CHAPTER 6

To develop a *Lagenaria siceraria* juice enriched with prebiotics and probiotics

6.1. Introduction

Probiotics are defined as live microbial supplements that are beneficial to the host by improving the gut's microbial balance [38]. The positive impact that food that contains living microorganisms (also known as probiotics) may have on human health is increasingly being brought to the attention of medical experts [3, 4]. Most fermented milk products and yoghurt contain probiotics. It has been shown that some probiotic strains of various species of Lactobacillus may have several beneficial impacts on one's health, including immunomodulation, an increase in one's resistance to infections, and a reduction in one's blood cholesterol levels [26]. Consuming dairy and non-dairy products that contain probiotic bacteria may help colonize the human gastrointestinal tract with beneficial bacteria known as probiotics. The growing number of vegetarians among consumers has resulted in an increase in interest in vegetarian alternatives to animal-derived probiotics. The increasing number of people who are lactose intolerant and who are concerned about the high cholesterol content of fermented dairy products has lately led to an increase in the demand among consumers for non-dairy probiotic products [28].

When it comes to the production of probiotic foods, the food substrate is an extremely important factor. To produce probiotic food products, a variety of food matrices of different kinds have been used. Matrixes derived from non-dairy sources, including fruits, vegetables, legumes, and grains, have been employed in the production of probiotic products. The next food category that will allow beneficial bacteria to establish themselves is beverages, and the probable substrates for these bacteria include chilled fruit juices, bottled water, and vegetable juices. Vegetables are a potential replacement to produce probiotic foods as a result of their broad availability and high nutrient content. Fermentation of vegetables in lactic acid enhances their safeness as well as their shelf life, their flavour, and their nutritional value. Altering the pH of the culture medium and adding nutrients to the growth medium are two ways that the structural features of plant source matrices may be changed [5]. Since these food matrices already include healthful components including minerals, vitamins, dietary fibres, and antioxidants, they may be an ideal substrate for probiotic microbes as a result of this property. In addition, the matrices do not include any of the allergens that are found in dairy products [39].

Prebiotics are indigestible components of food that have a favourable effect on the host by favourably influencing the proliferation of microorganisms that are already present in the

gastrointestinal tract [18, 20]. Prebiotics are also known as fermentable fibres. It is hypothesised that *Lagenaria siceraria*, which is a rich source of dietary prebiotics, might improve probiotic populations, which in turn impacts the physiology and metabolism of enteric microbes. This, in turn, results in the formation and release of potentially favourable bimolecular chemicals. It has been shown that the dietary fibres of *Lagenaria siceraria* enhance faecal steroid excretion [36]. Additionally, it has been documented that this plant may limit the formation of colon cancer and improve probiotic population [18]. Vegetables and synbiotics, which are a combination of prebiotics and probiotics, have been proposed as a new preventive and therapeutic option for a variety of diseases, including cancer, based on studies conducted on animals demonstrating that they reduce cancer risk factors in polypectomized patients and reduce cell proliferation in colon cancer patients. These findings suggest that vegetables and synbiotics could be used to treat and prevent cancer and other diseases. There are reports of research in which animal models with colons containing lactic acid–producing bacteria with prebiotics protected DNA damage caused by carcinogens [33,35]. These investigations were conducted in the colon. In a manner comparable to this, it has been shown that synbiotics inhibit the development of preneoplastic lesions and tumours in rats whose colons have been subjected to chemical carcinogens [21, 29, 34]. Both prebiotics and probiotics are more effective when used in conjunction with one another than when used individually [35,41]. Additionally, certain probiotic strains may be more effective when used in conjunction with one another. As a result, bottle gourd, which is known for having a high concentration of dietary fibre, has been used as a source of carbon, nitrogen, and oxygen for the development of beneficial lactic acid bacteria in the beverage.

Bamboo shoots have a broad variety of health benefits, significant medicinal value, and immense value as a food source, which is why the tribal people in the northeastern part of India ferment them. They serve as a reservoir for a wide range of important microorganisms, including strains of yeast and lactic acid bacteria (LAB). They provide colour, fragrance, flavour, taste, and texture to the finished product of the culinary endeavor. They can serve as functional meals due to the high concentration of probiotics that they contain. Fermented bamboo shoot-based meals are produced by a number of indigenous tribes in northeastern India; however, only a few of these foods are readily available on the local market. These include soidon, soibum, mesu, ekung, lung-siej, hirring, and eup [6]. Fermented bamboo shoot products are a rich source of protein, carbs,

fibre, and several essential micronutrients such as cadmium, manganese, copper, lead, cobalt, and copper and lead [31]. They have a high polyphenol content, which includes the types that are necessary for the action of antioxidants, such as coumaric acid, synergic acid, ferulic acid, chlorogenic acid, catechin, and hydroxybenzoic acid. In addition, they have high polyphenol content. The fermentation process of bamboo shoots is substantially impacted by lactic acid bacteria (LAB). The inclusion of LAB in the fermentation process not only enhances the flavour and fragrance, but it also contributes to the detoxification of certain undesirable compounds, including tannins and phytates, which may be present in the raw material [10].

Beer brewed from rice and fermented using conventional solid-state starters is a traditional alcoholic beverage that is widely consumed in several Asian countries [11, 12, 16]. The fact that they include a variety of proteins, carbs, vitamins, bioactive compounds, and various organic acids lends to their very high level of functionality and nutritional density [13, 27]. In contrast to wine, which is derived from grapes, or typical beers, which are made from barley, these beers are produced in a very different manner. As starters for fermentation, cake-like things produced from wheat or grits and medicinal herbs are utilized. These starters provide an ideal environment for a wide range of fungi, yeast, and lactic acid bacteria (LAB) to grow and flourish organically [13, 25, 27]. Before being used to make the finished product, the fermented mash is rough filtered, which contains a significant number of bacteria that were active throughout the fermentation process [27].

Hence, this research aimed at the production of probioticated *Lagenaria siceraria* juice using two strains of *Lactobacillus plantarum* and *Limosilactobacillus fermentum* isolated from rice beer and fermented bamboo shoots. The study includes determination of the viability of the strains, antimicrobial potential, antioxidant properties of the probioticated *Lagenaria siceraria* juice samples stored at 4 ◦C and at room temperature for four weeks. The purpose of the present study was to determine the suitability of a vegetable juice medium composed of bottle gourd as a raw material for production of probiotic vegetable juice by probiotic lactic acid bacteria.

6.2. Material and methods

6.2.1. Collection of samples

Rice beer (Zutho) and fermented bamboo shoots were collected from Nagaland and Manipur, India. The chemicals, solvents and microbial growth media were obtained from Sigma-Aldrich Corporation, USA and HiMedia, India.

6.2.2. Molecular Identification of the Isolates by 16S rDNA amplification and sequencing:

16S rDNA amplification

Molecular identifications of all the bacterial isolates were performed by amplification of 16S rDNA and sequencing of the amplified product. 16S rDNA sequences of all the isolated antagonistic bacteria were amplified by colony PCR with the help of universal primers [forward primer (27F) & reverse primer (1492. For colony PCR, single bacterial colony was first mixed in 5 µl of 200 mM NaOH and 95 µl of sterile distilled water in a sterile PCR tube and then it was kept for lysis at 95ºC for 10 minutes**.** The lysed products were used as template for PCR reaction. PCR reaction recipe for each 20µl volume reaction was: Dream Taq Buffer (10X) - 2.0 µl; 2 mM dNTPs - 1.2 µl; DMSO - 0.6 µl; forward primer (10 µM) - 1.0 µl; reverse primer (10 µM) - 1.0 µl; template DNA - 1.0 µl; Dream-Taq DNA polymerase (5U/µl) - 0.2 µl; nuclease-free water - 13µl. Cycling conditions used for PCR were: initial denaturation at 95°C - 5 min, annealing at 60°C – 30 sec, extension at 72°C – 1.30 min, final extension at 72°C – 10 min. Amplified PCR products were visualize on 0.8% agarose gel (1X TAE, 70V, 200 mA, and 45 min) and the amplified products were first observed in a Gel-Doc system (E-gel imager, Thermo-Fisher Scientific, Mumbai, India) and the picture of the gel was collected. The amplified products were extracted from the gel bands using QIAquick Gel Extraction Kit (QIAGEN). Sequencing of the PCR amplified products was carried out through Sanger sequencing method. The homologous sequences were retrieved using BLAST tool of NCBI and phylogenetic tree was constructed with the help of MEGA 6 and MEGA 7 software**.**

Sequencing PCR, clean up and Sanger sequencing

The sequencing PCR recipe for each 5 µl volume reaction was: Buffer- 1.0 µl; Big Dye- 1.0 µl; primer- 0.5 µl; and template DNA- 2.5 µl. The cycling conditions used for

sequencing PCR were 96°C- 1 min; 96°C - 10 sec; 52°C- 40 sec; 60°C- 4min; 4°C- ∞. The cycles were repeated till 35 times. After the PCR, the samples were cleaned up and prepare for Sanger sequencing. The PCR reaction products were transferred into sterile 1.5 ml microfuge tube. Master Mix I consists of 10 µl Mili-Q and 2 µl of 125 mM EDTA per reaction was prepared. Then 12 µl master Mix I was added to each PCR reaction product and contents were mixed properly. After that, Master Mix II consists of 2 µl of 3M NaOAc (pH 4.6) and 50 µl of ethanol per reaction was prepared. 52 µl of master mix II was added to each reaction. The contents were mixed well and incubated at room temperature for 15 mins. Then the reaction mix was spun at a speed of 12000g for 20 mins at room temperature and the supernatant was decanted. Then 250 µl of 70% ethanol was added and span at 12000g for 10 mins at room temperature, the supernatant was decanted. At last12-15 µl of Hi-Di form amide was added, transferred to sample tubes, covered with septa, denatured, spot chilled and proceeded for capillary electrophoresis. The sequences were retrieved using BLAST tool and aligned using CLUSTALW tool in order to find homologous sequences. Phylogenetic tree was constructed using MEGA 6 and MEGA 7 software by neighbour joining method.

6.2.3. General characteristics of the strains

The general characteristics of the strains were checked by performing the catalase test using 3 % H_2O_2. Durham's tubes containing phenol red broth was used to check the production of gas from glucose [9, 11]. Nessler's reagent was used to determine the production of ammonia from arginine [20]. Saturated ammonium sulfate solution was used for the gelatinase activity test [22]. On sterile blood agar, haemolysis activity was analysed and measured [19].

6.2.4. Growth characteristics at different pH, salt concentration and temperature

The growth characteristics of the strains were studied using De Man, Rogosa and Sharpe (MRS) broth. The pH of the media was adjusted to 3.9, 9.6 and 7.0 with 1 N HCl and 10 % NaOH solution. The strains were also grown in different salt concentration of 6.5, 10 and 18 % salt concentration using NaCl. The bacterial strains were incubated at 37℃ for 48 h. the LAB strains were also grown in different temperature of 10, 15 and 45 °C. The growth of the strains was evaluated spectrophotometrically at 600 nm [19].

6.2.5. Test for acid and bile tolerance

For acid tolerance test, MRS broth was adjusted to pH 1.5, 2.0 and 7.0 using 1.0 N HCl and NaOH respectively and inoculated with 18 h old LAB cultures at 10^8 c.f.u. /ml. Plating from each tube on MRS agar was done after an interval of 0, 1, 2, 3, 5, 6, 7, 8, 24 and 48 h after incubation at 37°C [11]. For bile tolerance test, 24 h old LAB cultures were inoculated in MRS broth supplemented with bile salts (1 to 14 %) at 10^8 c.f.u. /ml and incubated at 37 °C, followed by plating on MRS agar after 24 h [24].

6.2.6. Antibiotic susceptibility test

This was carried out using antibiotic discs, which were placed upon the antibiotic susceptibility test media Mueller−Hinton agar (MHA) plates, which were preinoculated with 48 h old cultures of the LAB strains [11]. Incubation was done for 24 h at 37 °C under anaerobic condition by using an anaerobic gas pack system (LE012, Himedia, India), following which the inhibition zones were measured using an antibiotic zone scale [11]. The antibiotics used were ampicillin, chloramphenicol, ciprofloxacin, erythromycin, penicillin, gentamycin, kanamycin, rifampicin, streptomycin, tetracycline, vancomycin, and linezolid.

6.2.7. Antibiosis activity tests

The antibacterial activity against *Escherichia coli* MTCC 40 and *Staphylococcus aureus* MTCC 3160 was tested by employing the agar spot-on-lawn test of Schillinger and Lucke [11, 37]. The LAB cultures were first spot inoculated on MRS agar plates and grown for 48 h under anaerobic condition. Then 0.25 ml culture of the indicator bacteria (18 h old) was mixed with 9 ml of antibiotic susceptibility test media (0.7 % agar) at 50 °C and poured over the spotted plates. The plates were incubated for 24 h at 37 °C, following which the inhibition zones were observed and expressed as the difference in diameter between the total zone of inhibition and the LAB growth spot. For antifungal activity, 2 cm long 48 h old streaks of the LAB cultures on MRS agar plates were overlaid with 10 ml of malt extract soft agar (0.7 %) containing 10^4 fungal (*Aspergillus niger* MTCC 281) spores/ml and incubated at 30 °C for 48 h. Clear zones of inhibition surrounding the bacterial streaks indicated inhibition [30].

6.2.8. Extraction of dietary fibre from the pomace of *Lagenaria siceraria*

Dietary fibre extraction using ultrasound assisted extraction method was done according to the method described by [5] with slight modification. To extract dietary fiber using ultrasound assisted extraction method, first, *Lagenaria siceraria* pomace powder was suspended in 0.5 M NaOH solution (1:25, solute/solvent) and stirred at an agitation speed of 500 rpm using laboratory stirrer (RQT-127D, Remi, Maharashtra, India) for 30 min at 50 °C. Further it was sonicated at 20 kHz for time varying from 10–20 min at temperature ranges 60– 80°C using probe type ultrasonicator (Q700-220 Digital Sonicator, Qsonica LLC, USA). The effect of ultrasonic amplitude (20%–50%) on yield was also investigated.

Then the mixture was neutralized using 0.5 M HCl. The extracted dietary fibre under optimum extraction condition was then dried at 40 °C overnight to obtain combined ultrasound-assisted and alkaline extracted dietary fibre.

The extracted dietary fibre under optimum extraction condition was then dried at 40°C overnight to obtain insoluble dietary fibre. Further, the supernatant was separated from insoluble dietary fibre. To the supernatant, 95% of four volume ethanol was added to precipitate soluble dietary fibre and the precipitate was then washed with 50%, 75% and 100% ethanol and dried at 40°C to obtain soluble dietary fibre.

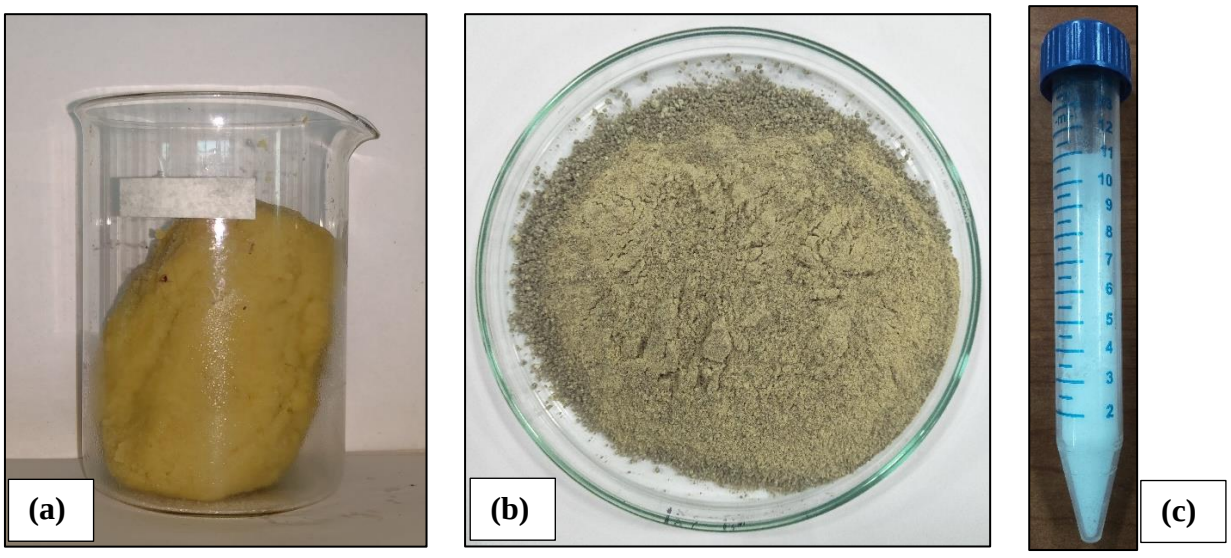

Figure. 6.1: Extraction of soluble dietary fibre from *Lagenaria siceraria* pomace

(a). *Lagenaria siceraria* pomace

(b). *Lagenaria siceraria* pomace dried and grounded powder

(c). *Lagenaria siceraria* pomace soluble dietary fibre

6.2.9. Cultivation of bacteria and growth measurement

Carbohydrate-free Man–Rogosa–Sharp (MRS) medium was used as a basal growth medium to study the ability of the isolated strains to utilize the *Lagenaria siceraria* soluble dietary fiber [23]. The fiber was taken in different concentration 1%, 2% and 5% (w/v). Basal MRS supplemented with different concentrations of *Lagenaria siceraria* soluble dietary fiber was divided into test tubes (5 ml in each), inoculated with overnight culture of the beneficial bacteria, about 10^6 colony-forming units/mL (c.f.u /ml), and incubated at 37°C in anaerobic jars—Oxoid Anaerobic System with Gas Pak H_2+CO_2 (Linegal Chemicals GmbH, Poland). The strains were incubated anaerobically for 48 h at 37°C. Numbers of bacteria were counted as log c.f.u /ml at 3 h intervals. All measurements were performed in triplicate parallels and repeated at least twice. The strains were also grown in standard Man–Rogosa–Sharp (MRS) medium to compare

6.2.10. Preparation of *Lagenaria siceraria* juice

Bottle gourds were washed and wiped with a absorbent paper, stripped and cut into uniform shapes (2×2×2 cm). Based on the method described by [8,15], blanching was performed with the help of distilled water (85ºC for 5 min). The sample was allowed to cool promptly with the assistance of chilled water as soon as the blanching was done. The BGJ was extracted from the sample with the help of the domestically used grinder (Bajaj Majesty 1.5 L JX4 Juicer Mixer Grinder). The juice was further centrifuged (Eppendorf Centrifuge 5430 R, Germany) at 5,000 rpm with duration of 15 min and filtered with the help of Whatman paper (No1) to eliminate the debris and suspended particles.

The BGJ was processed, using microwave temperature of 70˚C, microwave yield power of 750 W, ultrasound amplitude of 80%, and ultrasound exposure for 15 min [15]. The BGJ (100 ml) sample was initially treated in the microwave system (Milestone Technologies, NEOS GR Microwave Extraction System) at desired temperature of 70˚C for 20 s. The microwave treated samples were then moved to ultrasound system aseptically. The samples were chilled, and the temperature was brought down to 20ºC in the ultrasound-based system (QsonicaLLC.Q700-220) with cold water moving in circular

motion. With amplitude of 80% and process time of 15 min, the ultra-sonication was performed. The temperature of the samples was not allowed to rise above 30ºC.

6.2.11. *Lagenaria siceraria* juice and incorporation of prebiotic and probiotics

The samples of juice were given a probiotic enhancement by inoculating one 100 ml of *Lagenaria siceraria* juice with a culture of probiotic LAB [1]. Labels were placed on the samples that had been probiotically treated with strains of *Lactobacillus plantarum* and *Limosilactobacillus fermentum*. The inoculation samples were kept for a total of four weeks at temperatures of both 4°C and room temperature. The samples that were preserved were examined for viable cell content once a week. The pour plate method was used to test how well the probiotics in the probioticated *Lagenaria siceraria* samples were able to survive after being exposed to the environment. The number of viable counts was then multiplied by the reciprocal of the dilution factor and represented as a colony-forming unit. The viable counts were recorded by counting the visible colonies that were present on the culture medium (c.f.u.). A pH metre was used to determine the pH levels of the probioticated juice samples that had been kept at 4°C and at room temperature for one to four weeks. The approach described in [6] was used in the lactic acid generation analysis. The TPC and DPPH antioxidant activities of the probiotic juice were measured and analysed every week [15].

6.3. Results

6.3.1. Identification of the isolated strains

The LAB strains identified based on 16s rRNA sequencing were found to be *Lactobacillus plantarum* and *Limosilactobacillus fermentum.*

Lactobacillus plantarum

ACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTG
GCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACA
CCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGT
TTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTA
GATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAG
GGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC

AGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGC
GTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACAT
ATCTGAGAGAAACTGGTCAGGTATTGACGGTATTTAACCCGAAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC

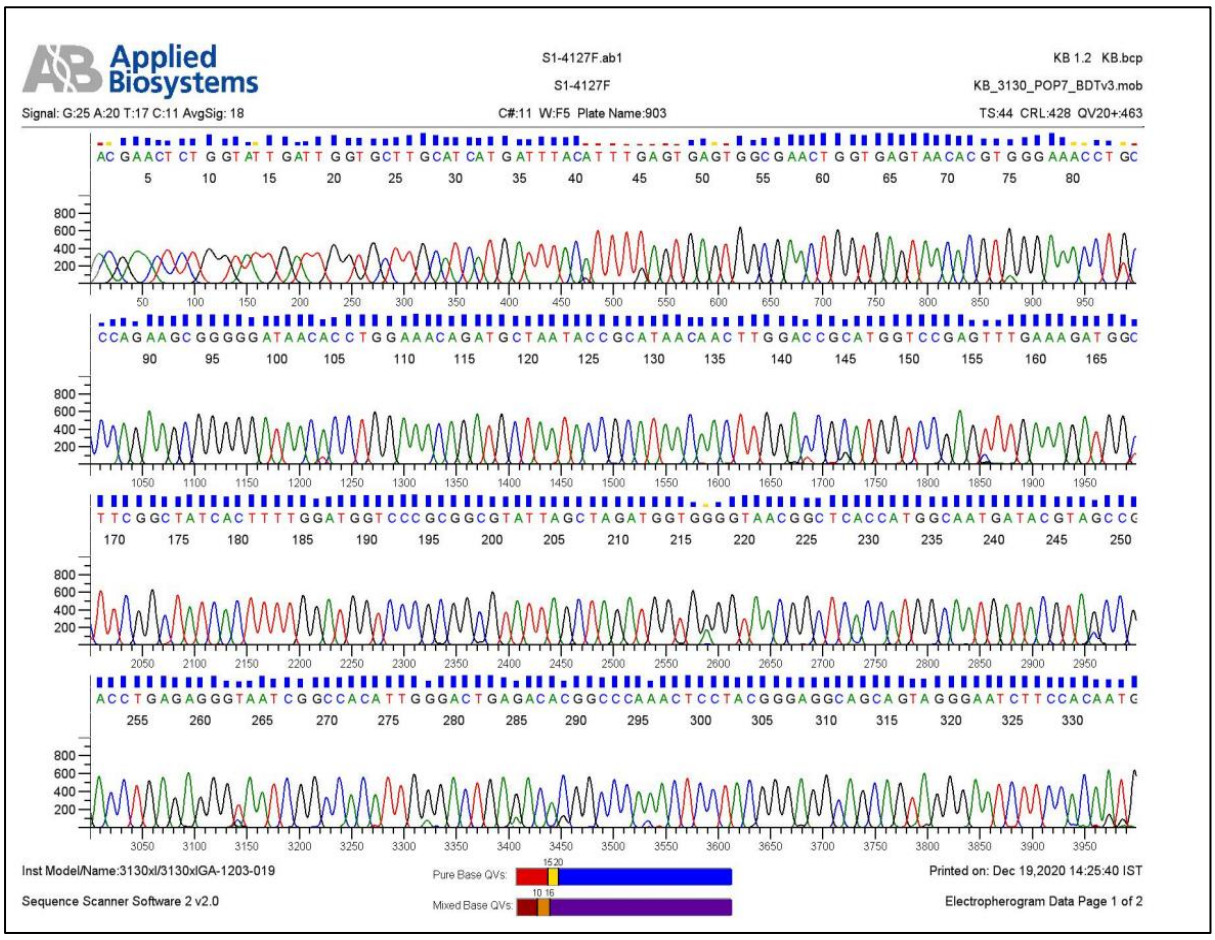

Figure. 6.2: Electropherogram data of *Lactobacillus plantarum* (Page1)

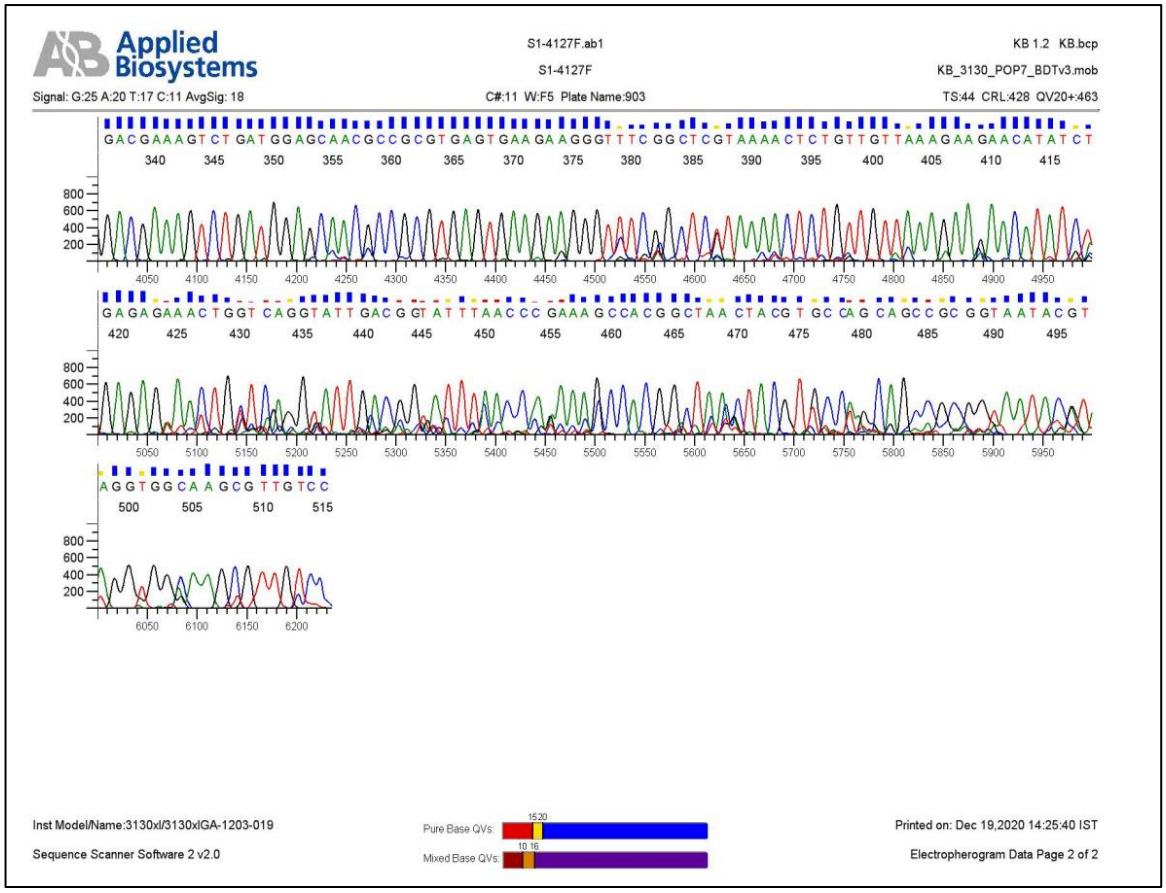

Figure. 6.3: Electropherogram data of *Lactobacillus plantarum* (Page2)

Limosilactobacillus fermentum

ACCCTAATCATCTGTCCCACCTTAGGCGGCTGGCTCCTAAAAGGTTACCCCA
CCGACTTTGGGTGTTACAAACTCTCATGGTGTGACGGGCGGTGTGTACAAGG
CCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCG
ACTTCCTGCAGGCGAGTTGCAGCCTGCAGTCCGAACTGAGAACGGGTTGGGG
GGGGGGGGCTA

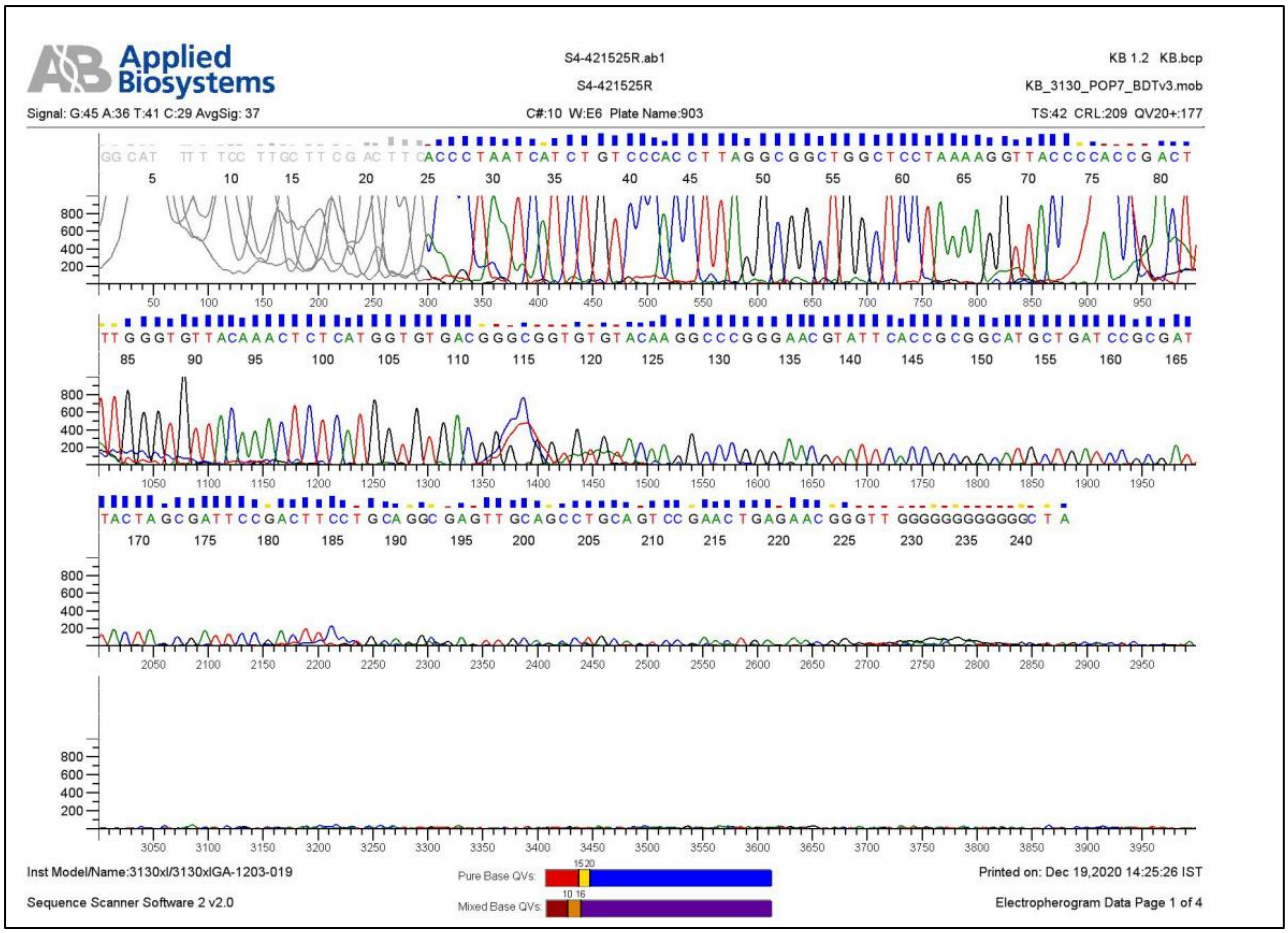

Figure. 6.4: Electropherogram data of *Limosilactobacillus fermentum*

6.3.2. General characteristics of the LAB strains

All the strains were found to be positive to produce catalase and negative for the production of gas from glucose, production of ammonia from arginine, gelatin hydrolysis and haemolytic activity.

6.3.3. Growth at different pH, NaCl concentration and temperature

The results for accessing the growth of the LAB strains under these conditions are shown in Table. It was observed that both the strains could grow at pH 7. However, at pH 9.6, the strains could not grow. At low pH of 3.9, *Lactobacillus plantarum* and *Limosilactobacillus fermentum* showed some growth. The strains could grow at 6.50 % NaCl but at 10 % NaCl and the highest concentration of 18 % NaCl the strains could not grow. At 10 °C the strains could not grow, both strains showed poor growth. At 15 °C. Whereas, at 45 °C the strains showed no growth (Table 6.1).

Table 6.1: Growth characteristics at different pH, Nacl concentration and temperature

	pH			Nacl			Temperature		
	3.9	7	9.6	6.50%	10%	18%	10	15	45
LP	+(w)	++	-	++	-	-	-	+(w)	-
LF	+(w)	++	-	++	-	-	-	+(w)	-

Good growth ++, Growth +, Poor Growth +(w), No Growth -

LP= Lactobacillus plantarum

LF= *Limosilactobacillus fermentum*

6.3.4. Tolerance of the LAB strains to acid and bile salts

Acid tolerance of the LAB strains at two different pH levels are shown in Table 6.2, 6.3. At the pH of 2.5 high resistances was shown by the strains *Lactobacillus plantarum* and *Limosilactobacillus fermentum.* The results for tolerance of the LAB strains to bile salt are shown in Table. It was seen that both of LAB strains were highly tolerant against bile salt. All the strains could maintain a count of above 7 log c.f.u./ ml up to a final concentration of 14 % bile salt (Table 6.2, 6.3).

Table 6.2: Acid tolerance

	pH 2.5											pH 1.5				
	0	1	2	3	4	5	6	7	8	24	48	0	1	2	3	4
LP	9.5	9.5	9.4	9.37	9.35	9.3	9.2	9.04	9	0.0	0.0	9.6	8.3	7.2	6.3	0
LF	9.2	9.2	9.0	8.22	8.16	8.04	7.8	7.4	7.1	0.0	0.0	9.5	7.1	7.0	0.0	0.0

LP= Lactobacillus plantarum

LF= *Limosilactobacillus fermentum*

Table 6.3: Bile Tolerance

%	8	10	12	14
LP	7.42	7.38	7.32	7.11
LF	7.91	7.83	7.75	7.25

LP= Lactobacillus plantarum

LF= *Limosilactobacillus fermentum*

6.3.5. Antibiotic susceptibility of the LAB strains

The antibiotic resistance test of LAB strains was performed, and it is important to be assessed to limit the transmission of antibiotic resistance genes to unrelated pathogenic or opportunistic bacteria. Based on the zones of inhibition, the LAB strains were designated as resistant (R), intermediate (I) and susceptible (S) as per Clinical and Laboratory Standards Institute guidelines [32]. Here, the resistant category includes the isolates which are not inhibited by the usually achievable concentrations of the antibiotic; the intermediate category includes those isolates where the antibiotics usually approach attainable MICs in tissue and blood, while the susceptible category implies those isolates which are inhibited by the usually achievable concentrations of antibiotics. The results for this test are shown in Table 4, and it was observed that both LAB strains were susceptible towards most of the tested antibiotics in varying degrees.

Table 6.4: Susceptibility of LAB strains to different antibiotics

	Zone of inhibition (mm)											
	AM10	C30	CIP5	E15	P10	GM50	K30	RA30	S25	TE10	VA10	LZD30
LP	35(S)	37(S)	16(I)	36(S)	34(S)	25(S)	0(R)	31(S)	13(I)	27(S)	0(R)	39(S)
LF	26(S)	32(S)	10(R)	32(S)	24(S)	19(S)	0(R)	28(S)	11(I)	26(S)	0(R)	31(S)

R- Resistant, I – Intermediate, S- Susceptible

As per clinical laboratory standards institutional guidelines

LP= Lactobacillus plantarum

LF= *Limosilactobacillus fermentum*

Note. AM10: Ampicillin (10 mcg); C30 –

Chloramphenicol (30 mcg); CIP5: Ciprofloxacin (5 mcg); E15: Erythromycin (15 mcg); P10: Penicillin (10 mcg); GM50: Gentamycin (50 mcg); K30: Kanamycin (30 mcg); RA30: Rifampicin (30 mcg); S25: Streptomycin (25 mcg); TE10: Tetracycline (10 mcg); VA10: Vancomycin (10 mcg); LZD30: Linezolid (30 mcg).

6.3.6. Antibiosis activity of the LAB strains

The results for antibiosis activity of the LAB strains are illustrated in Figure.6.5. It was observed that both the LAB strains were highly antagonist against both *E. coli* and *S. aureus*. In both the cases, the zones of inhibition were above 30 mm and some of the strains also exhibited zones of inhibition up to 40 mm. In the case of *A. niger*, the strains exhibited zones below 3 mm (Figure. 6.5).

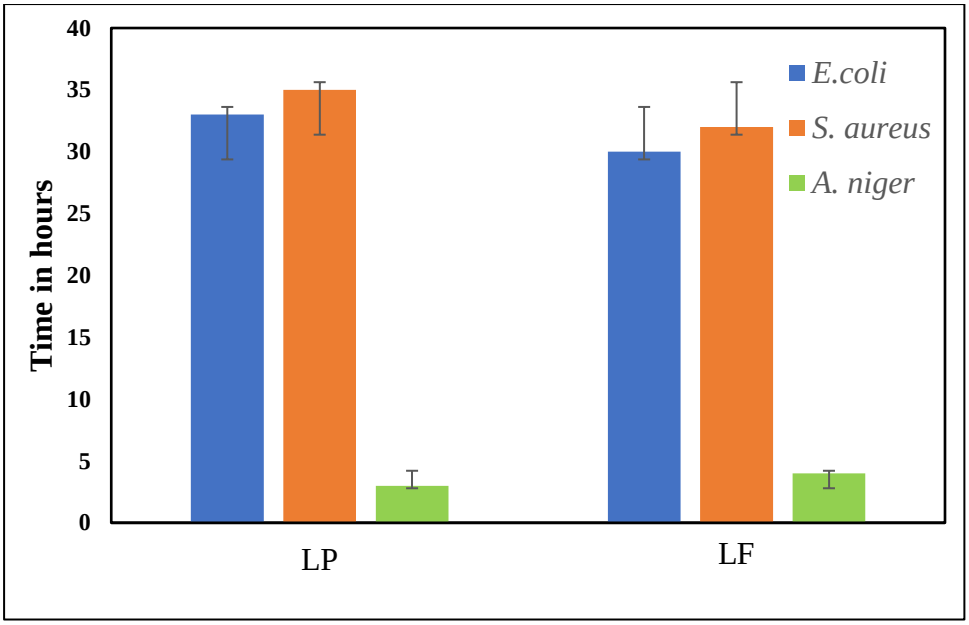

Figure. 6.5: Antibiosis activity of the LAB strains against *E. coli* MTCC 40, *S. aureus* MTCC 3160 and *A. niger* MTCC 281.

LP= Lactobacillus plantarum

LF= *Limosilactobacillus fermentum*

6.3.7. Cultivation of bacteria and growth measurement in carbohydrate free MRS broth media with different concentrations of *Lagenaria siceraria* soluble dietary fibre

The growth characteristics of the two probiotic strains were studied in standard MRS broth media and in carbohydrate free MRS broth media with different concentrations of *Lagenaria siceraria* soluble dietary fibre at 37℃. The growth curve of both the bacteria reached plateau phase within 15 h when it was grown in standard MRS media (Figure.6.6). The growth curves of the bacteria showed promising results when it was grown in carbohydrate free MRS media with 2% *Lagenaria siceraria* soluble dietary fibre. The bacteria grew differently by reaching plateau phase at 20h (Figure.6.7). There was poor growth in media where 1% and 5% *Lagenaria siceraria* soluble dietary fibre was used. It can be concluded that the soluble dietary fiber of *Lagenaria siceraria* (2%) was successful in providing the required nutrients to the two bacterial strains.

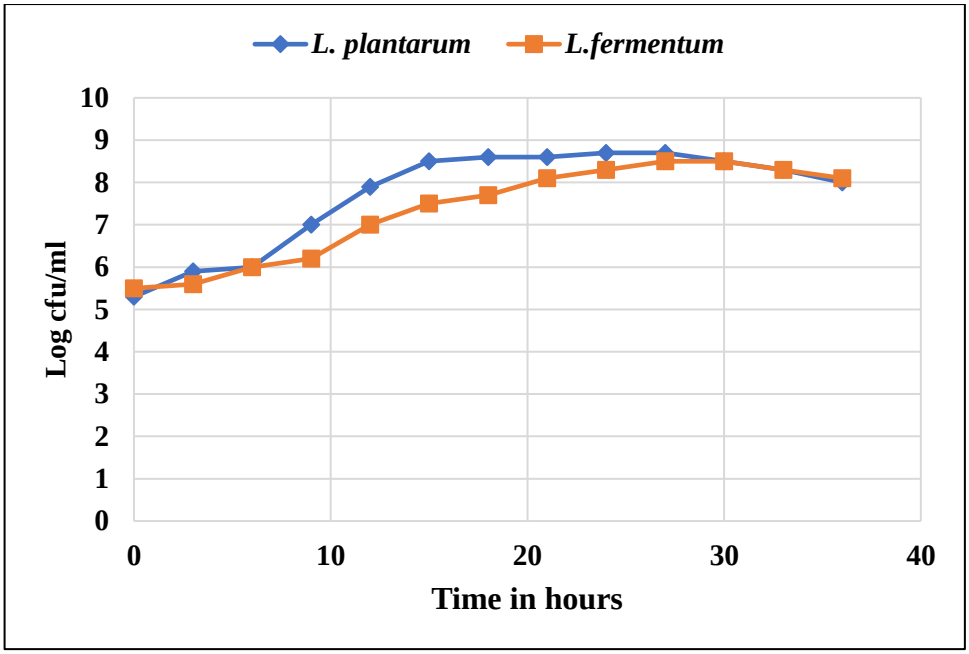

Figure. 6.6: Growth of bacteria in MRS media in time interval

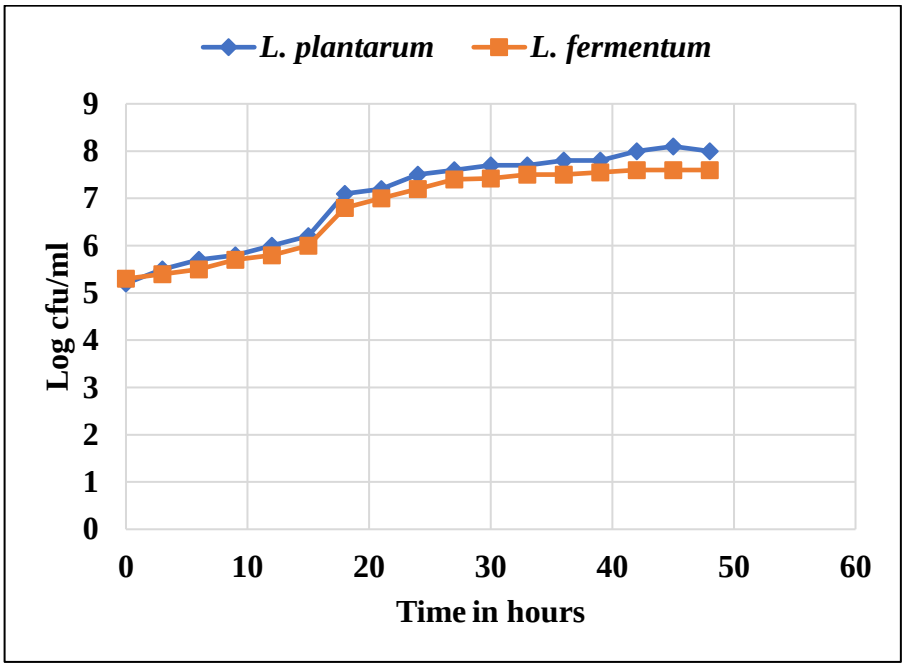

Figure 6.7: Growth of bacteria in carbohydrate free MRS media with 2% *Lagenaria siceraria* soluble dietary fiber

6.3.8. Preparation of probiotic *Lagenaria siceraria* juice

Lagenaria siceraria juice was pasteurized and probioticated with *Lactobacillus plantarum* making a cell count of log 10^8 c.f.u. /ml with and without 2% *Lagenaria siceraria* soluble dietary fiber**.** The samples were stored at 4℃ and at room temperature.

The cell viability of *Lactobacillus plantarum* were seen to decrease with the increase of time. It was seen that in the sample stored at room temperature for both samples with and without *Lagenaria siceraria* soluble dietary fiber, *Lactobacillus plantarum* counts were decreased by 5 logs within the first week of storage and no viable counts were available after the second week of storage (Figure.6.4).

In the sample stored at 4℃ the viable cell counts were stable. The cell viability of *Lactobacillus plantarum* when it was stored with 2% *Lagenaria siceraria* soluble dietary fiber, was seen to decrease by 1 log within the first week while it was seen to be stable at log 10^6 c.f.u. /ml (Figure. 6.8). after 4 weeks of storage. The viability count in the sample without *Lagenaria siceraria* soluble dietary fiber was seen to be much lower with log 10^2 c.f.u. /ml after 4 weeks of storage (Figure.6.4). This result concludes that the soluble dietary fiber aided in the survival of *Lactobacillus plantarum* during the storage at 4℃.

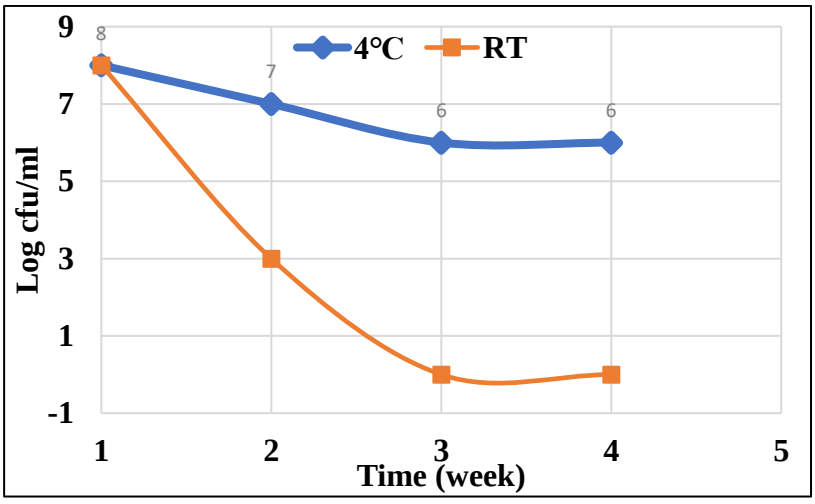

Figure.6.8: Change in viable cell counts of probiotic bacteria (log c.f.u. /ml) present in the probiotic juices with time.

The change in lactic acid concentrations were observed in the samples. The acidity increased significantly in samples stored at both 4℃ and at room temperature. The titratable acidity expressed as % lactic acid were seen to increase from 0.26 to1.15 % at 4℃ after 4 weeks of storage. And in the sample stored at room temperature it was seen to increase from 0.27 to1.3 % within the first two weeks (Figure.6.9).

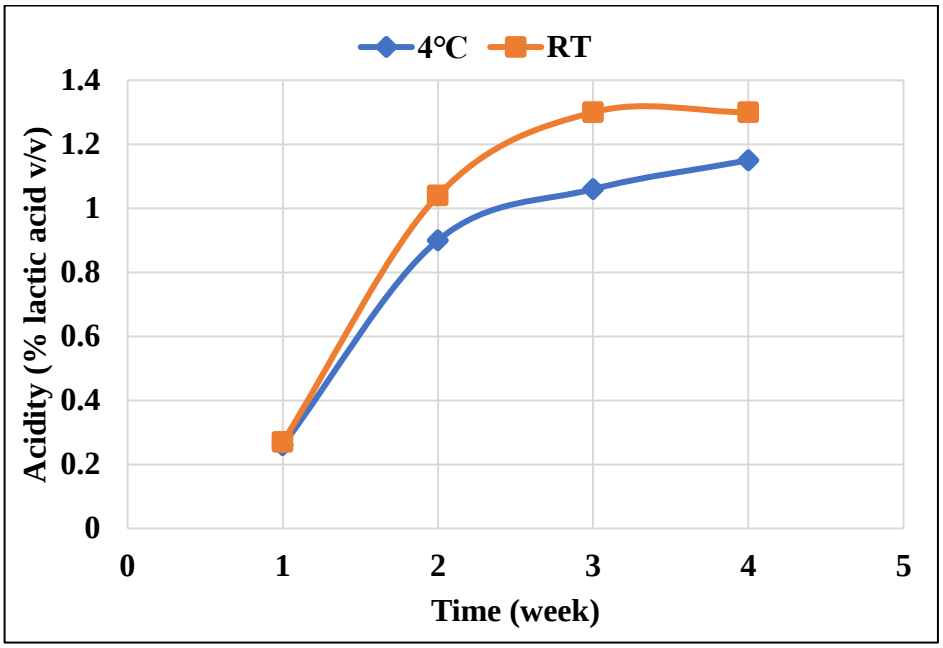

Figure. 6.9: Change in acidity expressed as % lactic acid (v/v) of the probiotic juices with time.

The pH of the samples was seen to gradually decrease in the storage period. The pH of the samples stored at 4℃ and at room temperature changed from 5.6, 5.6 to 4.2 and 3.1 after 4 weeks of storage (Figure.6.10.).

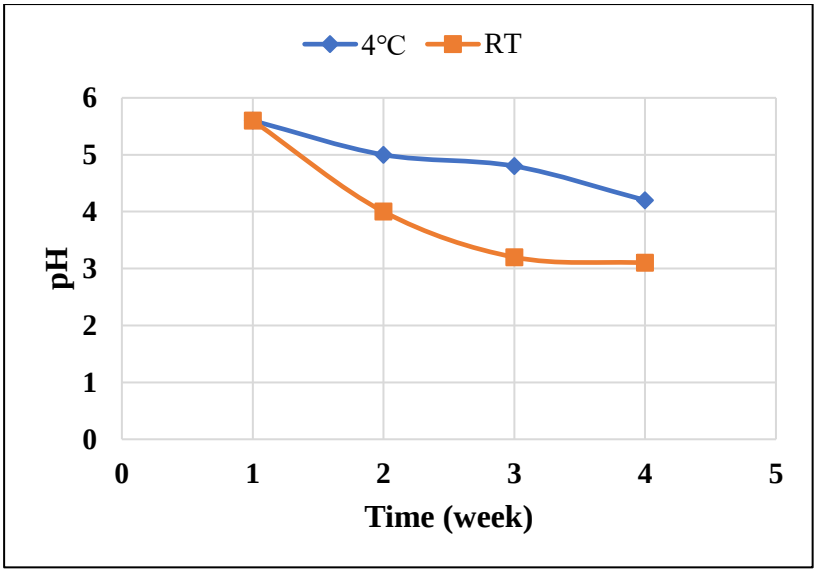

Figure. 6.10: Change in pH of the probiotic juices with time.

The change in the total phenolic content and DPPH free radical scavenging activity were seen to gradually decrease in the storage period. It was seen that the samples stored at 4°C significantly retained better values of DPPH and total phenolic content after 4 weeks of storage.

The total phenolic content of the samples stored at 4°C and at room temperature changed from 292.7, 290.85 to 285.5, 272.48 µg/g after 4 weeks of storage (Figure.6.11). The DPPH free radical scavenging activity of the samples stored at 4°C and at room temperature changed from 43,44 to 37 to 29% after 4 weeks of storage (Figure.6.12).

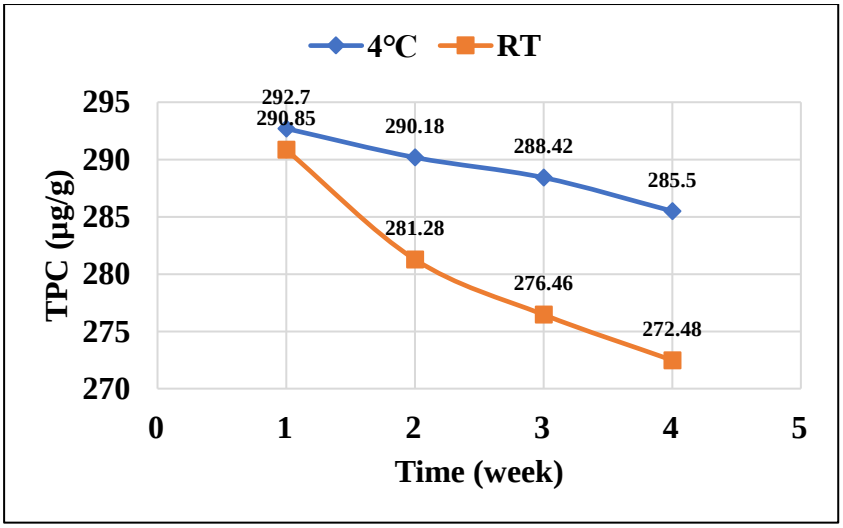

Figure 6.11: Change in TPC of the probiotic juices with time.

TPC

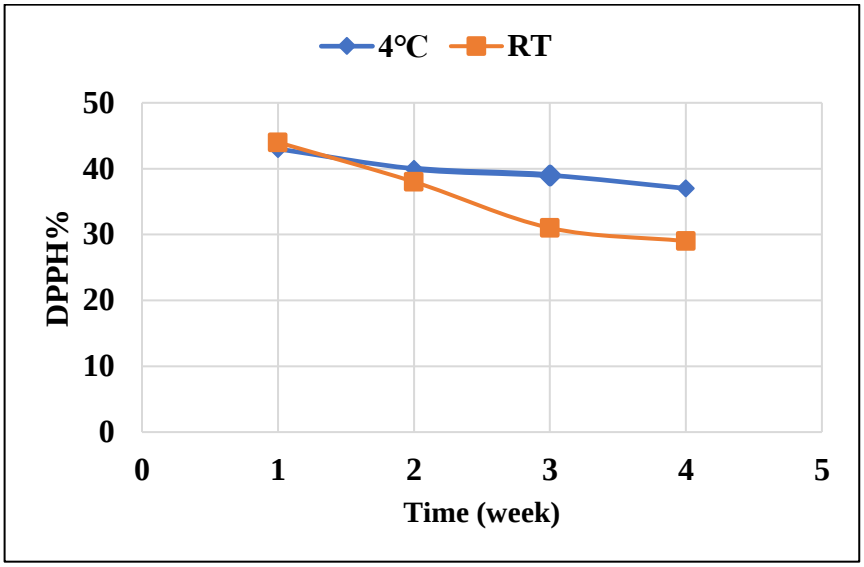

Figure 6.12: Change in DPPH free radical scavenging activity of the probiotic juices with time.

6.4. Conclusions

In vitro fermentation of the dietary fibers using carbohydrate free MRS media showed the significant survival and growth of *L. plantarum* and *L. fermentum* strain showing the potentiality of bottle gourd fiber as prebiotic. The isolated bacterial strain (*L. plantarum*) and bottle gourd fibers were incorporated in the pasteurized bottle gourd juice. The Storage study and the survivability of the strains in bottle gourd juice probiotic survivability of 10^6 c.f.u. /ml in the beverage.

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