



## Development of Probiotic Beverages Using Fingermillet [*Eleusinecoracana* (L.) Gaertn.] and Banana [*Musa* spp.] as Prebiotic Substrates

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### Authors' contributions

This work was carried out in collaboration among all authors. Author DMWDD conducted the laboratory experiments and wrote the first draft of the manuscript. Author JKRRS received the research grant and supervised the research. Authors CH, JG and SG were co-investigators and supervised the research. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** Development of probiotic beverages using previously isolated probiotic strains; *Lactobacillus plantarum* MF405176.1 and *Lactobacillus curieae* MF405178.1 in finger millet and banana flour substrates, respectively and monitor the microbiological, physicochemical and sensory properties of formulated probiotic beverages.

**Place and Duration of Study:** Food Technology Section, Industrial Technology Institute, Colombo, Sri Lanka. Between November 2017 to April 2018.

**Methodology:** Moisture content reduced ( $9 < 10\%$ ) finger millet (*ravivar.*) and banana (*ambalnadee* var.) flour were weighted separately (25 g each), suspended in individual containers consisting of 100 ml potable water ( $n=6$ ) and homogenised to obtain slurries. The slurries were sterilized ( $121 \pm$

1°C for 15 min) and cooled ( $35 \pm 1^\circ\text{C}$ ) prior to inoculation of starter cultures. Previously isolated, freeze dried probiotic strains; *L. plantarum* MF405176.1 and *L. curieae* MF405178.1 were inoculated in to finger millet and banana slurries, respectively at probiotic cell concentration of  $10^{10}$  CFU/ml. Slurries were allowed to ferment ( $37 \pm 1^\circ\text{C}$ ) until the pH reaches  $< 3.5$ . Throughout fermentation, pH was monitored hourly, while probiotic cell viability was measured at every 4h. Final products were evaluated for viable probiotic cell count, chemical composition (protein, fat and ash content), physical properties (pH, moisture, total soluble solids and titrable acidity), microbiological quality (aerobic plate count, Yeasts and Mould count, Coliform and *Escherichia coli*), shelf-life (for 5 weeks at  $4 \pm 1^\circ\text{C}$ ) and Sensory properties (color, odor, appearance, texture and overall acceptability using 9 point hedonic scale).

**Results:** Gradual increment of probiotic cell count with fermentation time was observed in both beverages. Compared to their respective controls, significant difference ( $P = .05$ ) in physical properties (total soluble solids, titrable acidity and pH) and significant increment ( $P = .05$ ) in chemical properties (fat and protein content) was observed in both beverages. Finger millet based probiotic beverage containing *L. plantarum* MF405176.1 exhibited better physical, chemical properties and higher acceptability. Further it demonstrated better shelf life compared to banana based beverage containing *L. curieae* MF405178.1. Both products could sustain the viability of probiotic starter cultures up to  $10^9$  CFU/ml even at the end of 5<sup>th</sup> week of shelf life period thus demonstrated the compatibility of finger millet and banana flour as ideal prebiotic substrates for development of probiotic food.

**Conclusion:** Study highlighted the prebiotic potentiality of finger millet and banana flour for the development of dairy free probiotic food. It confirms the behavior of new probiotic strains *L. plantarum* MF405176.1 and *L. curieae* MF405178.1 as starters in lactic acid fermentation.

**Keywords:** Banana; finger millet; lactobacillus plantarum; lactobacillus curieae; prebiotics; probiotics.

## 1. INTRODUCTION

Probiotics are 'live microorganisms, when administered in adequate amounts confer health benefit to the host' [1]. Application of probiotics as self-medications and as dynamic functional food and beverage are emerging due to its role in reduction and prevention of various adverse physiological conditions in human and animal [2]. Prebiotics are indigestible food components that promote the growth and activity of beneficial probiotic bacteria, thereby benefiting the host [3]. Other than *Lactobacillus*, several commercial probiotics, belonging to the genera of *Bifidobacterium*, *Streptococcus*, *Lactococcus* and certain species of *Enterococcus* are extensively used in probiotic food products and supplements. To make probiotic claims, food or supplement should contain minimum dose of  $10^7$  Colony Forming Unit (CFU)/g or ml of probiotic bacteria in food matrix [4]. At present global probiotic food market worth  $> 40$  billion USD and forecasted a growth of 11.7% per year [5,6]. Even though the global probiotic market is strongly linked with dairy industry, it is predicted that 33% share of the functional food market is of non-dairy origin. Drift towards vegetarianism, high prevalence of lactose intolerance in many populations around the world, high cholesterol content in dairy products and allergenic milk proteins are the key

contributory factors for the development of novel non-dairy probiotic foods and supplements [3,7]. Reduction of gastrointestinal, upper respiratory track and urogenital infections, improvement in lactose metabolism and inflammatory bowel disease, reduction of serum cholesterol and hypertension, suppression of *Helicobacter pylori* infection and depressive symptoms [8,9] are some of the major functions of probiotics. Further, they exhibit anti-carcinogenic, anti-diarrheal, anti-oxidant, anti-microbial and anti-aging properties [10,11]. In order to achieve competitive advantage over other commercial probiotic products, global non-dairy probiotic industry is progressing towards formulating dairy free, allergen free, religious (kosher, halal) professional brands, while shifting their delivery modes from traditional tablets and liquid concentrates to encapsulated products, oral shots and confectioneries [12]. Cereals and fruits are rich in prebiotics, abundantly available matrixes having the ability to undergo structural changes; therefore ideal substrates for the development of non-dairy probiotic food [13]. Among cereals, finger millet (*Eleusinecoracana* (L.) Gaertn.) being the 4<sup>th</sup> most important food crop in terms of area coverage [14] is commonly consumed in Africa and South Asia. Due to its high yield, superior adaption to diverse environmental conditions, resistance to pests

and diseases, finger millet has been denoted as the crop for future use [15]. Among fruits, banana (*Musa* species) being the 4<sup>th</sup> largest food crop of the world is abundantly cultivated in tropical and subtropical regions globally [16] and one fifth of the harvest is underutilized [17]. Both finger millet and banana are rich in resistant starch and dietary fiber [18,19] therefore appropriate as substrates for fermentation by probiotic bacteria. Presence of phenolic compounds in these two crops can attributed to the development of colour, oxidative stability, flavor and texture [20] of fermented food.

Considering the scope of finger millet and banana as non-dairy fermentable substrates, this study aimed to develop probiotic beverages by inoculating previously isolated probiotic Lactic Acid Bacteria (LAB); *Lactobacillus plantarum* MF405176.1 [3] and *Lactobacillus curieae* MF405178.1 [8] using finger millet and banana flour as substrates, respectively and monitor the microbiological, physicochemical and sensory properties of formulated probiotic beverages.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Finger Millet and Banana Flour

Finger millet var. *ravi* and banana var. *ambalnadee* was collected from the germplasm of the Field Crop Research and Development Institute, Mahailuppallama, Sri Lanka and Agriculture Research Centre, Girandurukotte, Sri Lanka, respectively. Finger millet seeds were washed with sterilized water and dried at  $35 \pm 2^\circ\text{C}$  in an oven (Memmert Universal Oven U, Germany) until the moisture content reduced to < 10%. Peels of the banana fruits were removed; flesh was cut in to 4 mm thickness slices and soaked in 1% (w/v) solution of Sodium Metabisulphite for 10 min to avoid enzymatic browning. Soaked banana pieces were dried in a tray dryer (JKO3RD KINKAI, China) at  $35 \pm 2^\circ\text{C}$  till the moisture content reduced to < 10%. Each dried sample was ground in a variable Speed Rotar Mill (Fritsch PULVERISETTE 14, Germany) and passed through a 0.5 mm sieve attached to the mill. The milled and sieved samples were packed separately in sterile bags, stored in cold room at  $4 \pm 1^\circ\text{C}$ .

### 2.2 Preparation of Freeze-dried Starter Cultures

Probiotic LAB strains *Lactobacillus plantarum* MF405176.1 and *Lactobacillus curieae*

MF405178.1 isolated from fermented finger millet and banana flour were selected for the study [3,8]. These strains demonstrated bioactivity and safety traits during previous studies. The strains were inoculated in to sterile MRS broth and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. Cells were harvested by centrifugation at  $10,000 \times g$  for 15 min at  $4 \pm 1^\circ\text{C}$  followed by suspending in 20 ml of 0.1 M sterile phosphate buffer. The mixtures were re-centrifuged at  $10,000 \times g$  for 15 min at  $4 \pm 1^\circ\text{C}$ . Each pellet was suspended in 20 ml phosphate buffer containing 2% unipectine (w/v), acryo protectant. Subsequently the mixtures of cells with cryo protectants were poured in to sterile petri plates and sealed with para films and foil. The plates were stored (Whilpool CF62TW, SA) at  $20 \pm 1^\circ\text{C}$  for 10 h and transferred to freeze drier (Christ LOC-1m, Germany) for 44 hand 15 min with 15 min initial freezing followed by 40 h of primary (when internal pressure reduced to 100 torr) and 4 h of secondary freezing at  $80 \pm 2^\circ\text{C}$ . Freeze-dried cells were packed in stomacher® bags, sealed well and stored at  $20 \pm 1^\circ\text{C}$  [21]. Growth kinetics of probiotic strains were measured by monitoring the optical density at 600 nm, followed by drawing the respective growth curves. Cell viability of freeze-dried cultures was investigated by plating on MRS agar followed by incubating at  $37 \pm 1^\circ\text{C}$  for 24 h and counting the colonies [22].

### 2.3 Fermentation and Viability Assessment of Starter Cultures

For primary lab scale product development, finger millet var. *ravi* with probiotic strain *L. plantarum* MF405176.1 and banana var. *ambalnadee* with probiotic strain *L. curieae* MF405178.1 were selected. Flours of finger millet and banana were weighted seperately (25 g each) and suspended in individual conical flasks containing cotton plugs. Potable water (100 ml) was added to each flask (n=6) and homogenised (Janke & Kunkel ultra turrex T25, Germany) to obtain the slurries. The slurries were sterilized at  $121 \pm 1^\circ\text{C}$  for 15 min and cooled to  $35 \pm 1^\circ\text{C}$  prior to fermentation. Freeze-dried starter cultures were inoculated in to respective slurries at a concentration of  $10^{10}$  CFU/ml and 5% (w/v) *kithul* (*Caryotaurens*) treacle was incorporated in to all fermentation slurries. Fermentation was performed at  $37 \pm 1^\circ\text{C}$  until pH of the slurry reach < 3.5 (20 h). Throughout the fermentation, pH of the fermented slurries was monitored hourly (Eutechcyberscan PH 11, Singapore). Samples were also drawn aseptically initially and after every 4 h to check

the cell viability. From the each fermented slurry, 1 ml was aseptically drawn and serially diluted in sterile saline, plated on MRS agar by following pour plate technique. Plates were incubated at  $37 \pm 1^\circ\text{C}$  for 48 h and the probiotic colonies were counted (Galaxy 230, Taiwan) and calculated as CFU/ml. Controls were prepared without inoculating probiotic strains.

## 2.4 Analysis of Physiochemical Characteristics and Microbiological Quality

Probiotic beverages were studied for chemical composition including protein (Kjeldhal's distillation unit, VELP, USA), fat (Soxtherm, Gerhardt, UK) and ash (Muffle furnace, Lenton, UK). Further, pH (Eutechcyberscan PH 11, Singapore), moisture (Mettler UE 400, Germany) and total soluble solids (Brixmeter 0 to 32%; Atago, Japan) of final products were also measured. Titrable acidity was measured as lactic acid (%) by titrating the fermented and control slurries with 0.1N NaOH using phenolphthalein as an indicator. To determine the microbiological quality, each sample was serially diluted up to  $10^{-12}$  in sterilized saline (0.85% NaCl, w/v) and analyzed for aerobic plate count [23], Yeasts and Mould count [24], Coliform [25] and *Escherichia coli* [26].

## 2.5 Shelf Life and Sensory Analysis

Shelf life of the fermented beverages was investigated weekly for 5 consecutive weeks under refrigerated conditions at  $4 \pm 1^\circ\text{C}$  (R-

NST2425 Singer, Sri Lanka). Viability of starter culture was measured as described earlier. Sensory properties (color, odor, appearance, texture and overall acceptability) were investigated using 12 trained panelists from institutional sensory panel of the Industrial Technology Institute, Sri Lanka. Score was based on a hedonic scale of 1 to 9 where: 1 = dislike extremely and 9 = like extremely [27].

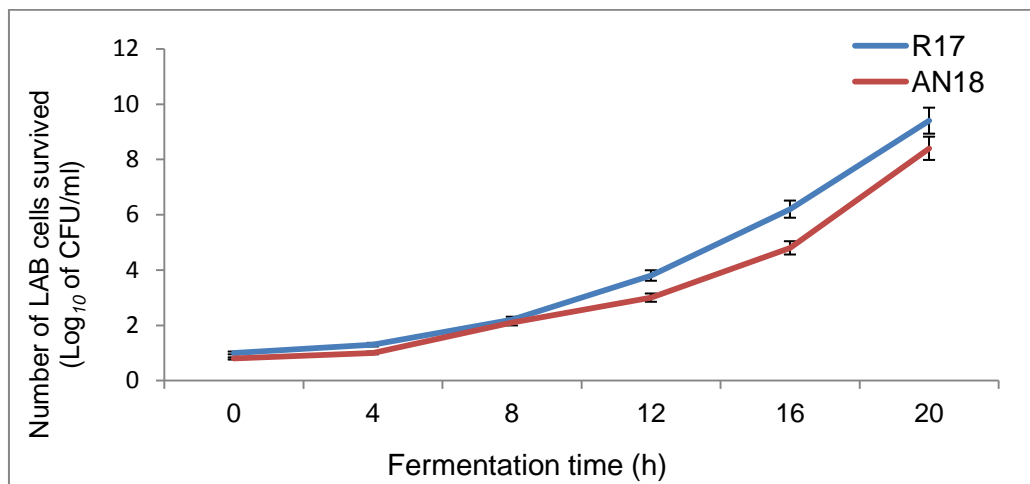
## 2.6 Data Analysis and Interpretation

All the experiments were conducted in triplicates and repeated once. The mean and standard error of the data obtained from parallel experiments were calculated using Minitab 14. The two-sample t-test was performed to analyze the data. Values of  $P=0.05$  considered as significant. Graphs were prepared using Microsoft excel, 2010.

## 3. RESULTS AND DISCUSSION

### 3.1 Viability of Probiotic Strains

Compatibility of finger millet and banana flour with inoculated probiotic strains was observed during the fermentation. This was proved by the gradual increment of probiotic cell count with fermentation time (Fig. 1). Probiotics role in suppression of the multiplication of undesirable and/or pathogenic bacteria could also contribute to this [28]. Cell viability of probiotic strains in their respective fermentation media did not demonstrate significant difference throughout fermentation.



**Fig. 1. Viability of probiotic starters during fermentation at 37°C**

Graph represented the mean values of three parallel experiments. R17 (*L. plantarum* MF405176.1) AN18 (*L. curieae* MF405178.1)

### 3.2 Physiochemical Characteristics and Microbiological Quality of the Probiotic Beverages

Compared to their respective controls, significant difference ( $P = .05$ ) in physical properties including total soluble solids, titrable acidity and pH was observed in both probiotic beverages. However, no significant difference in moisture content was observed. Similarly, a significant difference in ( $P = .05$ ) chemical properties including fat and protein content was observed in both probiotic beverages compared to controls. However, no significant difference in ash content was observed. Among the two probiotic beverages, beverage containing *L. plantarum* MF405176.1 exhibited better physical and chemical properties. This may be due to its' ability of digest prebiotics such as dietary fiber and resistant starch commonly preset in finger millet flour. Compared to respective controls, both beverages exhibited high protein and fat content at the end of fermentation; this may be due to the synthesis of metabolites by probiotic starter cultures. Finger millet based beverage containing *L. plantarum* MF405176.1 as the starter was detected with more proteins ( $2.50 \pm 0.06/100$  ml) compared to previously reported finger millet based fermented beverage containing commercial probiotic strain *Lactobacillus casei* 431 that is  $1.80 \pm 0.03/100$  ml [19]. Further, the products were free from yeast and mould, Coliform and *E. coli* contaminations (Table 1).

### 3.3 Sensory Analysis of the Probiotic Beverages

Beside its' functional properties, symbiotic probiotic food also need to produce sensorial acceptability. Finger millet based probiotic beverage containing *L. plantarum* MF405176.1 showed higher acceptability compared to banana based beverage containing *L. curieae* MF405178.1 (Fig. 2). Further standardization of the product in order to improve the sensory properties is essential. Since the probiotic strains were not yet evaluated for toxicity in humans, flavor of the beverages were not assessed.

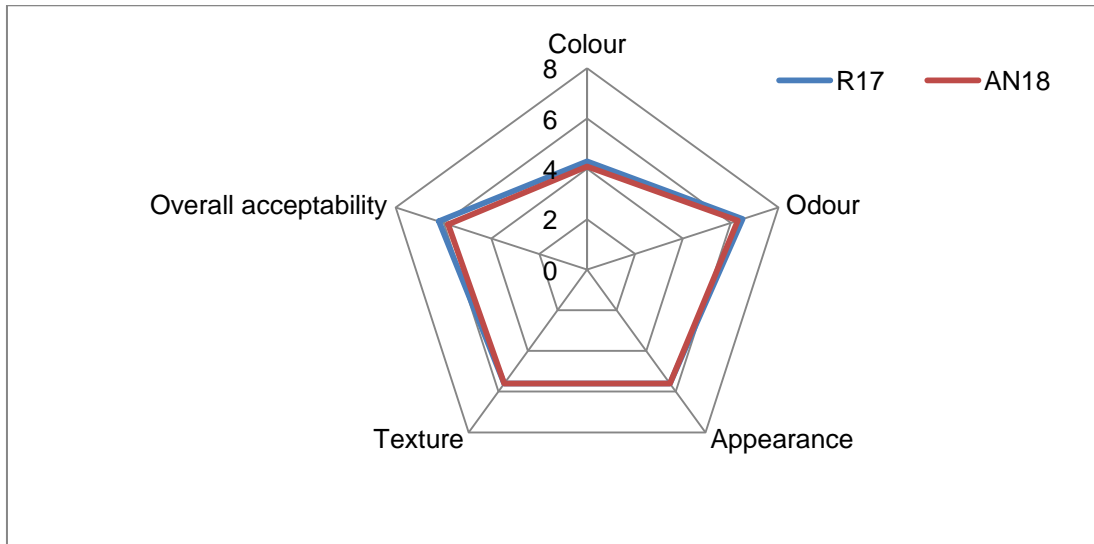
### 3.4 Shelf Life Analysis of the Probiotic Beverages

Probiotic beverages containing *L. plantarum* MF405176.1 and *L. curieae* MF405178.1 exhibited  $\log_{10} 9.63 \pm 1.01$  CFU/ml and  $\log_{10} 8.33 \pm 1.08$  CFU/ml of cell count, respectively, at the beginning of the shelf life study. At the end of 1<sup>st</sup> week, they exhibited  $\log_{10} 8.90 \pm 0.35$  CFU/ml and  $\log_{10} 7.00 \pm 0.29$  CFU/ml, respectively. Subsequently at the end of 2<sup>nd</sup> week,  $\log_{10} 8.46 \pm 0.56$  CFU/ml and  $\log_{10} 7.42 \pm 0.92$  CFU/ml was detected. At the end of 3<sup>rd</sup> week, they exhibited  $\log_{10} 8.30 \pm 0.59$  CFU/ml and  $\log_{10} 7.33 \pm 0.82$  CFU/ml viability, respectively. At the end of 4<sup>th</sup> and 5<sup>th</sup> week, cell viability among the beverages reduced gradually (Fig. 3). However interestingly it was observed that both the products even at the end of 5<sup>th</sup> week is able to retain the viability of

**Table 1. Physiochemical characteristics and microbiological quality of the probiotic beverages**

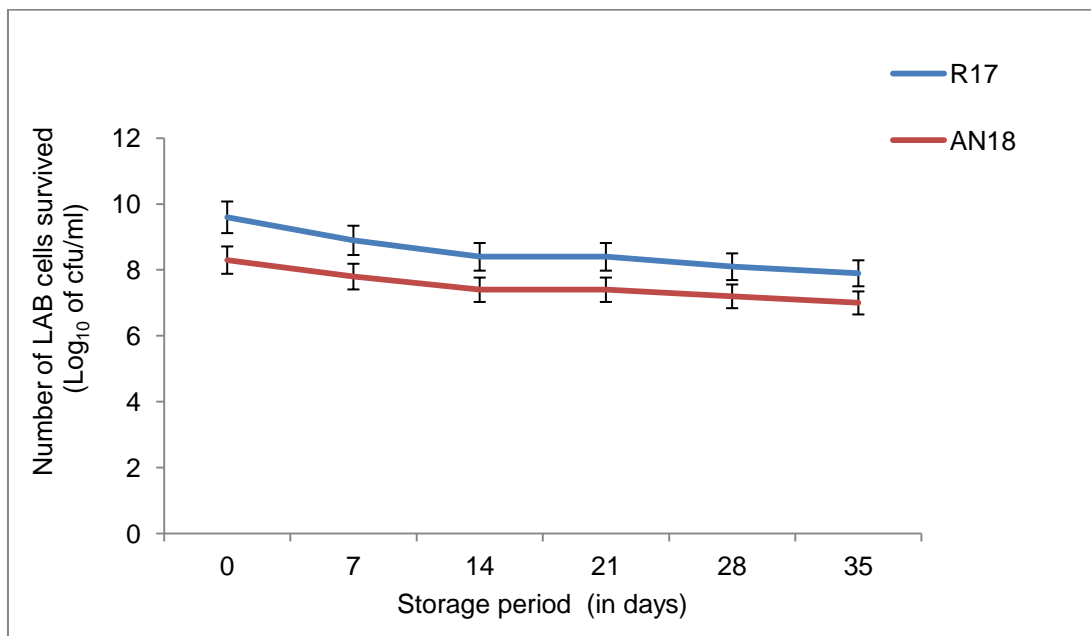
Parameters tested	Finger millet based beverage		Banana based beverage	
	Control	R17	Control	AN18
<b>Physical</b>				
Moisture (%)	74.00 $\pm$ 1.15	82.33 $\pm$ 0.67	73.00 $\pm$ 0.58	81.67 $\pm$ 0.88
Total soluble solids (%)	8.83 $\pm$ 0.03	6.50 $\pm$ 0.00	7.80 $\pm$ 0.00	6.13 $\pm$ 0.03
Titrable acidity (%)	0.38 $\pm$ 0.03	1.27 $\pm$ 0.03	0.20 $\pm$ 0.04	0.88 $\pm$ 0.05
pH	6.80 $\pm$ 0.00	3.48 $\pm$ 0.00	6.10 $\pm$ 0.00	3.51 $\pm$ 0.00
<b>Chemical (/100 ml)</b>				
Ash	0.57 $\pm$ 0.02	0.76 $\pm$ 0.01	0.42 $\pm$ 0.01	0.71 $\pm$ 0.00
Fat	0.08 $\pm$ 0.00	0.14 $\pm$ 0.01	0.10 $\pm$ 0.00	0.25 $\pm$ 0.01
Protein	1.67 $\pm$ 0.03	2.50 $\pm$ 0.06	1.07 $\pm$ 0.03	1.97 $\pm$ 0.07
<b>Microbiological (CFU/ml)</b>				
Total plate count	9.17 $\pm$ 0.91 $\times$ 10 <sup>1</sup>	5.83 $\pm$ 0.87 $\times$ 10 <sup>1</sup>	8.83 $\pm$ 0.54 $\times$ 10 <sup>1</sup>	5.67 $\pm$ 0.96 $\times$ 10 <sup>1</sup>
Yeast and mould	ND	ND	ND	ND
Coliform	ND	ND	ND	ND
<i>E. coli</i>	ND	ND	ND	ND
<i>Salmonella</i>	ND	ND	ND	ND

Data is expressed as mean  $\pm$  SEM, n=6. R17 (*L. plantarum* MF405176.1) AN18 (*L. curieae* MF405178.1) Control: without starter culture. ND: Not Detected.



**Fig. 2. Sensory analysis of the probiotic beverages**

Data is expressed as mean  $\pm$  SEM, n=6. R17 (beverage containing *L. plantarum* MF405176.1) AN18 (beverage containing *L. curieae* MF405178.1)



**Fig. 3. Viability of probiotic starter cultures during storage at 4°C**

Data is expressed as mean  $\pm$  SEM, n=6. R17 (beverage containing *L. plantarum* MF405176.1) AN18 (beverage containing *L. curieae* MF405178.1)

the probiotic cells up to  $10^9$  CFU/ml; which is the key requirement to claim a food as a probiotic product. Finger millet based probiotic beverage containing *L. plantarum* MF405176.1 has better shelf life compared to banana based probiotic beverage containing *L. curieae* MF405178.1. Superior substrate adaptability and substrate compatibility of *L. plantarum* MF405176.1 might have contributed to this trait.

Finger millet flour is reported as one of the most commonly consumed ingredient in traditionally fermented food such as *ogi*, *uji*, *togwa*, *ogi-baba*, *kwunu-zaki*, *degue* and *mangisi* in Africa. Microorganisms involved in fermentation of these foods were identified as *L. plantarum*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus* and *Enterococcus* [29]. Furthermore,

production of *Koozhu*, a finger millet based fermented food product in India reported to contain *Lactobacillus* and *Pediococcus* as starter cultures [30]; thus validate the compatibility of finger millet flour as an ideal substrate for the development of probiotic food [31]. Even though many traditional fermented foods are made in the domestic level; lack of large-scale industrial utilization has discouraged farmers in raising millet crops [32]. Therefore, it is important to investigate and develop a feasible processing technology for nutritional improvement as well as to harvest health benefits and promote their utilization as food on large scale. Typical grain texture and hard seed coat of finger millet is attributed to its' superior keeping quality. However this makes them hard to process in convenient form. Unavailability of the proper processing technologies to develop ready-to-use or ready-to-cook value added products is the major obstacle that limits the diversified use of finger millet [33]. Banana being an abundant low-cost ingredient, several authors have successfully proved the compatibility of banana flour in food product development [34,35,36] including probiotic food [37,38].

#### 4. CONCLUSION

This study emphasizes the potentiality of finger millet and banana as ideal substrates for the development of novel, dairy free probiotic food. Study also demonstrates the behavior of novel probiotic strains *L. plantarum* MF405176.1 and *L. curieae* MF405178.1 as starter cultures during fermentation. Tested probiotic strains could sustain the cell viability during its shelf life period thereby fulfilling the basic requirement to claim as probiotic food. Further, decrease of pH being one of the main limiting factors for spoilage and pathogenic microbial growth thereby contributes to increase food safety as well as sensory properties. Fermentation further enhances the nutritional values and improves the digestibility of prebiotic substrates. Such probiotic food would be beneficial for all age groups including children, adults and senior citizens.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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