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Fortification of Fruit Juices by Probiotic Lactic acid Bacteria Producing Siderophore Isolatedfrom Infant Faecal Matter

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Abstract: This study was undertaken to determine the suitability of different fruit juices as a raw material for production of probiotic juice by two lactic acid bacteria (*Lactobacillus brevis*, *Lactobacillus rhamnosus*). The fruit juices were fermented at 30ºC for 72 hr and changes in the microbial population, pH, sugar contentand titratable acid were observed during the fermentation period. The viable cell counts reached upto 10^8CFU/ml and pH decreased from 5.2 to 3.3. The viability of all strains was also determined at 4ºC and it was observed that the viable cell counts of the lactic acid bacteria in the fermented fruit juices remained constant up to 4 weeks. The above two isolates were also found to produce siderophores in a range of 50 - 90 % of siderophore units. The fruit juices proved to be a suitable media for the production of a fermented probiotic drink and may serve as a healthy beverage for vegetarians, particularly diabetics after conducting certain sensory evaluation tests.

Key words: Probiotics, Lactic acid bacteria, Fruit juices, Siderophore, Infant faecal matter.

Introduction

Consumers believe that certain foods can have a positive impact on long-term and current health. This has helped to facilitate an acceptance of term "Functional foods" which are foods or dietary components that may provide a health benefit beyond basic nutrition. The fermented foods used by humans can be traced back to centuries. The medicinal as well as flavor enhancing properties of fermented foods are may be due to the presence of bacteria known as probiotics[15]. Probiotics are live microorganisms with the potential of settling mainly in host (humans/ animals) intestine and comprising certain health advantages for it [7]. Probiotics aid in digestion and nutrient assimilation. These bacteria are also known for their beneficial effects for the immune system and health [9]. There is a genuine interest inthe development of fruit-juice-based functional beverages with probiotics, because they have taste profiles that are appealing to all age groups and areperceived as healthy and refreshing foods [3]. Probiotics food products are also regarded as a significant part of functional foods market, so that they comprise between 60 % and 70 % of the total functional food market [15]. However, with an increase in consumer vegetarianism throughout developedcountries, thereis also a demand for vegetarian probiotic products.In recent years, consumer demand for non-dairy based probiotic products has increased due to the problems of lactose intolerance and cholesterolcontent associated with the consumption of fermented dairy products [27]. In this respect, fruits offer an alternative for the production of probiotic foods due to their large distribution and nutritive value. Lactic acid fermentation can help to improve the safety, shelf life, and nutritionaland sensory properties of fruits [27]

Microbes require 0.4 - 1.0 M iron for their optimum growth and to run their number of crucial biochemical reactions including reduction of the

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oxygen for synthesis of ATP, reduction of ribotide precursors of DNA, etc. Although iron is highly abundant in earth's crust, dissolved iron concentrations at various locations like the surface waters of the open ocean are low (0.4 mM)[13]. It predominantly occurs in ferric [Fe (III)] form, which is abundant but biologically unavailable. This scarcity made microorganisms to synthesize specific molecules known as siderophores. Siderophores are relatively low-molecular-mass (500 - 1000 Da) iron-chelating ligands that are synthesized by most microorganisms under iron-limited conditions, they bind to ferric ions with high affinity and solubilize the iron in order to make it biologically available [2]. Hence, probiotic microorganisms with all the special abilities will not only give the advantage of all the probiotic features but also corrects the deficiencies of iron.

Fruits and vegetables are rich in functional food components such as minerals, vitamins, dietary fibers, and antioxidants. Fruit juices are often supplemented with oxygen-scavenging ingredients such as ascorbic acid, thus promoting anaerobic conditions that facilitate probiotification [3]. Furthermore, fruits and vegetables do not contain any allergens that might prevent usage by certain segments of the population. In recent year, fermentation of different fruit juices by probiotic lactic acid bacteria was studied by several authors [12,28]. Recent studies report that fruit juices could serve as suitable media for cultivating probiotic bacteria. The calcium and vitaminfortified juices, which are consumed casually by the consumer for health benefits. This marks the peak sale of the fruit juices in the market.The objective of the study is to increase the fermentation efficiency of *L. brevis* and *L. rhamnosus* in fruit juices. Hence, commonly consumed fruits like litchi, white grape, apple, pears, orange, black grape and pineapple were taken as a proper medium for lactic acid fermentation and the probiotic juices obtained could serve as a health beverage for consumers those are allergic to dairy products.

Materials and methods

Isolation and screening for siderophore production

Infant faecal matter (6 months - 2 yrs old infant) were collected from different hospitals, local houses in Baripada, Mayurbhanj district, Odisha. They were diluted by serial dilution method (10 fold) and plated using MRS agar media (Mann Rogassa Sharpe) [21] by spread plate and pour plate method. Plates were then incubated at 37°C for 48 hr and after the incubation period the colonies were picked based upon their morphological appearance and characterized by various biochemical tests. The isolates were preserved as frozen glycerol stocks(-80°C)and maintained on MRS agar slants at 4°C and working cultures were prepared by propagating them in MRS broth.

Inoculum of all the eighty different isolates were prepared in MRS broth and incubated in a rotary shaker maintained at 37°C, 150 rpm for 24 hr. Siderophore production was studied using modified succinate medium [11]. One ml inoculum of each isolate was centrifuged at 10,000 g, 4°C for 10 min, the supernatant was discarded and the pellet was washed twice with PBS (pH 7.3). Fermentation was then carried out by suspending the pellet into 20 ml of succinate medium and incubated at 37°C, 150 rpm for 120 hr. The sample was collected after every 24 hr, centrifuged at 10,000 g, 4°C for 10 min. The supernatant was used for carrying out qualitative and quantitative analysis.

Qualitative detection of siderophore.

Qualitative detection of siderophore was carried out using universal Chrome Azurol Assay (CAS) [19]. The culture supernatant obtained after fermentation was mixed in equal volumes with CAS reagent and observed for the change of colour. A reference was prepared using uninoculated succinate medium as control.

Quantitative detection of siderophore.

Quantitative detection of siderophore was carried out as per the method described elsewhere [14]. The culture supernatant obtained after fermentation was mixed in equal volume with CAS reagent and the % of siderophore units was assessed by taking the OD at A_{630} nm using UV-VIS spectrophotometer (UV-VIS117, Systronics, India). An uninoculated succinate medium was used as reference.

% of siderophore Units= Ar-As/Ar x 100

Where, Ar = Absorbance of the reference; As=Absorbance of sample

Strain identification

Identification of isolates producing maximum siderophore was carried out by complete 16S rRNA gene sequence analysis and phylogenetic studies (Macrogen Inc., Korea). Universal primers 518F (52 -CCAgCAgCCgCgg TAATA Cg-32) and 800R (52-TACCAgggTATCTAA TCC- 32) were used for the amplification of 16SrRNA gene of the isolates. Evolutionary analyses were conducted in MEGA 5 software [26]. Evolutionary history was inferred using the Neighbor-Joining method [18] and the evolutionary distances were computed using Tajima-Nei method[19].

Statistical analysis

Mean data of two independent experiments with three replicates of different characterization studies were used for the evaluation of results. Correlation analysis was also performed to find out the linear association and to compare the factor level difference among the variables.All the analysis was carried out by using SPSS software 19.0 version (SPSS Inc., IBM, NewYork, USA).

Acid tolerance

Isolates were grown overnight in MRS broth at 37°C followed by centrifugation at 8000 g for 5 min. Cell pellet was harvested and washed twice in sterile phosphate buffered saline (PBS) pH 7.3 and resuspended in 1 ml of PBS and the strains were further diluted 1:100 in PBS at pH 1, 2, 3 and 4. Samples were then incubated at 37 ^{0}C and viable bacterial cells were determined at 0, 60, 120 and 180 min time interval by plating on MRS agar plates. Growth of bacteria was expressed in $\log_{10}$ CFU/ml and then survival % of strains was calculated.

Bile salt tolerance

Bile salt tolerance was determined by inoculating 100 µl overnight grown culture of the isolates into 900 µl MRS broth supplemented with 0.3 %, 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, 3 %, 3.5 % and 4 % bile salt (Ox gall, Hi-media) and was incubated at 37°C for 24 hr. The viable bacteria were enumerated by plating 100 µl of culture onto the MRS agar plates incubated at 37°C for 24 hr. Growth of bacteria was expressed in $\log_{10}$ CFU/ ml and survival % of strain was then calculated.

Fermentation of probiotic fruit juices

The fruits like white grape, black grape, orange, pears, apple, pineapple are purchased from the local market. The selected fruits were washed thoroughly with running tap water, rinsed with distilled water and blotted dry. The seeds were separated manually from pulp. The juice is then extracted by hand pressing and straining the above prepared material through double fold muslin cloth. These juices were filter sterilized (0.22 mm) and used as substrates for further studies. Fermentation experiments were conducted in conical flasks (250 ml), each containing 150 ml of fruit juice. All samples were inoculated ($\geq 10^5$CFU/ml) with 24 hr culture and incubated at 30°C for 72 hr.

Physico-chemical and microbiological analyses

Samples were taken at 24 hr intervals for chemical and microbiological analysis. p^H was measured wih a p^H meter. Total acidity, expressed as % lactic acid, was determined by titrating with 0.02 N NaOH to p^H 8.2. Sugar content was analyzed as glucose by the phenol sulfuric acid method[4]. Viable cell count (CFU/ ml) were determined by standard plate countmethod with lactobacilli MRS medium after 48 hr incubation at 30°C.

Effect of low temperature on cell viability in probiotic fruit juices

After 72 hr of fermentation at 30°C, the fermented samples (75 ml) were stored at 4°C for 4 weeks. The viability and bacterial load were determined by viable plate count method at weekly intervals.

Antagonistic Activity of fermentedJuice

The antagonistic activity of the fermented juice was studied against certain pathogenic species (*Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus*). Actively growing culture of the test organisms were mixed 2.5 %

(2.5×10^7 cfu/ml) with melted nutrient agar and poured in sterile petri dishes and allowed to solidify. Agar cups of 1cm diameter were punched at the centre of the plate using a sterile gel puncher. The fermented substrate was pipeted out (0.1 ml) into the well. When the mixture solidfied, the plates were first incubated at 4°C for 60 min to allow the test material to diffuse in agar and then incubated at 37°C for 18 hr. After incubation, the diameter of the clear zone around the wells was measured [24].

Results and discussion

Strain isolation and identification

Selection of isolates was based on the macroscopic differences in the colony morphology and also on the collection of samples from different sources. A total number of 80 isolates were screened for siderophore production. Based upon the qualitative detection of siderophore production 2 isolates, IFM5 (1) and IFM2 (2) were chosen for further studies. Morphologically isolate IFM5 (1) and IFM2 (2) appeared white circular smooth surfaced and microscopically they appeared as Gram +ve rods.

16S r-RNA gene analysis resulted isolates with expected base pairs 989 and 781 bp for IFM2 (2) and IFM5 (1), respectively. After performing a BLAST search isolates IFM2 (2) and IFM5 (1) exhibited close association with known *Lactobacillus* sp. The IFM2 (2) and IFM5 (1) strain showed high similarities with *L. brevis* and *L. rhamnosus*, respectively. These results were further confirmed by constructing phylogenetic trees separately for IFM2 (2) and IFM5 (1) (Fig 1).

Qualitative Determination of Siderophores

Detection of siderophore was carried out using universal CAS assay. This assay is based on the principle of higher affinity of siderophores to acquire iron from its complex with weak chelator in the reagent due to which it undergoes decolourization. A positive siderophore production is confirmed by change of colour from blue to golden yellow. Out of 80 isolates, two isolates IFM2 (2) and IFM5 (1) gave +ve results for siderophore production.

Isolates, IFM2 (2) and IFM5 (1) were producing siderophores in the range of 50 - 90 % siderophore units, hence used for further studies (data not shown).

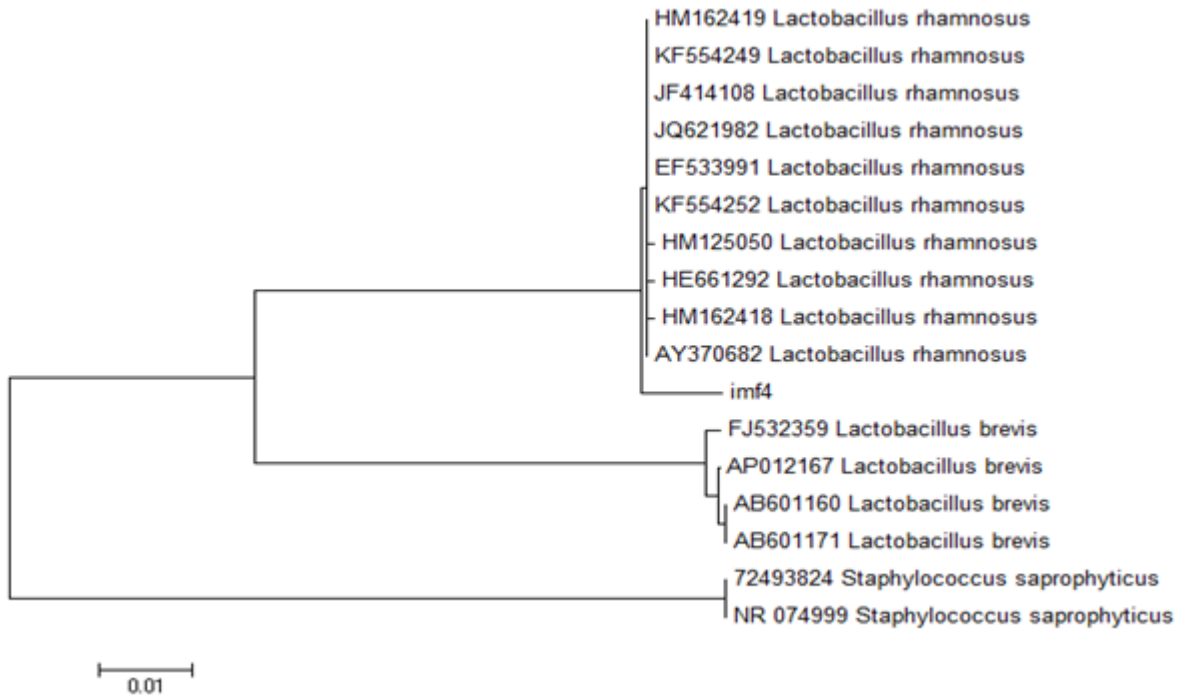

Fig. 1. Phylogenetic tree of two Lactobacillus isolates (A)IFM3 and (B)IFM4

Acid tolerance

The effect of pH (1.0 - 4.0) on *Lactobacillus* strains was tested and the number of viable cells and survival percentage at each pH value was determined (Table 1). Among the isolates evaluated for acid tolerance more than 70 % viability was observed at pH 3 and nearly 50 % viability at pH 2 and 40 % viability at pH 1 after 1hr of incubation.Low pH is known to be an effective barrier to entry of bacteria into the intestinal tract [1]. The probiotic strains are likely to be buffered by food or other carrier molecules when exposed to extremes of pH in the stomach. It has been reported that the survival rate in the stomach might increase in the presence of foodstuff, which may protect the LAB from the effect, by pepsin and acid [6]. Acidity of human gastrointestinal tract varies from 1.5 to 4.5 therefore the *in vitro* studies were mostly performed at a pH range of 1.0 - 5.0. Zheng *et al*. [29] reported that *L. plantarum* isolated from kefir reduced viability after exposure to pH 2 which was consistent to our results.

Bile salt tolerance

The survival percentage at different concentrations of bile salts (0.3 - 4 %) were studied. The results are shown in Table 2. The results indicatedthat IFM5 (1) showed high survival rate (72 %) at 2 % bile salt concentration and IFM2 (2) showed high survival rate (95 %) at 0.5 % bile salt concentration. Bile salt plays a fundamental role in the specific and non-specific defense mechanisms of the gut, the magnitude of its inhibitory effects is determined primarily by the concentration of bile salts [5, 29]. The physiological condition of bile salts in the small intestine is between 0.2 - 2.0 [8,10]. Sabir *et al.* [17]reported that *Lactobacillus helviticus* CD6 showed 100 %, 98 % and 85 % survibility at varying bile salt concentration such as 0.2, 0.3 and 0.5 % for 24 h, whereas, it failed to survive in 1.0, 1.5 and 2.0 % concentration. However, in the present study, our isolates were able to survive upto 2.0 % bile salt concentration with 60 % -80 % viability rate and gradually decreased as the concentration was increased. This behaviour might be due to that bile salt causes the increase in permeability of bacterial cell membranes, as the membranes are

Table 1. Isolates incubated at different pH (6.8 to 1.0) and the number of viable cells (log CFU/ml) and survival percentage

Isolates	Control pH 6.8	Log CFU/ml pH 4	*Survival Percentage (%)	Log CFU/ml pH3	Survival Percentage (%)	Log CFU/ml pH 2	Survival Percentage (%)	Log CFU/ml pH 1	Survival Percentage (%)
L. rhamnosus	10.6	6.9± 0.1	67	5.8±0.5	54	4.5 ±0.2	36	4 ±0.0	31
L. brevis	12.1	9.1± 0.2	71	5.8 ±0.5	66	5.4 ±0.6	41	4.5 ±0.3	33

Data are shown as the mean ± standard deviation (n=5)
Percent inhibition = final (CFU/ml)/control (CFU/ml) x 100

Table 2. Viable cell count of two isolates at different concentration of bile salt

Isolates	Bile salt concentration (%)	Viable cell count (log cfu/ml)
L. rhamnosus	0.0	6.9×10^8
	0.3	5.9×10^8
	0.5	5.8×10^8
	1.0	5.2×10^8
	1.5	4.9×10^8
	2.0	3.9×10^8
	2.5	3.7×10^8
	3.0	2.6×10^8
	3.5	2.2×10^8
	4.0	1.5×10^8
L. brevis	0.0	6.4×10^8
	0.3	5.6×10^8
	0.5	5.2×10^8
	1.0	4.9×10^8
	1.5	4.9×10^8
	2.0	4.1×10^8
	2.5	3.5×10^8
	3.0	2.8×10^8
	3.5	2.8×10^8
	4.0	2.5×10^8

composed of lipids and fatty acids [10]. Further it allowed to predict the potential of the isolates as a probiotic, since it survived 0.5% bile concentration which was higher than the physiological condition in the duodenum [1].

Physico-chemical and microbiological analyses

Fruit juice could serve as a good medium for cultivating probiotics [16]. It was observed that all the fruit juices, without any added nutrients served as good culture media and matrixes for the growth of *L.brevis* and *L. rhamnosus*.Probiotification of fruit juices with the twoisolatesshowed a decrease in pH, increase in acidity and an improvement in the utilization of sugars as determined at different intervals like 24, 48 and 72 hr and the results are presented in Table 3. Similar results were observed by Kumar *et al.* [3] when probiotication of mango and sapota juice was carried out using *L. plantarum* NCDC LP20.

Antagonistic activity of fermented juices

The probicated juices were evaluated for antagonistic activity against *E.coli*, *Salmonella typhimurium* and *Staphylococcus aureus* and compared with tetracycline as control. The inhibition zone as well as the activity index are given in Table 4. The inhibitory action of probiotic bacteria against the pathogens may be due to the accumulation of secondary metabolites such as lactic acid, acetic acid, ethanol, carbon dioxide, formic acid, benzoic acid, hydrogen peroxide, diacetylacetin and bacteriocin [13]. Probiotics have shown to process inhibitory activities mostly towards Gram-(+ve) as well as Gram (-ve) pathogens and closely selected bacteria due to the bactericidal effect of protease sensitive bacteriocins [22]. The results of our present study agree with who inferred that antimicrobial sub-stances produced by *Lactobacillus* have a great potential for inhibiting the growth of pathogenic microorganisms [23].

Effect of low temperature on cell viability of probioticlactic acid bacteria

The changes observed for the cell viability of the selectedstrains during the cold storage are presented in Table 5. Results indicatedthat the

Table 3. Physico-chemical analysis of fermented fruit juices

Name of the Fruits	Strain name	pH	OD (600 nm)	Total Sugar	Titratable Acidity
Litchi	IFM5(1)(0-72 hr)	5.20-2.40	0.240-1.911	13.6-10.0	0.6-1.5
	IFM2(2)(0-72 hr)	5.65-2.29	0.856-1.258	13.8-11.5	0.3-1.5
Pineapple	IFM5(1)(0-72hr)	3.65-1.98	0.837-1.708	15.8-12.6	0.8-1.2
	IFM2(2)(0-72 hr)	3.35-1.16	0.370-1.721	15.4-11.8	0.5-2.0
Applege	IFM5(1)(0-72 hr)	4.22-3.83	0.701-1.781	18.4-15.6	0.7-1.2
	IFM2(2)(0-72 hr)	4.84-2.23	0.781-1.792	18.2-14.9	0.1-1.8
Pears	IFM5(1)(0-72 hr)	4.58-2.22	0.769-1.737	15.1-11.6	0.7-1.2
	IFM2(2)(0-72 hr)	3.83-2.26	0.729-1.703	15.4-12.8	0.3-1.3
Orange	IFM5(1)(0-72 hr)	4.00-2.42	0.816-1.969	18.9-16.5	0.1-1.5
	IFM2(2)(0-72 hr)	3.65-2.72	0.816-1.769	18.4-15.8	0.2-1.8
Black grape	IFM5(1)(0-72 hr)	3.90-2.25	0.781-1.202	17.8-15.8	0.1-1.2
	IFM2(2)(0-72 hr)	4.1-2.20	0.804-1.329	17.5-14.5	0.5-1.7
White grape	IFM5(1)(0-72 hr)	3.73-1.92	0.804-1.747	19.8-16.8	0.6-1.2
	IFM2(2)(0-72 hr)	3.85-1.04	0.816-1.769	19.5-17.5	0.8-1.8

IFM5(1)-*L.rhamnosus*
IFM2(2)-*L.brevis*

Table 4. Antagonistic effects of fermented fruit juices

Fermented juices	Zone of Inhibition (mm) *Salmonella typhimurium*	*Bacillus subtilis*	*E. coli*	*S. aureus*
Litchi	10±0.5 (0.616)	7±0.2 (0.431)	1±0.05 (0.072)	12±0.8 (0.640)
Pineapple	14±0.4 (0.862)	7±0.2 (0.431)	5±0.3 (0.363)	6±0.2 (0.320)
Apple	8±0.1 (0.492)	6±0.2 (0.369)	8±0.1 (0.580)	7±0.2 (0.373)
Orange	4±0.1 (0.246)	7±0.2 (0.431)	3±0.1 (0.217)	5±0.3 (0.266)
Pears	5±0.2 (0.308)	4±0.1 (0.246)	7±0.2 (0.508)	2±0.05 (0.106)
White Grape	9±0.1 (0.554)	2±0.05 (0.123)	6±0.2 (0.435)	4±0.1 (0.213)
Black Grape	8±0.1 (0.492)	1±0.05 (0.061)	5±0.2 (0.363)	8±0.1 (0.427)
Tetracycline (Control)	16.23±0.86	16.23±0.86	13.77±1.74	18.73±0.27

Values are means of three replicates (±standard deviation)
Values in parenthesis are Activity Index of fermented juice against pathogens
Activity Index = Inhibition zone of the test sample divided by inhibition zone of a standard drug

microbial population of *L. brevis*and *L. rhamnosus*, didn't lost its viability. For maximum health benefits, the minimum number of probiotic organisms in a food product should be 10^6 CFU/ml [20, 3].

Statistical analysis

The correlation analysis was used for the measurement of the linearassociation between variables. Pearson's correlation coefficients ($r2$) amongthe analytical variables are presented in (Table 6 a and b).

All the analytical parameters were significantly correlated with each other. For example, the pH was significantly correlated to titratable acidity and LA.

Table 5. Effect of low temperature (4°C) during 4 weeks on the viability of *L. brevis* and *L. rhamnosus*

Fermented fruit juices	Time (weeks)	Log (CFU/ml) *Lactobacillus brevis*	Log (CFU/ml) *Lactobacillus rhamnosus*
Litchi	1	6.7×10^8	7.8×10^8
	2	5.8×10^8	7.2×10^8
	3	5.2×10^8	6.5×10^8
	4	4.9×10^8	6.1×10^8
Pineapple	1	9.9×10^8	8.6×10^8
	2	8.7×10^8	8.4×10^8
	3	7.6×10^8	7.8×10^8
	4	7.2×10^8	7.0×10^8
Apple	1	4.5×10^8	5.6×10^8
	2	3.6×10^8	5.2×10^8
	3	3.2×10^8	4.6×10^8
	4	2.9×10^8	4.1×10^8
Orange	1	8.9×10^8	6.9×10^8
	2	8.1×10^8	6.2×10^8
	3	7.5×10^8	5.8×10^8
	4	6.8×10^8	5.2×10^8
Pears	1	5.8×10^8	4.8×10^8
	2	5.5×10^8	3.8×10^8
	3	4.6×10^8	3.2×10^8
	4	4.2×10^8	2.6×10^8
White grapes	1	9.2×10^8	10.5×10^8
	2	8.9×10^8	9.9×10^8
	3	7.8×10^8	9.2×10^8
	4	6.9×10^8	8.6×10^8
Black grapes	1	9.5×10^8	8.6×10^8
	2	9.1×10^8	8.3×10^8
	3	8.6×10^8	7.2×10^8
	4	7.5×10^8	6.5×10^8

Conclusion

The two lactic acid bacteria (*Lactobacillus brevis* and *Lactobacillus rhamnosus*) were found to be capable of rapidly utilizing the different fruit juices for cell synthesis without pH adjustment. They decreased the pH to as low as 3.3 and increased the acidity to as high as 1.8 % and the viable cell counts reached 10^8/ml after fermentation at 30°C. During 4 weeks of cold storage at high acidic conditions both the lactic acid bacteria remain viable. From the results obtained in this study, it is concluded that fruit juices may be exploited as a fermentation medium for the delivery of probiotic LAB to lactose-intolerant people and those who are allergic to milk-based products. Fermentedfruit products are believed to be cholesterol-free, lowcost and healthy beverages, which could serve toprovide better health and nutritional benefits to the population but after many sensory evaluation analyses. Further, food even fortified with iron may not be in soluble form and if colon harbors probiotic microbes producing siderophores would give bonus to human health by correcting the deficiencies of

Table 6 (a). Correlation analysis of various physio-chemical parameters of fermented juices by *L. rhamnosus*

		Fermented apple juice	
pH	**Viable cell count**	**Total sugar**	**Titratable acidity**
1.000	-0.829**	0.880**	-0.806**
	1.000	-0.971**	0.939**
		1.000	-0.920**
			1.000
		Fermented orange juice	
pH	**Viable cell count**	**Total sugar**	**Titratable acidity**
1.000	-0.628*	0.749**	-0.887**
	1.000	-0.874**	0.870**
		1.000	-0.936**
			1.000

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

Table 6 (b). Correlation analysis of various physico-chemical parameters of fermented juices by *L. brevis*

		Fermented apple juice	
pH	**Viable cell count**	**Total sugar**	**Titratable acidity**
1.000	-0.830**	0.863**	-0.929**
	1.000	-0.910**	0.936**
		1.000	-0.975**
			1.000
		Fermented orange juice	
pH	**Viable cell count**	**Total sugar**	**Titratable acidity**
1.000	-0.483	0.788	-0.360
	1.000	-0.911**	0.980**
		1.000	-0.950**
			1.000

**Correlation is significant at the 0.01 level (2-tailed)

iron required for metabolic process such as formation of red blood cells, DNA repair, etc. Iron deficiency is more common in Indian population, which leads to birth defects, anaemia, cancer, etc. Hence, probiotic microorganisms with all the special abilities will not only give the advantage of all the probiotic features but also corrects the deficiencies of iron. However, studies such as this is a prerequisite to exploit the biotechnological potential of the probiotic bacteria more specially the LAB probiotics.

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