



# **Molecular Characterisation and Antagonistic Activity of Fructophilic Lactic Acid Bacteria Isolated from Selected Fruits**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

Fructophilic lactic acid bacteria have very unique biochemical characteristics when compared to other LAB in relation to their hexose sugar metabolism and utilization of oxygen. The aim of this study was to isolate and identify lactic acid bacteria from fresh fruits and determine their antibacterial activity against pathogenic and spoilage organisms. 84 fresh fruit samples comprising of Mango, Apple, Oranges, Watermelon, Guava, Lime were purchased from major fruit markets. Lactic acid bacteria were isolated from fruits using modified Man Rogosa Sharpe media and phenotypically identified using Gram staining, biochemical tests and API 50CH Kit. Polymerase Chain Reaction (PCR) analysis and 16SrRNA sequences were used to confirm their identity. Antibacterial activity of the cell free supernatant of the LAB was carried out against four pathogenic and two spoilage bacteria respectively. The result obtained indicate that one hundred and ninety fructophilic lactic acid bacteria were isolated and identified into four groups: *Pediococci*, *Lactobacilli*, *Enterococci* and *Leuconostoc* respectively. *Lactobacillus plantarum* had the highest frequency of occurrence while *Lactobacillus alimentarium* had the least. Out of 190 FLAB isolates obtained, only 98 showed inhibitory activity against the pathogenic and spoilage organisms tested, only 23 of the tested

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isolates were able to produce bacteriocin as shown by the effect of enzyme treatments. This study clearly suggests that Fructophilic LAB share similar traditional attributes with those obtained from fermented and dairy food, hence the need to utilize them in food processing and preservation.

**Keywords:** *Fructophilic lactic acid bacteria; characterisation; antagonistic activity.*

## 1. INTRODUCTION

Fructophilic lactic acid bacteria (FLAB) are a special group of LAB, which like fructose as a growth substrate [1]. They are found in fructose-rich niches, e.g. flowers, fruits, and fermented foods made from fruits.

Fruit have good record from public health stand point. Many fruit possess natural defence mechanism. Fruits contain organic acids in quantities adequate enough to contribute a pH value of 4.6 or lower. Fruit juice are popular drinks as they contain antioxidants, vitamins, and mineral that are essential for good nutrition and play a vital role in the prevention of heart disease, cancer and diabetes. They contain essential nutrients which support the growth of acid tolerant bacteria, yeast and moulds [2].

Lactic acid bacteria and microorganisms most frequently used as probiotic agents also exist as the part of indigenous microflora of fruits [3]. Lactic acid bacteria (LAB) are a group of Gram-positive, non-sporulating, anaerobic or facultative aerobic cocci or rods, which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrates [4].

Lactic acid bacteria have been isolated extensively from fermented and dairy food products; most of the LAB used as probiotics and other functions were obtained from dairy and fermented starchy substrates, the recent rise in consumption of fresh fruits and plant-based produce has necessitated to search for new strains of lactic acid bacteria from fresh fruits. This study was undertaken to characterise fructophilic strains of lactic acid bacteria using phenotypic and molecular methods and determine if they share the same traditional attributes with those obtained from dairy and fermented food products.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

A total of 84 fresh fruits samples comprising of oranges 16 (*Citrus sinensis*), banana 12 (*Musa*

*parasidiaca*), lime 12 (*Citrus aurantifolia*), apple 12 (*Malus domestica*), Pineapple 12 (*Ananas comosus*), watermelon (*Citrullus lanatus*) and mango 10 (*Mangifera indica*) and Guava 10 (*Psidium guajava*) were purchased from fruit markets from January to March, 2016 and June to August, 2016. The samples were packed in a sterile polythene bag and transported to the laboratory for microbiological analysis.

### 2.2 Isolation of Fructophilic Lactic Acid Bacteria (FLAB)

Isolation of "FLAB" was carried out using the method of Mahrosh et al. [5] and Endo et al. [6] respectively. To analyze the target micro flora "FLAB", the fruit samples were thoroughly washed with tap water followed by a final rinse with normal saline (0.9%). Each of the fruits was processed separately. The epicarp of the fruits was peeled off using a sterile laboratory knife. Then, 25 g of the fruits were weighed after which were manually chopped with a sterile cutter and then crushed to form a paste with the use of a sterile laboratory mortar and pestle.

Then 10 mls of normal saline was added and was homogenized, then serial dilution of the diluted juice was carried out. 1 ml of the juice extract was aseptically inoculated into 9 ml of De Man Rogosa and Sharpe (MRS) broth modified with addition of 20% Fructose (Oxoid, UK) in MacCartney bottles. Each bottle was labeled accordingly and incubated at 37°C for 18-24 hours.

After incubating at 24 hours, 1 ml of culture was inoculated into modified MRS plates using pour plate method. Then the plates were incubated for 18 hours aerobically, sub culturing was done repeatedly to obtain pure culture. Pure culture that are Gram positive, catalase negative and non-spore former are stored in 20% glycerol broth at -20°C for further microbiological analysis.

### 2.3 Phenotypic Identification of LAB

Characteristics like margin, color, luster, consistency, elevation etc were examined for each isolate.

Suspected Lactic Acid Bacteria were phenotypically identified using biochemical tests and API 50 CHL Kit (Biomerieux, France) according to the methods of Schillinger and Lucke, [7].

## 2.4 Molecular Identification of LAB Isolates

Isolation of genomic DNA was done using Genomic DNA isolation Kit Norgen biotec cat24700. Universal primers developed by LGC Genomics Germany were used for The Forward (5-AGAGTTTGATCCTGGCTCAG-3) and Reverse universal primers (5-ACGGHTACCTTGTTACGACTT-3)

The sequences were aligned in the national center for biotechnology information (NCBI) database using the standard nucleotide-nucleotide homology search BLAST (the basic local alignment search tool) (<http://www.ncbi.nlm.nih.gov/BLAST>). The sequences of the isolates were deposited in GenBank, under the accession numbers MG850837 to MG3850860 Phylogenetic tree of aligned sequences was constructed using neighbor-joining method of Saitou and Neil, [8].

## 2.5 Antibacterial Activity

Antibacterial activity of LAB against spoilage and pathogenic bacteria was performed using agar well diffusion method as described by Savadago et al. [9]. Type culture (ATCC) of *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* while *Escherichia coli* and *Bacillus cereus* isolated from spoilt fruits were used as indicator organisms; their identity was confirmed using biochemical tests.

## 2.6 Test Organisms

Stock culture of indicator organisms were maintained on nutrient agar slants in the refrigerator. Cell free supernatants of LAB were screened for antagonistic activity against the

indicator bacteria inoculated on Mueller Hinton (Oxoid, UK) media and incubated at 37°C for 18-24 hours. Zone of inhibition was measured in millimeters after incubation.

## 2.7 Effect of Enzymes on Antimicrobial Activity

The effect of enzymes on antimicrobial activity of fructophilic lactic acid bacteria isolated in this study was carried out using the method of Chen et al. [10]. Cell free supernatant of the isolates was treated with the following enzymes proteinase K (pH 7), trypsin (pH 7) and catalase (pH 7) respectively procured from Sigma, Germany while sterile distilled water was used as control. After treatment with the various enzymes, antimicrobial activity was detected by agar well diffusion method.

## 2.8 Statistical Analysis

Data are expressed as mean and standard deviation. Statistical significance was determined using analysis of variance where  $P \leq 0.05$  was considered significant.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [26]. The tree with the highest log likelihood (-3265.1849) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 25 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1299 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [27].

## Lactic Acid Bacteria and Their Accession Number:

S/N	Code	Source	Lactic acid bacteria	Accession number
1	UIB12	BANANA	<i>Lactobacillus plantarum</i>	MG850848
2	UIB19	BANANA	<i>Lactobacillus plantarum</i>	MG850855
3	UIO1	ORANGE	<i>Lactobacillus plantarum</i>	MG850837
4	UIW02	WATER MELON	<i>Lactobacillus plantarum</i>	MG850838
5	UIM7	MANGO	<i>Pediococcus pentosaceus</i>	MG850843
6	UIA9	APPLE	<i>Pediococcus pentosaceus</i>	MG850845

S/N	Code	Source	Lactic acid bacteria	Accession number
7	UIO21	ORANGE	<i>Pediococcus pentosaceus</i>	MG850857
8	UIA15	APPLE	<i>Enterococcus faecium</i>	MG850851
9	UIL20	LIME	<i>Enterococcus faecium</i>	MG850856
10	UIB4	BANANA	<i>Enterococcus faecium</i>	MG850840
11	UIP8	PINEAPPLE	<i>Enterococcus faecium</i>	MG850844
12	UIL11	LIME	<i>Enterococcus faecium</i>	MG850847
13	UIO17	ORANGE	<i>Enterococcus faecium</i>	MG850853
14	UIB18	BANANA	<i>Enterococcus faecium</i>	MG850855
15	GY2	GUAVA	<i>Leuconostoc mesenteroides</i>	Number not given yet

### 3. RESULTS AND DISCUSSION

Fresh fruit samples were processed for enumeration of fructophilic lactic acid bacteria. All the isolates obtained in this study are gram positive, all are negative for catalase, oxidase, indole, methyl-red, voges-proskauer, endospore/spore staining, variable results were obtained for production of ammonia from Arginine, most of the LAB isolates were cocci while others are bacilli, the colonies appear white or creamy on the plates, colonies were small, raised and shining (Table 1.1).

Most of the LAB isolates obtained in this study were able to ferment D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-raffinose, while variable results were obtainable 1.2) for other sugars like D-arabinose, D-melastine (T Fig. 1 shows the frequency of occurrence of lactic acid bacteria isolated from fresh fruits; *Lactobacillus plantarum* had the highest, followed by *Enterococcus sp*, *Pediococcus pentosus*, *Pediococcus acidilacto*, *Lactobacillus fermentum*, *L. delbruecki*, *Streptococcus thermophiles*, *L. pen*, *L. lactis*, *Leuconostoc mesenteroides*, *L. brevis*, *L. helveticus* and the least frequency was found *Lactobacillus alimentarius*. All the fresh fruits samples in this study harbor all the LAB isolates except *L. alimentarius*. The LAB isolates were phenotypically identified using their reaction to sugars on API 50CHL galleries.

24 FLAB isolates were subjected to 16rRNA analysis, the isolates had 97-99% homology with type strain in NCBI, they were identified into the *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Pentococcus pentosaceus*. The morphology and biochemical characteristics of the fructophilic lactic acid bacteria obtained in this study were similar to LAB isolates obtained from dairy and fermented food products as reported by Panda et al. [11]. The occurrence of LAB from fresh fruits has been reported by Trias et al. [12]; Abubakar and Al-adiwish [13] isolated LAB with proteolytic

activity from fruits; Chen et al. [10] isolated LAB from ripe Mulberries; Emernini et al. [14] isolated *Lactobacillus pentosus*, *Weissella species* and *Lactobacillus plantarum* from fruits with *L. plantarum* with the highest frequency of occurrence, a report similar to what was obtained in this study. *Lactobacillus plantarum*, *L. fermentarium* and *Leuconostoc mesenteroides* obtained from fruits in this study have been previously isolated from unpasteurized orange juice by Parish and Higgins [15] LAB has equally been isolated from fruits Pulp process by-products by Garcia et al. [16]; *L. brevis*, *L. paracasei*, *L. plantarum* and *L. fermentum* respectively were obtained from this study; Chen et al. [17] isolated LAB belonging to *Lactobacillus* and *Weissella* from banana fruits in Taiwan in which only 36 had bacteriocin activity from 164 LAB isolates, Di Cagno et al. [18] isolated *Lactobacillus plantarum* and *Lactobacillus rossiae* from Pineapple fruits., Yang et al. [19] obtained LAB from fruits residue of banana leaf and stem, pineapple peel and pineapple peel in which *Lactobacillus plantarum* had the highest frequency of occurrence, LAB had also been recovered from corn-lime juice by George-Okafor and Anosike [20] The occurrence of *Enterococcus faecium* in the fruits sampled is similar to the report of Bello et al. [21] reported that LAB obtained from fresh pepper and tomatoes had inhibitory activity against common food pathogens.

#### 3.1 Antagonistic Activity

98 isolates out of 190 FLAB isolates had varying inhibitory activity against six indicator bacteria, as shown in Table 2.1 The LAB isolates in this study showed remarkable antagonistic activity similar to those obtained from dairy and fermented source as reported by Ogunbanwo et al. [22].; the degree of inhibition exerted against the test organisms used in this study is similar to what was obtained from LAB from fruits as reported by Trias et al. [12]. Medouakh et al. [23] had earlier reported the inhibition of *Helicobacter pylori* by cell-free supernatants of *Lactobacillus spp*

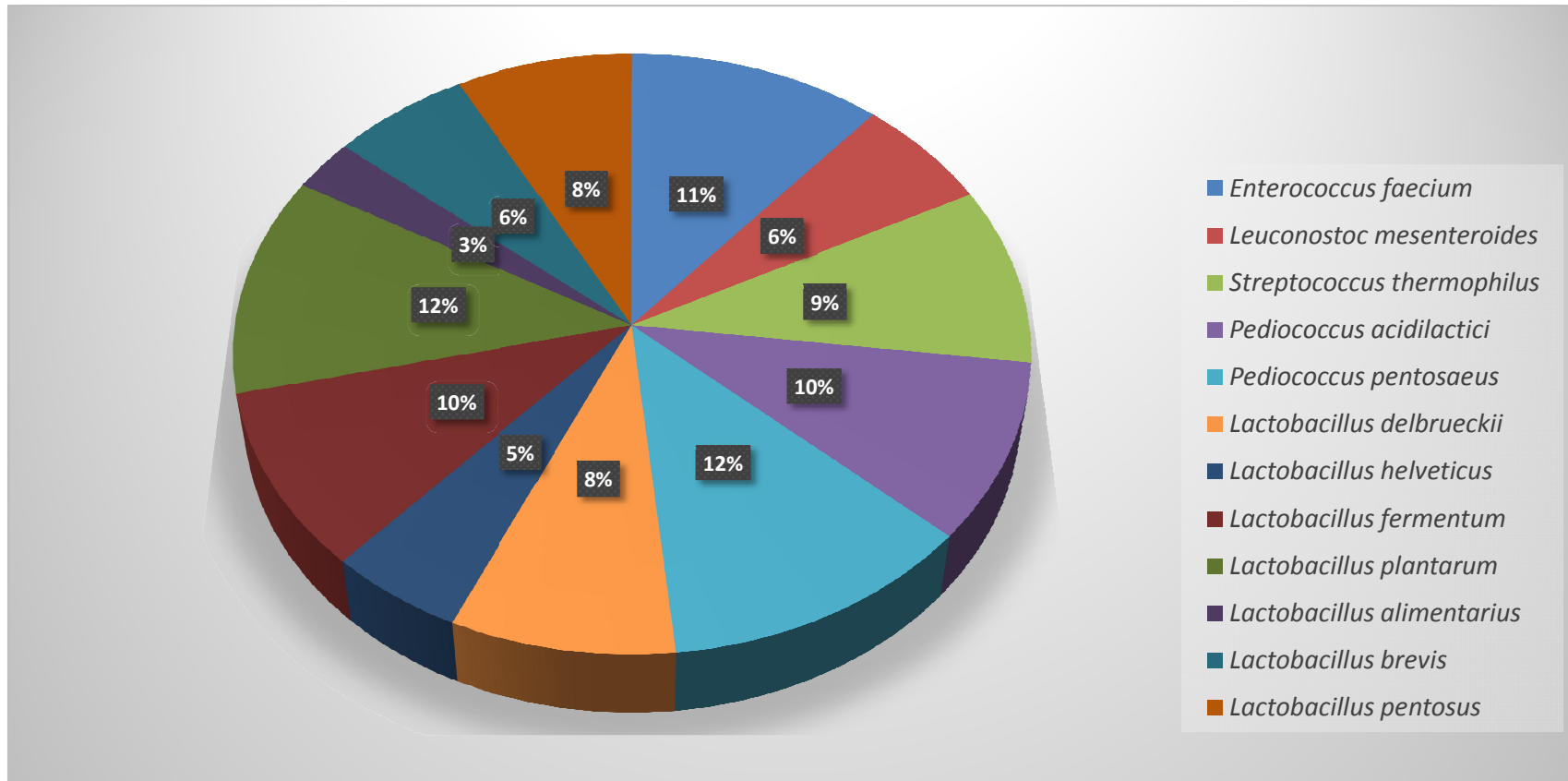


Fig. 1. Percentage occurrence of FLAB isolated from fresh fruits

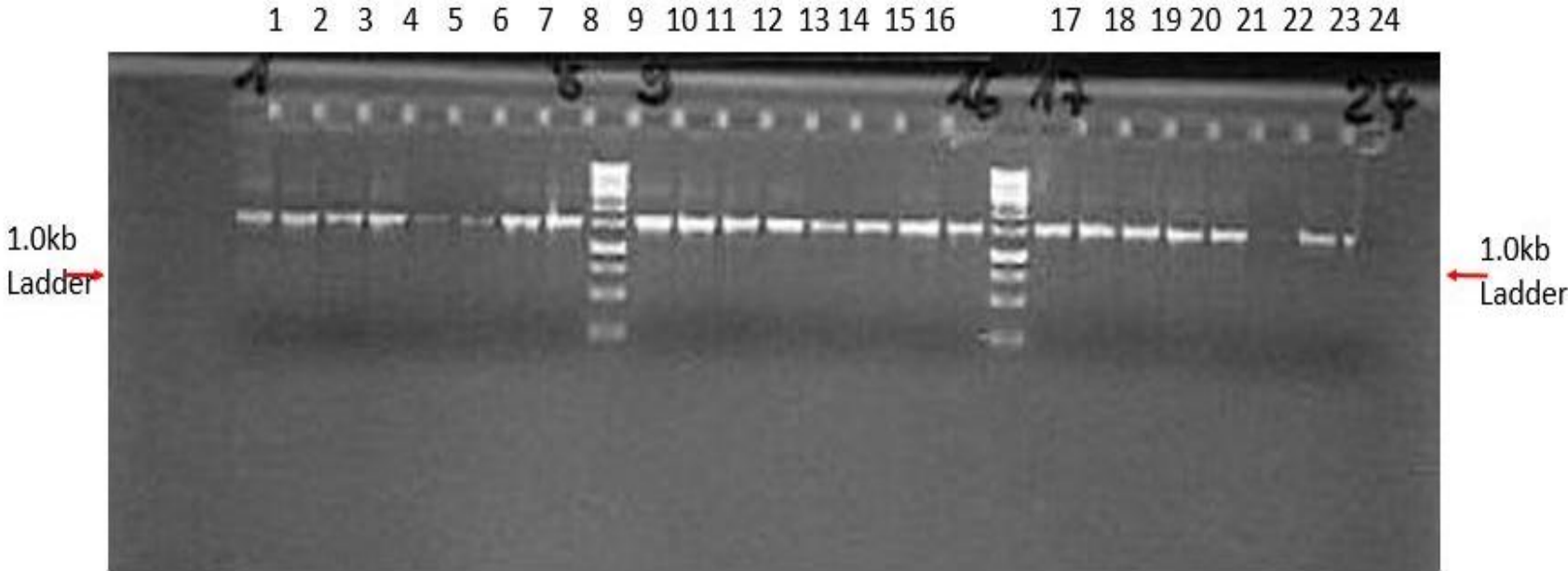


Plate 1: Amplified PCR product of 16S rRNA gene on 1% agarose gel

Table 1.1. Morphological and biochemical characteristic of antimicrobial producing lab isolates from fresh fruits

S/N	ISOLATE CODE	GRAM REACTION	SHAPE	CATALASE	INDOLE	OXIDASE	MP	VP	PRODUCTION OF AMMONIA FROM ARGinine	ENDOSPORE STAINING	COLOUR	MARGIN	ELEVATION	LUSTER	CONSISTENCY
1	OD1	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
2	OD2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
3	OD3	+	C	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
4	OD4	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
5	OY1	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
6	OY2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
7	OY3	+	C	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
8	OY4	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
9	OY5	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
10	OY6	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
11	OY7	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
12	OY8	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
13	OY9	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
14	OY10	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
15	OLG1	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
16	OLG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
17	OLG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
18	OLG4	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
19	OLG5	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
20	OG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
21	OG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
22	OG3	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
23	LLG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
24	LLG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
25	LLG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous

S/N	ISOLATE CODE	GRAM REACTION	SHAPE	CATALASE	INDOLE	OXIDASE	MP	VP	PRODUCTION OF AMMONIA FROM ARGinine	ENDOSPORE STAINING	COLOUR	MARGIN	ELEVATION	LUSTER	CONSISTENCY
26	LLG4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
27	LD1	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
28	LD2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
29	LY1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
30	LY2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
31	LY3	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
32	LY4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
33	LG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
34	LG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
35	LG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
36	MD1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
37	MD2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
38	BLG 1	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
39	BLG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
40	BLG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
41	BLG4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
42	BY1	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
43	BY2	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
44	BY3	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
45	BY4	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
46	BY5	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
47	BD1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
48	BD2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
49	BD3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
50	BD4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
51	PLG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
52	PLG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous



S/N	ISOLATE CODE	GRAM REACTION	SHAPE	CATALASE	INDOLE	OXIDASE	MP	VP	PRODUCTION OF AMMONIA FROM ARGININE	ENDOSPORE STAINING	COLOUR	MARGIN	ELEVATION	LUSTER	CONSISTENCY
53	PLG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
54	PLG4	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
55	PLG5	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
56	PLG6	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
57	PLG7	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
58	PD2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
59	PY1	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
60	PY2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
61	PY3	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
62	PY4	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
63	PG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
64	PG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
65	PG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
66	WD1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
67	WD2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
68	WD3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
69	WD4	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
70	WG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
71	WG2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
72	WG3	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
73	WG4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
74	WG5	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
75	WG6	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
76	WO1	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
77	WO2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
78	ALG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
79	ALG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous

S/N	ISOLATE CODE	GRAM REACTION	SHAPE	CATALASE	INDOLE	OXIDASE	MP	VP	PRODUCTION OF AMMONIA FROM ARGININE	ENDOSPORE STAINING	COLOUR	MARGIN	ELEVATION	LUSTER	CONSISTENCY
80	ALG3	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
81	ALG4	+	R	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
82	ALG5	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
83	ALG6	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
84	MJ2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
85	AD1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
86	AD2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
87	AD3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
88	AD4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
89	AD5	+	C	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
90	AD6	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
91	AY1	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
92	AY2	+	R	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
93	AY3	+	C	-	-	-	-	-	-	-	Small	Creamy	Raised	Smooth shining	Butyrous
94	AG1	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
95	AG2	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
96	AG3	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
97	AG4	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous
98	AG5	+	C	-	-	-	-	-	+	-	Small	Creamy	Raised	Smooth shining	Butyrous

Table 1.2. API sugar fermentation of antimicrobial producing lactic acid bacteria isolates

S/N	Isolates	D-arabinose	L-arabinose	D-ribose	D-galactose	D-glucose	D-fructose	D-mannose	L-rhamnose	L-sorbose	Dulcitol	Inositol	Methyladmannopyros	Methyladglucopyraos	Inulin	D melezitose	D raffinose	Starccch	Glycogen	D turanose	D tagatose	L arabitol	Gluconate	2 keto gluconate	5 keto gluconate	PROBABLE IDENTITY
1	OD1	-	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus acidophilus</i>
2	OD2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Leuconostoc mesenteroides</i>
3	OD3	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Enterococcus sp</i>
4	OD4	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus plantarum</i>
5	OY1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus pentosus</i>
6	OY2	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactococcus lactis</i>
7	OY3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Leuconostoc mesenteroides</i>
8	OY4	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus delbruecki</i>
9	OY5	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Pediococcus acidilacti</i>
10	OY6	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	+	+	-	-	<i>Streptococcus thermophilus</i>
12	OY8	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	+	-	-	<i>Enterococcus faecium</i>
13	OY9	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Pediococcus acidolacti</i>
15	OLG1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Lactobacillus brevis</i>
16	OLG2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Lactococcus lactis</i>
17	OLG3	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Pediococcus acidilacti</i>
18	OLG4	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Lactobacillus helveticus</i>
19	OLG5	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Streptococcus spp</i>
20	OG1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	+	+	-	-	<i>Lactococcus lactis</i>
21	OG2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus rhainnosus</i>
22	OG3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus casei</i>
25	LLG3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Pediococcus pentosaceus</i>
26	LLG4	-	+	+	+	+	+	+	W	-	-	-	+	-	+	+	+	-	-	-	+	-	+	-	-	<i>Streptococcus spp</i>
27	LD1	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus plantarum</i>
28	LD2	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Lactobacillus fermenta</i>
30	LY2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	-	-	<i>Lactobacillus brevis</i>
31	LY3	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Lactobacillus pentosus</i>

S/N	Isolates	D-arabinose	L-arabinose	D-ribose	D-galactose	D-glucose	D-fructose	D-mannose	L-rhamnose	L-sorbose	Dulcitol	Inositol	Methyladmannopyros	Methyladglucopyraos	Inulin	D melezitose	D raffinose	Starcch	Glycogen	D turanose	D tagatose	L arabitol	Gluconate	2 keto gluconate	5 keto gluconate	PROBABLE IDENTITY	
33	LG1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	<i>Lactococcus lactis</i>	
34	LG2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Pediococcus denosis</i>
35	LG3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Enterococcus spp</i>
36	MD1	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	<i>Leconostoc mesenteroides</i>
37	MD2	-	+	+	+	+	+	-	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus plantarus</i>
38	BLG1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus fermentum</i>
39	BLG2	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus fermentum</i>
40	BLG3	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	+	+	-	-	+	-	-	<i>Pediococcus pentosaceus</i>
41	BLG4	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactococcus lactis</i>
42	BY1	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus helveticus</i>
43	BY2	-	+	+	+	+	+	+	W	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus brevis</i>
44	BY3	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus pentosus</i>
45	BY4	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus acidophilus</i>
47	BD1	-	+	+	+	+	+	+	W	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Pediococcus dannosus</i>
48	BD2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	<i>Enterococcus faecum</i>
49	BD3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Enterococcus faecum</i>
50	BD4	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Enterococcus faecium</i>
51	PLG1	-	+	+	+	+	+	+	W	-	-	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	<i>Enterococcus faecium</i>
52	PLG2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Streptococcus thermophile</i>
53	PLG3	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	-	+	-	-	+	-	-	<i>Streptococcus spp</i>
54	PLG4	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus brevis</i>
55	PLG5	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus pentosus</i>
56	PLG6	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus plantarum</i>
57	PLG7	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus fermentum</i>
58	PLG8	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus fermentum</i>
59	PY1	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus platarum</i>
61	PY3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus brevis</i>
62	PY4	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	<i>Leuconostoc mesenteroides</i>
63	PG1	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus fermentum</i>

S/N	Isolates	D-arabinose	L-arabinose	D-ribose	D-galactose	D-glucose	D-fructose	D-mannose	L-rhamnose	L-sorbose	Dulcitol	Inositol	Methyladmannopyros	Methyladglucopyraos	Inulin	D melezitose	D raffinose	Starcch	Glycogen	D turanose	D tagatose	L arabitol	Gluconate	2 keto gluconate	5 keto gluconate	PROBABLE IDENTITY
64	PG2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	<i>Pediococcus spp</i>
65	PG3	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	<i>Enterococcus spp</i>
66	WD1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus acidophile</i>
67	WD2	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus brevis</i>
68	WD3	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	<i>Enterococcus faecium</i>
69	WD4	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	<i>Enterococcus faecium</i>
70	WG1	-	+	+	+	+	+	+	W	-	-	-	+	-	+	+	-	-	-	+	+	-	+	-	-	<i>Pediococcus acidolactic</i>
71	WG2	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus platarium</i>
72	WG3	-	+	+	+	+	+	+	W	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	<i>Lactobacillus plantarum</i>
73	WG4	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	<i>Leuconostoc</i>
74	WG5	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	<i>Streptococcus thermophilis</i>
75	WG6	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Lactobacillus delbruecki</i>
76	WO1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Lactobacillus plantarum</i>
77	WO2	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus plantarum</i>
78	ALG1	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Enterococcus spp</i>
79	ALG2	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Pediococcus spp</i>
80	ALG3	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	+	+	-	+	-	-	<i>Lactobacillus thamnusus</i>
81	ALG4	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Lactobacillus brevis</i>
82	ALG5	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus planterun</i>
83	ALG6	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Lactobacillus brevis</i>
84	ALG7	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Pediococcus pentosaceus</i>
85	AD1	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Pentococcus damnosus</i>
86	AD2	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Streptococcus spp</i>
87	AD3	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Enterococcus feacalis</i>
88	AD4	-	+	+	+	+	+	+	W	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	<i>Leuconostoc mesenteroides</i>
89	AD5	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	<i>Pediococcus pentosaceus</i>
90	AD6	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	<i>Lactobacillus delbruecci</i>
91	AY1	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Lactobacillus rhennoles</i>
92	AY2	-	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	<i>Lactobacillus acidophilus</i>

S/N	Isolates	D-arabinose	L-arabinose	D-ribose	D-galactose	D-glucose	D-fructose	D-mannose	L-rhamnose	L-sorbose	Dulcitol	Inositol	Methyladmannopyros	Methyladglucopyraos	Inulin	D melezitose	D raffinose	Starcch	Glycogen	D turanose	D tagatose	L arabitol	Gluconate	2 keto gluconate	5 keto gluconate	PROBABLE IDENTITY
93	AY3	-	+	+	+	+	+	+	W	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-	<i>Lactobacillus betis</i>
94	AG1	-	+	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	<i>Enterococcus spp</i>
95	AG2	-	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	<i>Pediococcus spp</i>
97	AG4	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	<i>Lactococcus latis</i>
98	AG5	-	+	+	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-	+	-	-	+	-	-	<i>Lactobacillus pentosus</i>

Table 2.1. Categorization of antimicrobial activity of lactic acid bacteria

Antimicrobial activity	Diameter of zone of inhibition	Number of isolates
VERY STRONG	>25	18
STRONG	>15	39
MODERATE	<15	24
WEAK	<10	17
		<b>98</b>

Table 3. Antimicrobial activity of selected lactic acid bacteria against test bacteria

S/N	Isolate code	Isolate	ZONE OF INHIBITION (MM)					
			A	B	C	D	E	F
1	ORG1	<i>Lactobacillus plantarum</i>	28.67±2.51	29.33±2.51	30.0±2.00	28.00±1.00	28.67±2.51	27.67±2.30
2	WG3	<i>Lactobacillus plantarum</i>	29.33±2.51	30.33±3.05	27.33±1.53	28.00±4.35	29.00±2.00	28.33±2.08
3	BY2	<i>Enterococcus faecium</i>	29.00±2.00	29.00±2.64	27.67±2.30	29.00±3.60	29.33±2.51	29.00±2.64
4	BY3	<i>Enterococcus faecium</i>	30.67±2.08	28.00±1.00	29.33±3.05	28.67±3.51	27.67±3.78	28.67±3.51
5	AG1	<i>Enterococcus faecium</i>	28.33±1.52	28.00±4.35	29.33±3.05	28.67±3.78	29.00±3.60	28.00±1.00
6	MJ2	<i>Pediococcus pentosaceus</i>	28.33±3.21	28.00±1.73	29.67±2.08	28.33±3.21	28.00±2.64	27.67±3.78
8	AG2	<i>Pediococcus pentosaeus</i>	27.33±3.21	27.33±0.57	29.67±0.57	28.00±3.60	29.00±2.00	29.67±2.08
9	BLG	<i>Lactobacillus plantaran</i>	28.33±0.57	30.33±1.52	29.33±2.51	29.00±3.60	29.67±0.577	29.00±1.73
10	ALG2	<i>Enterococcus faecium</i>	28.67±0.57	28.67±3.21	28.00±3.46	27.67±1.52	26.33±1.52	30.33±1.52

S/N	Isolate code	Isolate	ZONE OF INHIBITION (MM)					
			A	B	C	D	E	F
11	OG2	<i>Enterococcus faecium</i>	26.67±1.52	27.33±1.52	30.00±1.73	28.67±0.57	28.67±3.21	27.67±1.52
12	BD3	<i>Enterococcus faecium</i>	28.67±3.51	28.00±3.46	28.67±4.04	29.67±2.08	28.33±0.57	28.00±2.64
13	BY3	<i>Lactobacillus plantarum</i>	26.67±1.52	28.67±2.52	27.67±2.51	27.67±2.30	25.33±0.57	26.67±1.52
14	LY2	<i>Lactobacillus faecium</i>	28.00±2.64	27.33±4.04	30.67±1.52	28.33±3.24	27.33±2.30	29.33±3.21
15	PG2	<i>Pediococcus pentosaceus</i>	31.00±2.64	28.33±2.51	28.00±1.00	27.33±0.57	28.67±1.52	28.33±3.51
16	OY3	<i>Leuconostoc mesenteroides</i>	28.00±3.00	29.67±2.51	30.33±1.52	29.67±2.08	29.33±3.21	27.67±3.78

KEY: A *Staphylococcus aureus*; B *Bacillus cereus*; C *Salmonella typhi*; D *Klebsiella pneumoniae*; E *Pseudomonas aeruginosa*; F *Escherichia coli*

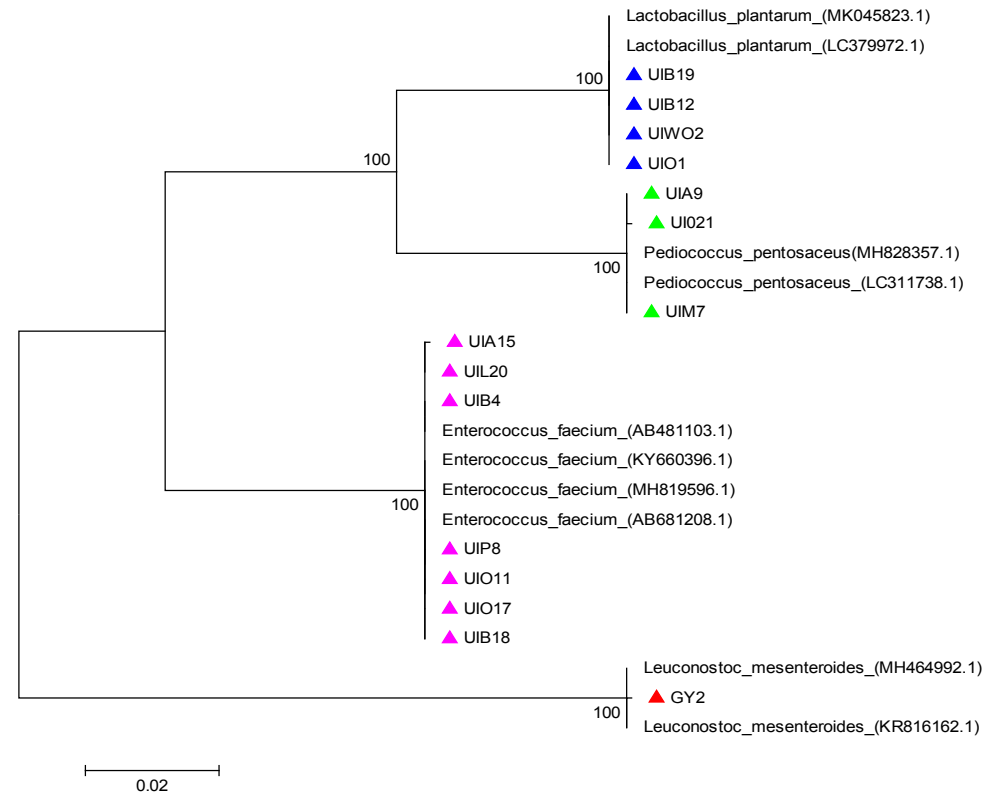


Fig. 2. Molecular Phylogenetic analysis by Maximum Likelihood method

**Table 4. Effect of enzyme treatments on selected fructophilic lactic acid bacteria**

		Enzyme treatments			
		Diameter of the zone of inhibition {Mm}			
		Control	Proteinase K	Trypsin	Catalase
1	<i>Lactobacillus plantarum</i>	15	00	00	14.3
2	<i>Pediococcus pentosaeus</i>	11.5	00	00	9.9
3	<i>Enterococcus faeciun</i>	11.3	00	00	11.5
4	<i>Leuconostoc mesenteroides</i>	12.3	00	00	8.8
5	<i>Lactobacillus pentosus</i>	11.5.	00	00	11.1
6	<i>Lactobacillus fermentum</i>	12.3	00	00	7.5

obtained from goat's milk; Zhou et al. [24] reported the inhibition of *Aeromonas hydrophilia* by *Lactococcus lactis* obtained from fresh milk [11]. Okereke et al. [25] reported the inhibition of *S. aureus*, *E. coli* and *Bacillus cereus* by bacteriocin-producing LAB isolates, a report that is in agreement to with this study as some of the isolates are bacteriocin-producers.

#### 4. CONCLUSION

Lactic acid bacteria obtained from fresh fruits in this study share similar characteristics with those obtained from dairy and fermented products as obtained from the Blast and sequencing data, they equally possess antibacterial properties with production of bacteriocin that can be harnessed as bio preservatives in fruit juices.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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