardiovascular disease, weight loss, arthritis and brain function [16].

In nature, lactic acid bacteria are found in various habitats such as fermented foods, vegetables, fruits and the human digestive tract. It is known that lactic acid bacteria are generally regarded as safe [5] for consumption so that it can be used to increase human health. Lactic acid bacteria produce antimicrobial substances such as organic acids, diacetyl, hydrogen peroxide and bacteriocins [11, 12] which are suspected to be associated with the preservation and characteristic flavor of fermented food [13].

The antimicrobial activity of bacteriocins produced by lactic acid bacteria isolated from fermented foods has been demonstrated against some food-borne pathogens [14, 15]. The use of lactic acid bacteria as a natural preservative can be in the form of starter cultures or metabolites produced by lactic acid bacteria such as bacteriocins, which have a relatively narrow spectrum of bactericidal activity [17]. Classical antibiotics which have shown resistance in food born infections such as *Salmonella* Typhimurium [18, 19] and *S.* Entritidis [20] and bacteriocins may be a suitable alternative, in being easily degraded by the digestive enzymes without the risk of disruption of normal tract ecology [17].

Limited studies have been done in Cameroon to isolate and identify genotypically bacteriocin-producing LAB from fermented foods. More studies are needed to explore other food sources and expand the spectrum of bacteriocinogenic LAB that can inhibit food born *Salmonella* pathogens. Also data on relationship among isolates from fermented foods in Cameroon is limited. Molecular identification using 16srRNA gene sequencing is an accurate method used to identify LAB and has proven to be reliable because it can identify species to strain level [21]. Extraction of DNA, Polymerase Chain Reaction (PCR) and phylogenetic analysis have been used successfully to identify isolates from fermented food [22] and evaluation of relationship among isolates from local fermented samples has been carried out in studies out of Cameroon [23] . The aim of this study was to isolate, and carry out molecular identification and phylogenetic analysis using 16srRNA sequencing method, of bacteriocin-producing LAB from fermented foods in four main cities in Cameroon against *Salmonella* Typhimurium and *S.* Enteritidis, and to assess the relationship among isolates from local fermented food samples.

II. MATERIALS AND METHODS

2.1 Collection of samples and isolation of lactic acid bacteria

A total of 112 fermented food samples (Corn pap, Kumkum,Water fufu, Soya bean yogurt) were bought from different selling shops in local food markets of Bamenda, Buea, Douala, and Yaounde-Cameroon. All the samples were put in sterile bottles and preserved in ice packs and stored at 4°C for a maximum of 24 h before the analysis.

Then 10g of each samples was homogenized in 90ml sterile water for 3-4 minutes and furthermore, ten-fold serial dilutions was done for each sample to 10^{-5}. This was done by diluting 1 ml of the sample in 9 ml of physiological saline (0.85% NaCl). To isolate LAB, 0.1 ml of appropriate dilution was inoculated into de Mann, Rogosa and Sharpe (MRS) agar plates [23] and spread properly with a sterile glass rod. The plates were incubated anaerobically at 30 °C for 24 - 48 h. Suspected colonies with distinct morphology were picked up with sterile wire loop and purified successively on the MRS agar plates by restreaking on the same medium and were also identified by the Gram staining and catalase tests according to the assay methods of described previously [25]. Gram positive and catalase negative isolates were selected as presumptive LAB and stored on the MRS agar slants at 4 °C for further investigations [26].

2.2 Indicator Pathogens

The food-borne indicator pathogens chosen as test strains for testing the antimicrobial activity of bacteriocins produced by isolated LAB were, American Type Culture Collection cultures of *Salmonella enterica subsp. enterica*

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serovar Typhimurium (ATCC®14028™) and *Salmonella enterica subsp. enterica* serovar Enteritidis (ATCC® 13076™). They were obtained from the culture collection of Microbiological and Biomedical Laboratories, Manassas, Va, 20108, USA. The pathogens were subcultured in nutrient agar [27] for 24 h before use.

2.3 Determination of Bacteriocin Production by the Isolated LAB

Each presumed LAB isolate was inoculated from slants at 4^0C into 5ml of MRS broth and incubated at 30^0C for 24h. Cell free supernatants (CFS) were collected by centrifugation at (6000rpm for 10min. at 4 °C) of the overnight broth cultures. The pH of the CFS was adjusted to pH 7.1 with 1M NaOH to eliminate the effect of organic acids and inhibition due to hydrogen peroxide was also removed by the addition of enzyme catalase (Sigma-Aldrich Corporation, USA) at a final concentration of 1.0 mg/ml for 1 h. The Agar well diffusion methods [28] was modified and used to test for the antimicrobial activity of the bacteriocin against test organisms. The treated CFS was introduced into wells made on nutrient agar plates containing test pathogen appropriately diluted at 0.5 McFarland standards and incubated for 24h at 30°C. Clear zones around the wells indicate inhibition by bacteriocin.

To confirm that the inhibition was due to bacteriocin, CFS was treated with 1mg/ml of proteolytic enzymes such as proteinase k [29]. The controls consisted of distilled water (negative control) and untreated CFS (positive control). The samples and controls were incubated at 30°C for 2 h and the absence of bacteriocin activity was determined by the agar-well diffusion (AWD) assay against the indicator strains. All experiments were done in triplicate.

2.4. Molecular Identification of Bacteria

2.4.1. Genomic DNA Extraction.

DNA Extraction Kit obtained from BIO BASIC INC., CANADA was used to extract DNA from lactic acid bacteria and extraction was done according to the manufacturer's procedure. Genomic DNA quality was assessed by 0.7% agarose gel electrophoresis. The DNA samples were quantified and verified for purity using a spectrophotometer by measuring absorbance at A260/A280 >1.8 and then stored at -20^0C for PCR amplification and sequencing.

2.4.2 PCR Amplification and Sequencing of Amplicons:

Bacteria primers for 16S rRNA gene were obtained from CAMMA, Albany, NY, USA, and used for PCR amplification of the LAB. Both 17 bases MP096 forward primer (5'-ATG CAA GTC GAG CGA AC-3') and reverse primer (5'-TGT CTC AGT TCC AGT GTG GC-3') for 16SrRNA were employed using a Thermocycler and the procedure was done according to manufacturer's protocol.

The PCR products were purified by standard method and amplicons were separated according to molecular weights by 1.5% agarose gel electrophoresis using 50-2,000 bp ladder as the gold standard [30].The nucleotide sequences of the PCR amplified 16SrRNA products were determined by using the Sanger chain termination method [31].

2.5 Statistical Analysis

Statistical analysis using one-way ANOVA was done to compare significance of mean inhibition zones among the isolates at a significance level of $P<0.05$.

The identity of the isolates were analysed by using BLAST program and comparing sequences with those deposited in the standard NCBI GenBank(*BLAST;http://blast.ncbi.nlm.nih.gov/Blast. cgi*). Sequences were aligned using CLUSTALW. Then, a phylogenetic tree was constructed to determine the closest bacterial species by Neighbour Joining statistical method using MEGA X software version 10.0.5 (Tamura et al., 2004). Distances and clustering with the Neighbour-Joining method was determined using bootstrap values based on 1000 replicates. The trees were rooted using *Fusobacterium ulcerans* strain NCTC 12111 as the outgroup strain.

III. RESULTS AND DISCUSSION

Isolation of lactic Acid Bacteria isolates

A total of 70 LAB (62.5%) were isolated from 112 food samples such as corn pap, kumkum, soyabeans yogurt and water fufu, in the cities of Bamenda, Buea, Douala, Yaounde-Cameroon. The isolates were gram positive, catalase negative after isolation and purification on culture plate using MRS agar at 30C for 24hrs, and were later tested for bacteriocin production (table 1).

Bacteriocin test for Lactic acid bacteria

Results in table 1 showed that 23 strains (32.9%) out of the 70 presumptive LAB isolates were good bacteriocin producers having antimicrobial activity against two indicator pathogenic strains (*Salmonella enterica subsp. enterica* serovar Typhimurium ATCC 14028™ and *Salmonella enterica subsp. enterica* serovar Enteritidis ATCC 13076™) using the agar well diffusion method. The strains produced significant inhibition zones of >7mm ($P<0.05$) with the highest inhibition produced by WFS strain (14.5mm and 12mm against *S.* Typhimurium and *S.* Enteritidis respectively) as shown in table 2. This finding corroborates with studies carried out on antimicrobial activity of bacteriocin from LAB isolates which demonstrated significant inhibition against food borne pathogens including *Salmonella spp* [14, 15, 32]. Antimicrobial compounds from traditionally fermented foods have shown activity against a wide range of pathogens for several years. The development of biopreservatives using lactic acid bacteria and their metabolites provides protection of food against pathogens, as these bacteria produce antimicrobial substance including bacteriocin [33, 34]. This study has also shown that strains of *Lactobccilus species*, *Pediococcus acidilactici* and Pediococcus cellicola isolated from corn pap, kumkm, Soyabeans yogurt and water fufu can greatly inhibit *S.* Typhimurium *and S.* Enteritidis which have shown antibiotic drug resistance [18, 19, 20]. Therefore these sources can be exploited for

isolation of these LAB isolates for the development of probiotics and for food preservation.

Table 1: The number and percentage of lactic acid bacteria (LAB) isolated from fermented foods (N=112)

Parameter	Number	Percentage
Positive culture of LAB on MRS agar	70	62.5%
PCR positive presumptive LAB	70	62.5%
Positive bacteriocinogenic LAB	23	32.9%

Table 2: Average diameter of inhibition zones of the inhibitory culture supernatant, on test indicator strains, using the agar well diffusion assay.

			Zone of Inhibition (mm) ± SD	
SN	**Food Type**	**LAB Isolates**	***Salmonella* Typhimurium**	***Salmonella* Enteritidis**
1	corn pap	SAL5	7.8 ± 1.15	8.5 ± 1.32
2	corn pap	FCP6	11.3 ± 1.12	9.7±1.14
3	corn pap	DLA1	7 ± 1.16	7± 1.33
4	corn pap	N2	11.8 ± 0.25	8.3 ± 0.28
5	corn pap	K25	10.5 ± 1.17	9.8 ±0.38
6	corn pap	M2	11.5 ± 1.15	8.5 ± 0.21
7	corn pap	CJIL	9.8 ± 0.14	9.5 ± 0.12
8	corn pap	MRLa	**14** ± 1.22	**11** ± 0.45
9	corn pap	WFS	**14.5** ± 0.21	**12** ± 0.11
10	water fufu	M1	7.8 ± 0.11	7.5 ± 0.32
11	water fufu	FCJas	10 ± 1.15	7.3 ± 0.14
12	water fufu	W1	10.3 ± 0.13	8 ±1.81
13	water fufu	W2	8.5 ± 2.01	6.8 ± 0.11
14	soyabean yoghurt	YI	12.5 ± 1.24	10.5± 0.31
15	soyabean yoghurt	SAL1	9.8 ± 0.78	7.3 ± 0.00
16	soyabean yoghurt	LAB1	11.5 ± 0.33	**11** ± 1.23
17	soyabean yoghurt	NY15	12.3 ± 1.71	9.8 ± 0.92
18	soyabean yoghurt	CJ3	10.5 ± 0.95	7.8 ± 0.43
19	kumkum	SBY	7.3 ± 1.35	9.5 ± 0.16
20	kumkum	CB1	13 ± 2.12	**11** ± 0.24
21	kumkum	NFY3	11±1.92	6.8± 0.25
22	kumkum	K2	12.5 ± 1.34	8 ± 0.45
23	kumkum	DLA2	12 ± 0.42	8 ± 0.37

P<0.05

Distribution of isolated bacteriocinogenic Lactic acid bacteria in fermented Foods

The 23 LAB isolates were found to belong to 2 genera; the predominant genus is *Lactobacillus* with 20 isolates, followed by *Pediococcus (3 isolates)*. Nine (9) of these isolates were from Corn pap (coded; FCP6, SAL5, DLA1, N2, K25, M2, CJIL, MRLa, WFS), five (5) from Kumkum (coded; NFY3, SBY, DLA2, K2, CB1), five from Soya bean yogurt (coded; CJ3, LAB1, NY15, SAL1, Y1) and four (4) from Water fufu (coded; M1, FCJas, W1, W2). The result also showed that the predominant LAB isolates were found to belong to the species, *L. plantarum* with 9 LAB isolates (39.1%) from three fermented foods(table 3) in all four localities with *Lactobacillus plantarum* strain JCM 1149, found to be the predominant strain (table 4) isolated from three localities (Buea, Bamenda, Douala). This finding is in agreement with the study carried out by Tatsandjeu and colleagues, in Ngaoundere-Cameroon, who isolated LAB from fermented cassava product against food born pathogens and Lactobacillus was predominant [14]. Also, this study is in accordance with research done by Hama and colleagues, who isolated LAB from fermented foods in 2019, in Burkina Faso and discovered that *Lactobacillus plantarum* was the predominant species [35]. The fermentation of Corn pap is enhanced by lactic acid bacteria especially the Lactobacilli such as; *Lactobacillus plantarum, Lactobacillus lactis, Lactobacillus acidophilus, lactobacillus cellobiosus, Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus casei* [3]. Some cassava fermented food as Water fufu, Kumkum, bobolo, Myondo are largely spread in central and west Africa especially in Cameroon and the involved microorganisms include; *Lactobacillus, Leuconostoc, Lactococcus, Enterococcus* and *Bacillus* [7]. In this study, *Pediococcus* species were isolated from Corn pap, kumkum and Soyabeans yogurt (table 4). This agrees with the finding that Pediococci are used as probiotics, and are commonly added as beneficial microbes in the creation of cheeses and yogurts [36]. Many studies indicate that soya beans is a good substrate for probiotic bacteria [37]. Several health benefits have been reported for traditional yogurt [38, 39, 40]. Bacteriocins apart from their advantage of having antimicrobial property

against food born infections and being easily degraded by the digestive enzymes without the risk of disruption of normal tract ecology [17] and improve gut function, they have shown to be heat stable [41]. During fermentation, LAB release into the medium antimicrobial substances such lactic acid, hydrogen peroxide, baceriocins [14].

Table 3: Distribution of bacteriocinogenic Lactic acid bacteria at species level in fermented food samples

	Corn pap	Waterfufu	Kumkum	Soy yogurt	
Isolate	**Frequency%**	**Frequency(%)**	**Frequency (%)**	**Frequency%**	**Total**
L. pentosus	1(11.1%)	0(0%)	0(0%)	1(20%)	2 (8.7%)
L. plantarum	5(55.6%)	2(50%)	2(40%)	0(0%)	9(39.1%)
L. salivarus	1(11.1%)	0(0%)	0(0%)	1(20%)	2 (8.7%)
L. paraplantarum	1(11.1%)	2(50%)	0(0%)	0(0%)	3(13.1%)
Uncultured L. spp	0(0%)	0(0%)	2(40%)	2(40%)	4(17.4%)
P. acidilactici	1(11.1%)	0(0%)	0(0%)	1(20%)	2 (8.7%)
P. cellicola	0(0%)	0(0%)	1(20%)	0(0%)	1 (4.3%)
Total	9	4	5	5	23

Table 4: Distribution of Bacteriocinogenic Lactic acid bacteria (LAB) strains in Fermented Foods from four localities in Cameroon

LAB Isolates	**Identification(16SrRNA)**	**Sample Type**	**Sampling Location**				
			Buea	**Bamenda**	**Douala**	**Yaounde**	**Total**
NY15	*L. salivarus* strain JCM 1231	Soy Yogurt				**1**	**1**
MRLa	*L. salivarus* strain JCM 1231	Corn pap		**1**			**1**
M1	*L. plantarum* strain 07B144	Waterfufu	**1**				**1**
FCP6	*L. plantarum* subsp. *argentoratensis* strain MMBO7	Corn pap				**1**	**1**
N2	*L. plantarum* strain JCM 1149	Corn pap	**1**				**1**
DLA2	*L. plantarum* strain JCM 1149	Kumkum			**1**		**1**
M2	*L. plantarum* strain JCM 1149	Corn pap		**1**			**1**
CJ3	Uncultured *L. sp.* clone 184a	Soy Yogurt	**1**				**1**
SBY	Uncultured *L. sp.* clone 184a	Kumkum		**1**			**1**
SAL1	Uncultured *L. sp.* clone 184a	Soy Yogurt		**1**			**1**
NFY3	Uncultured *L. sp.* clone 184a	Kumkum	**1**				**1**
CJIL	*L. plantarum* strain CXG9	Corn pap		**1**			**1**
LAB1	*L. pentosus* strain HBUAS56231	Soy Yogurt	**1**				**1**
SAL5	*L. plantarum* strain CIP 103151	Corn pap	**1**				**1**
FCJas	*L. paraplantarum* strain DSM 10667	Waterfufu		**1**			**1**
W1	*L. paraplantarum* strain DSM 10667	Waterfufu		**1**			**1**
WFS	*L. paraplantarum* strain DSM 10667	Corn pap			**1**		**1**
CB1	*L. plantarum* strain UNIFG30	Kumkum				**1**	**1**
DLA1	*L. pentosus* strain MP.VNM.5	Corn pap			**1**		**1**
W2	*L. plantarum subsp.* argentoratensis strain ED116	Waterfufu			**1**		**1**
K25	*P. acidilactici* strain OHER2	Corn pap	**1**				**1**
Y1	*P. acidilactici* strain MKK21	Soy Yogurt				**1**	**1**
K2	*P. cellicola* strain Z-8	Kumkum		**1**			**1**
Total			**7**	**8**	**4**	**4**	**23**

Molecular Identification and Phylogenetic Analysis

The 16SrRNA gene of the presumptive LAB isolates were amplified by PCR and sequenced and we obtained on average between 800bp and 1300bp sequences (figures 1a, b, c) characteristic of LAB gene segment [42] deposited in the GenBank. Results of the nucleotide BLAST search and phyolgenetic analysis using MEGA X (10.0.0 version) identification, revealed 23 bacteriocin-producing LAB isolates which had >90% sequence homology (E-values<$0.003x10^{-16}$) to those deposited in the GenBank with accession numbers (Table 5). The PCR allows good discrimination of isolates while the 16SrRNA gene sequencing and phylogenetic analysis were performed for molecular identification of isolates [43]. Molecular methods are more accurate methods for identifying bacteria than phenotypic methods and new molecular methods such as

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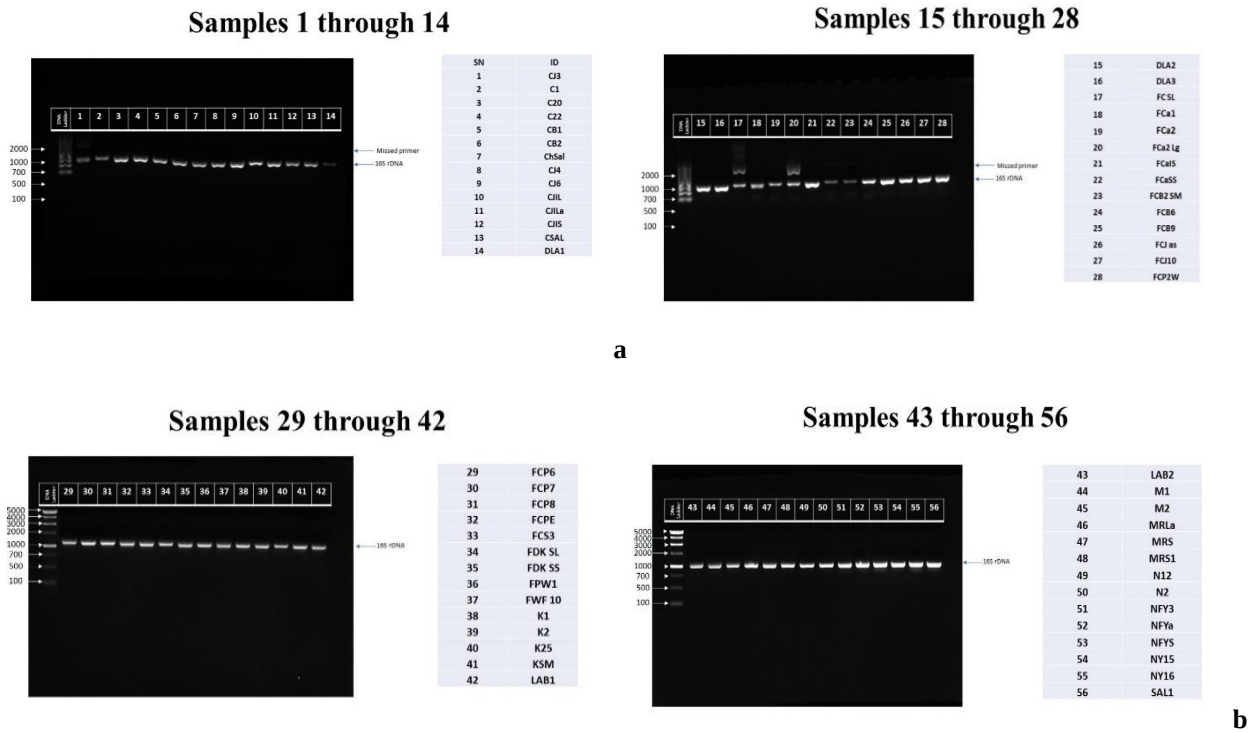
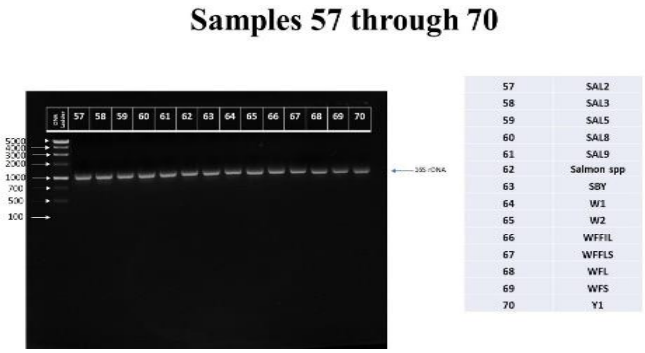
16SrRNA gene sequencing have been employed recently to identify microbes. This method has led to an increase in strains number [44].

The strains were placed in a cluster consisting of the genera Lactobacillus and Pediococcus. Strains in the cluster of the genus Lactobacillus are shown in the phylogenetic trees (Fig. 2a and 2b) which formed well-defined clusters with 20 type strains with over 90% homology with *Lactobacillus species* recorded in the GenBank; M1 had close similarity to *Lactiplantibacillus plantarum* strain 07B144; NY15 and MRLa had similarity to *Lactobacillus salivarius* strain JCM 1231; FCJas, W1, and WFS were closely related to *Lactobacillus paraplantarum* strain DSM 10667); FCP6 (*Lactobacillus plantarum subsp. argentoratensis* strain MMB07); Strains N2, M2 and DLA2 had closest match to *Lactobacillus plantarum* strain JCM 1149; SBY, SAL1, NFY3 and CJ3 were closely linked to Uncultured *Lactobacillus sp*. clone 184a; CJIL was closely smilar to *Lactiplantibacillus plantarum* strain CXG9; LAB1 was similar to *Lactobacillus pentosus* strain HBUAS56231; SAL5 (*Lactobacillus plantarum* strain CIP 103151), DLA 1 *(Lactobacillus pentosus* strain MP.VNM.5), CB1 (*Lactobacillus plantarum* strain UNIFG30) and strain W2 was closely related to *Lactobacillus plantarum subsp. argentoratensis* strain ED116; with bootstrap values at 1000 replicates.

Strains in the cluster of the genus *Pediococcus* (Figure 3) revealed 3 type strains which had over 90% similarity with Pediococcus bacteria deposited in the GenBank; K2 was closely related to *Pediococcus cellicola* strain Z-8; K25 was closely similar to *Pediococcus acidilactici* strain OHER2; and Y1 had the closest match to *Pediococcus acidilactici* strain MKK21, with bootstrap support values at 1000 replicates. Although the partial sequence of the 16S rRNA gene provides sufficient phylogenetically meaningful information, identification of the bacteria will be more accurate if the full sequence of the 16S rRNA gene is employed [45]. Thus, based on the identification by partial 16S rRNA gene, primers were designed to amplify the whole 16S rRNA gene of the respective strains and the full sequence was used for the identification of the LAB

The phylogenetic relationship among the samples were also determined using the maximum likelihood method with query cover above 90% of samples at 1000 replicates (Figure 4). Results showed genetic relationship of isolates from Yaounde, Douala and Bamenda to ancestral isolate from Buea. Samples from Bamenda showed the closest relation to samples from Buea, followed by samples from Douala. Samples from Yaounde showed closer similarity to samples from Douala and Bamenda than samples from Buea. Therefore, closely related isolates, though isolated from different regions, are genetically similar and may possess similar properties than those further from each other in the phylogenetic tree. This study is similar to the work carried out by Saeed and colleagues to determine the evolutionary relationship and similarity among local samples from different regions using phylogenetic analysis by Maximum Likelihood method and minimum evolution method [23]. Lactic acid bacteria like any other bacteria have the ability and capability to exchange genetic materials from the environment through the horizontal gene transfer to be more adapted and survive in the new environment [46].





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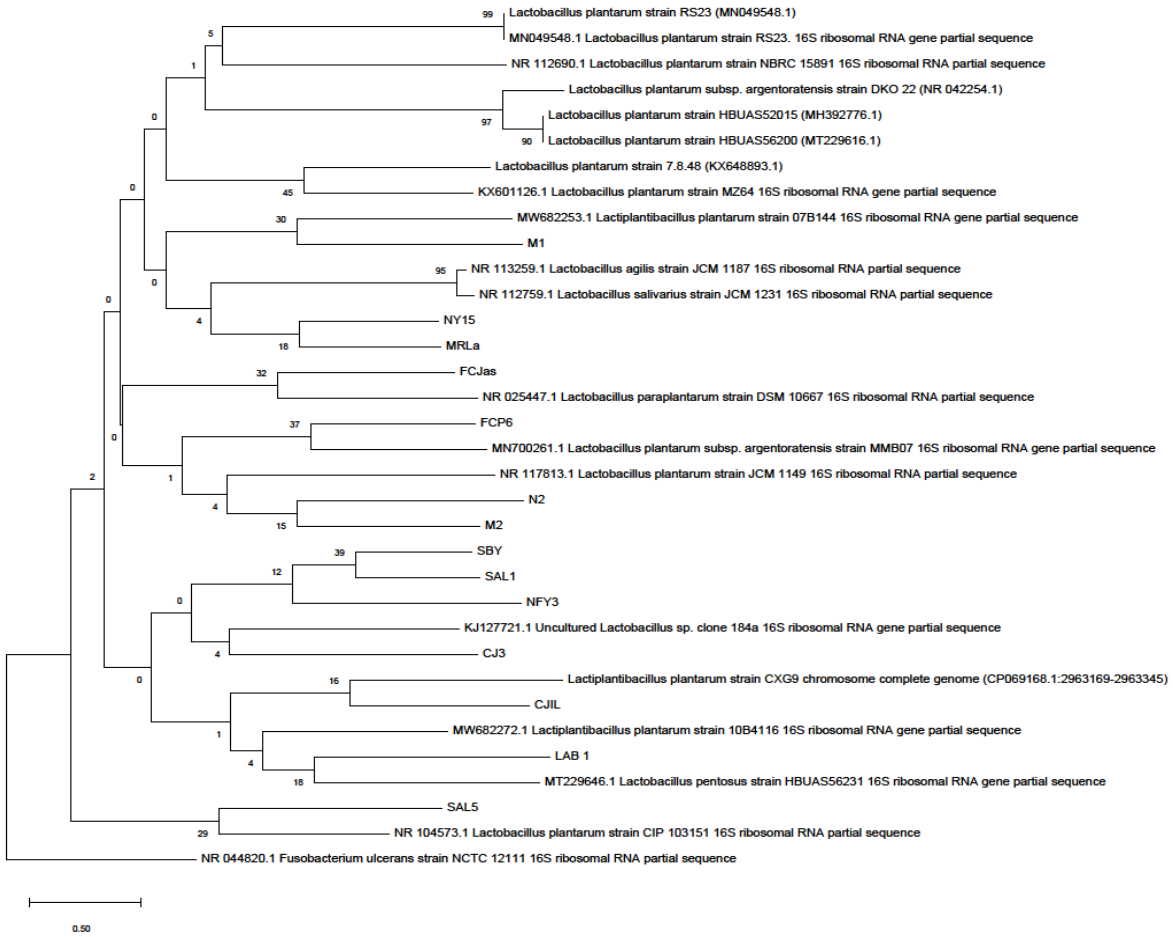
Figures 1a, 1b, 1c: Agarose gel (1.5%) electrophoresis showing amplified 16SrRNA gene of presumed LAB isolates; DNA Ladder (marker); Lanes 1-70, PCR products of LAB isolates.

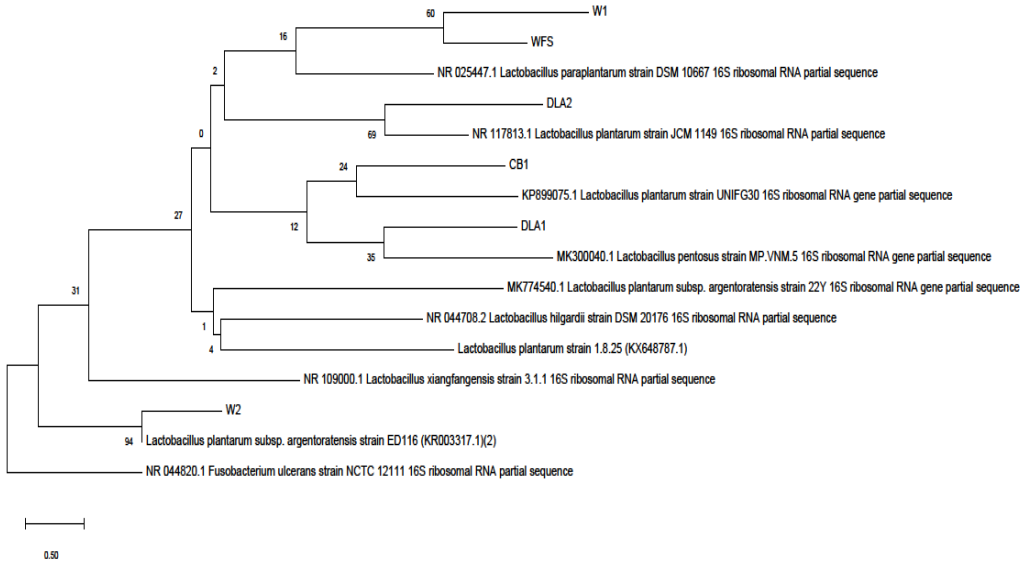
Table 5: Phylogenetic neighbors of bacteriocinogenic Lactic acid bacteria on the basis of similarity to the partial 16S rDNA sequence

SAMPLE SEQUENCE CODE	E-Value	PERCENTAGE SIMILARITY	IDENTIFICATION (16SrDNA Sequencing)	ACCESSION NUMBER
M1	0.001×10^{-33}	93%	*Lactiplantibacillus plantarum* strain 07B144	MW682253.1
NY15 MRLa	$O.01 \times 10^{-27}$ 0.003×10^{-26}	92% 91%	*Lactobacillus salivarius* strain JCM 1231	NR 112759.1
FCP6	0.003×10^{-23}	91%	*Lactobacillus plantarum* subsp. argentoratensis strain MMB07	MN700261.1
N2 M2 DLA2	0.002×10^{-31} 0.001×10^{-30} 0.003×10^{-31}	93% 91% 92%	*Lactobacillus plantarum* strain JCM 1149	NR 117813.1
CJ3 SBY SAL1 NFY3	0.001×10^{-20} 0.0002×10^{-19} 0.03×10^{-21} 0.01×10^{-21}	91% 93% 90% 91%	Uncultured *Lactobacillus sp.* clone 184a	KJ127721.1
CJIL	0.001×10^{-25}	95%	*Lactiplantibacillus plantarum* strain CXG9	(CP069168.1:2963169-2963345)
LAB 1	0.004×10^{-17}	91%	*Lactobacillus pentosus* strain HBUAS56231	MT229646.1
SAL5	0.002×10^{-29}	90%	*Lactobacillus plantarum* strain CIP 103151	NR 104573.1
FCJas W1 WFS	0.01×10^{-21} 0.003×10^{-20} 0.04×10^{-21}	90% 91% 92%	*Lactobacillus paraplantarum* strain DSM 10667	NR 025447.1
CB1	0.003×10^{-16}	93%	*Lactobacillus plantarum* strain UNIFG30	KP899075.1
DLA1	0.001×10^{-24}	90%	*Lactobacillus pentosus* strain MP.VNM.5	MK300040.1
W2	0.003×10^{-32}	96%	*Lactobacillus plantarum* subsp. argentoratensis strain ED116	KR003317.1
K25	0.0023×10^{-23}	91%	*Pediococcus acidilactici* strain OHER2	MW435849.1
Y1	0.0045×10^{-17}	93%	*Pediococcus acidilactici* strain MKK21	KY494432.1
K2	0.0013×10^{-22}	90%	Pediococcus cellicola strain Z-8	NR 043290.1

.



a



b

Figures 2a and 2b: Phylogenetic trees showing the position of isolates and related *Lactobacillus* strains based on 16SrRNA gene sequences. These trees were constructed using the neighbour joining method (MEGA X 10.0.5) at 1000 replicates. *Fusobacterium ulcerans strain* NCTC 12111 was used as an outgroup organism.

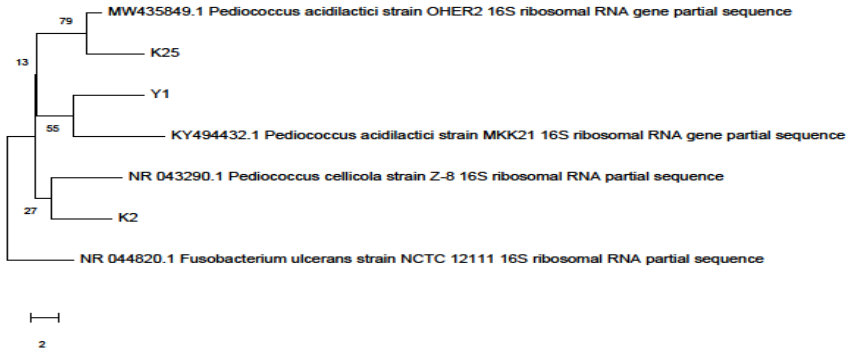


Figure 3: Phylogenetic trees showing the position of isolates and related *Pediococcus* strains based on 16SrRNA gene sequences. These trees were constructed using the neighbour joining method (MEGA X 10.0.5) at 1000 replicates. *Fusobacterium ulcerans strain* NCTC 12111 was used as an outgroup organism.

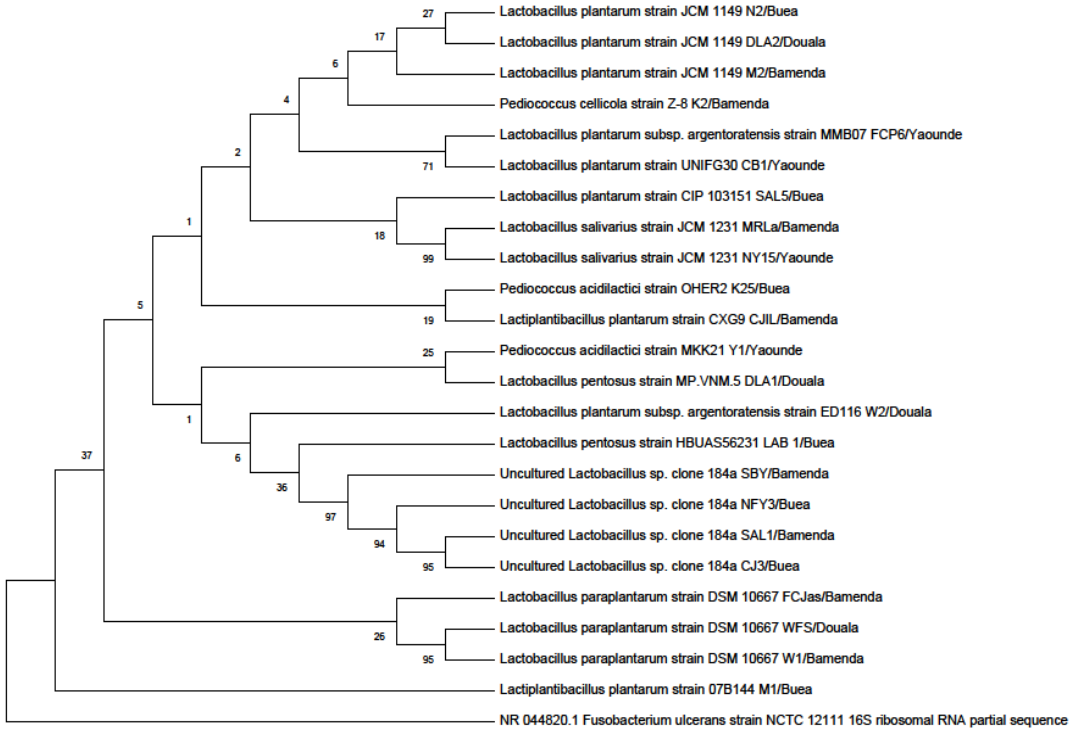


Figure 4: Phylogenetic tree showing the phylogenetic location of Lactic acid bacteria strains isolated from locally fermented foods in Cameroon, based on 16SrRNA gene sequences. This tree was constructed using the neighbour joining statistical method (MEGA X.0.5) at 1000 replicates. *Fusobacterium ulcerans strain* NCTC 12111 was used as an outgroup organism

IV. CONCLUSION

On the basis of the present results, it can be concluded that the fermented foods; Corn pap, Kumkum,Water fufu and soya beans yogurt are potential sources that could be exploited for isolation of different species and strains of bacteriocin-producing LAB against the food born *Salmonella enterica* pathogens. These isolated lactic acid bacteria strains can be employed in food industries as preservatives and probiotics and their bacteriocins can be used in medicine to formulate cocktail drugs against *Salmonella enterica* pathogens to combat the increasing *Salmonella* antibiotic multi-drug resistance. The distribution of samples from Bamenda, Douala, and Yaounde could be likened to have ancestry to an isolate from Buea.

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