

# Production of Probiotic Mango Juice by Fermentation of Lactic Acid Bacteria

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In this study, the probiotification of mango juice was carried out by lactic acid bacteria fermentation. Mango juice fermentation was performed at 30°C for 72 h under micro-aerophilic conditions. The microbial population, pH, titrable acidity, sugar, and organic acid metabolism were measured during the fermentation period and the viability of the strains was determined under the storage conditions at 4°C for 4 weeks. The lactic acid bacteria reduced the pH to as low as 3.2 from 4.5 within 72 h of fermentation. The substrate concentration was reduced to 5.8% (w/v) from 12% (w/v). *Lactobacillus plantarum* exhibited the fastest utilization of sugar and reduction of pH in the mango juice when compared to the other strains used. The viability of the cells was maintained at  $1.0 \times 10^7$  CFU/ml throughout the storage period. From this investigation, it can be concluded that mango juice is suitable for the production of probiotic beverage.

**Keywords:** Mango juice, lactic acid bacteria, fermentation, probiotic beverage

## Introduction

Probiotics are live organisms, which provide a benefit to the host when provided in adequate quantities. Probiotification of foods is one of the methods used to produce functional foods. Probiotics have been shown influence on the growth and/or metabolic activity of the gut microbiota and, thereby, its composition and functions [2]. They provide several health benefits including reduction in the level of serum cholesterol, improvement of gastrointestinal function, enhancement of immune system, and reduction in risk of colon cancer and should be in high concentration typically  $10^6$  CFU/g of product [1, 8, 9, 14, 18, 21]. In present

scenario, probiotics have been added to yogurt and other fermented dairy products. However, an increased demand for non-dairy probiotic products comes from vegetarianism; lactose intolerance, milk cholesterol content, milk protein allergy, and others factors [16]. This fact has led to development of probiotic products from various food matrices including fruits and vegetables [13, 29]. Different studies have been carried out to explore the suitability of fruit juices such as tomato, beet and cabbage juices as raw materials for the production of probiotic drinks. Various lactic acid bacteria have been employed as probiotic bacterial cultures. Results have indicated that all the strains are capable of growth in the fruit juices and the microbial population increases significantly after 24 h of fermentation [7, 23, 27-29].

Mango is the most cultivated and occupied 60% of the total area under fruits. Mango contains a high concentration of sugar (16–18% w/v) and acids with organoleptic properties, and also contains antioxidants like carotene (as provitamin A, 4,800 IU) are helpful as cancer-preventing agents. Mango juice along with aromatics is recommended

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as a restorative tonic; it contains good concentrations of vitamin A and C, which are useful in heat apoplexy. The surplus amounts are preserved in the form of dried slices, pickles, canned pulp, as juice and powder and also as wine through fermentation [17]. An alternative and profitable method of utilizing the surplus mangoes would be the probiotification if it could become widely accepted. In view of the above, the aim of the present research is to investigate the suitability of the mango juice for probiotification, evaluate the growth rate and substrate metabolism of selected probiotic lactic acid bacteria, and evaluate their viability in cold storage conditions. This study would give an opportunity to introduce a novel probiotic fruit (mango) drink with inherent health benefits.

## Materials and Methods

### Mango processing

Mangoes used in this study were procured from local market of Kadapa and were washed with 1% HCl and peeled off manually. Pulp was recovered manually and up to 50 mg/l potassium metabisulfite was added in accordance with the degree of maturation and hygienic state of the mango fruits. Samples were treated with previously optimized enzyme concentration and conditions (0.8% and pH 5.0) of Trizyme P50, pectinolytic enzyme procured from Triton Chemicals (Mysore, India). Juice was extracted as mentioned earlier by Reddy and Reddy [17].

### Microorganism and fermentation

Probiotic lactic acid bacteria, *Lactobacillus acidophilus* (MTCC10307), *Lactobacillus delbrueckii* (MTCC911), *Lactobacillus plantarum* (MTCC9511) and *Lactobacillus casei* were obtained from Microbial Type Culture Collection Center (MTCC), Chandigarh, India. The cultures were grown at 30°C for 24 h in MRS (de Man, Rogosa, and Sharpe) broth and were used as an inoculum. Stock culture was prepared by adding sterile glycerol (50%, v/v) to the activated culture. The glycerol stock culture was stored frozen (-20°C) in sterile screw cap tubes containing 10 ml of the culture suspension. Fermentation experiments were conducted in 250 ml Erlenmeyer flasks each containing 100 ml of pasteurized mango juice. All samples were inoculated with 5% (>10<sup>5</sup> CFU/ml) of 24 h culture and incubated at 30°C for 72 h. The variation of viable cell number, pH and reducing sugar contents was monitored periodically during incubation.

### Analytical methods

The reducing sugars were measured by adding 0.3 ml of appropriately diluted sample to a tube, applying 0.9 ml of dinitrosalicylic acid (DNS) reagent, thoroughly mixing, heating in boiling water for 5 min, and cooling on ice [10]. The absorbance was measured at 546 nm with a UV spectrophotometer (UV-1601, Shimadzu Co., Kyoto, Japan). The reducing sugar content was determined using a glucose standard curve.

The pH was measured directly using a pH meter (Elico, India). For acidity measurement, 3 drops of 1% phenolphthalein was added to 10 ml of samples, and titrated by addition of 0.1 N NaOH. Then, the acidity (%) was calculated by inserting the amount of NaOH added into the following equation:

$$\text{Acidity (\%)} = (\text{NaOH added} \times \text{NaOH factor} \times 0.006) / \text{Sample volume} \times 100$$

Viable cells (CFU/ml) were determined by the standard plate count method using MRS medium. To determine the effect of cold storage on viability after completion of fermentation, the fermented samples were stored at 4°C for 4 weeks and the viability of probiotic bacteria was measured during storage time. The microbial population was measured at weekly intervals and expressed as CFU/ml. All of these determinations were carried out in three replications.

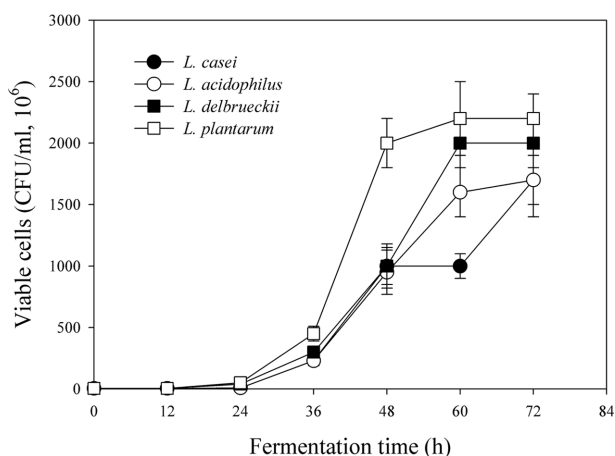
### Statistical analysis

All experiments were carried out in triplicate, and each sample was analyzed in duplicate. The results are expressed as mean ± S.D. (standard deviation). The Microsoft Excel statistical computer package was used to analyze the experimental data (Microsoft, USA).

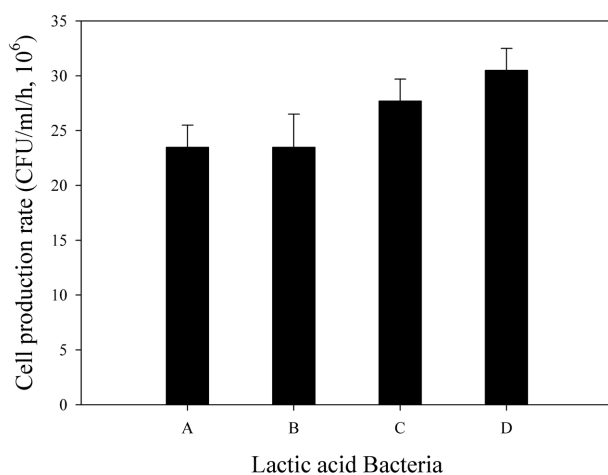
## Results and Discussion

### Growth of lactic acid bacteria during mango juice fermentation

The four lactic acid bacteria used in this study showed good growth in mango juice and found capable of rapidly utilizing mango juice for cell synthesis and lactic acid production without nutrient supplementation. Viable cell counts and cell production rate of the fermentation process for each strain are presented in Fig. 1 and Fig. 2, respectively. A small decline in the microbial population was observed



**Fig. 1.** Viable cell counts of *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, and *L. casei* during mango juice fermentation at 30°C with initial pH of 4.5.



**Fig. 2.** Cell production rate of *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, and *L. casei* during mango juice fermentation at 30°C with initial pH of 4.5. (A) *L. casei*, (B) *L. acidophilus*, (C) *L. delbrueckii*, (D) *L. plantarum*.

for all strains from the inoculation to the first 12 h of the fermentation. This may be due to the stress induced from the differences between the pre-culture medium and the fermentation medium which resulted in decrease of the growth rate at the earlier stage of fermentation process. The MRS broth, as the pre-culture medium, has a pH of about 5.6 but the initial pH of the mango juice was relatively lower (approximately 4.5). Yanez *et al.* [26] also reported that low pH of the medium can lead to the decrease in the maximum growth rate and an extended length of the lag phase. However, after passing the lag phase, the bacterial population of *L. casei*, *L. acidophilus*, *L. delbrueckii* and *L. plantarum* increased to  $1.5 \times 10^9$ ,  $1.7 \times 10^9$ ,  $2.0 \times 10^9$  and  $2.2 \times 10^9$  CFU/ml, respectively (Fig. 1). As expected from the results of viable cell counts, Fig. 2 shows that the highest cell production rate was observed from the culture of *L. plantarum* ( $3.1 \times 10^6$  CFU/ml·h). The second highest cell production rate was ranked by the culture of *L. delbrueckii* ( $2.8 \times 10^6$  CFU/ml·h). From these results, it can be concluded that *L. plantarum* and *L. delbrueckii* had a better growth rate in mango juice. Mousavi *et al.* [11] have been reported that *L. plantarum* was grown well in the selected four lactic acid bacteria in pomegranate juice.

#### Substrate consumption during mango juice fermentation

Substrate (sugar) utilization during mango juice fermentation by *L. acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii* was given in Table 1. The lactic acid cultures rapidly fermented mango juice and reduced the level of sugar. *L. plantarum* consumed the sugar at a much faster rate than *L. acidophilus*, *L. casei*, and *L. delbrueckii*. For example, *L. plantarum* reduced the sugar level from an initial value of

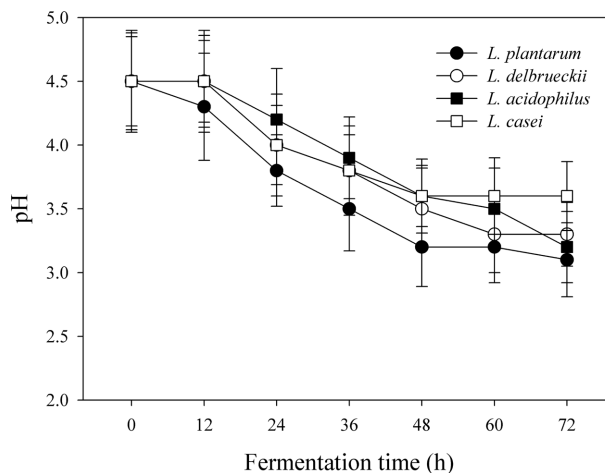
**Table 1.** Substrate consumption pattern during mango juice fermentation by *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, and *L. casei* at 30°C with initial pH of 4.5 and initial glucose concentration of  $12.0 \pm 0.9\%$ .

Time (h)	Glucose concentration (%)			
	<i>L. casei</i>	<i>L. acidophilus</i>	<i>L. delbrueckii</i>	<i>L. plantarum</i>
0	12.0 ± 0.9	12.0 ± 0.9	12.0 ± 0.9	12.0 ± 0.9
12	11.2 ± 0.8	11.2 ± 1.1	11.5 ± 1.5	11.0 ± 0.8
24	10.4 ± 0.8	10 ± 1.2	9.7 ± 1.0	9.2 ± 0.6
36	8.6 ± 0.9	8.5 ± 0.9	7.8 ± 0.6	6.8 ± 0.5
48	7.7 ± 0.4	7.2 ± 0.5	7.0 ± 0.4	6.4 ± 0.5
60	7.1 ± 0.8	7.0 ± 0.8	6.7 ± 0.5	6.0 ± 0.7
72	7.0 ± 0.5	7.0 ± 1.0	6.2 ± 0.4	5.8 ± 0.5

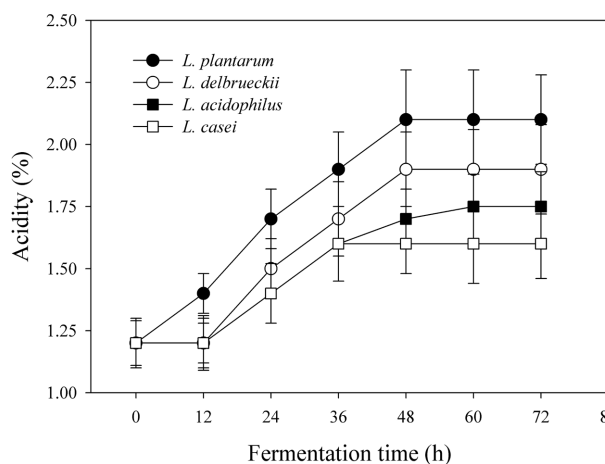
12% to 9.2%, 6.4%, and 5.8 % after 24, 48, and 72 h of fermentation, respectively. As shown in Fig. 1, the four lactic acid cultures grew rapidly in mango juice and reached to a viable cell population of greater than  $1.0 \times 10^9$  CFU/ml after 40 h of fermentation at 30°C. Extending the fermentation time from 48 to 72 h did not result in a significant increase in viable cell counts. This could be due to the low pH and high acidity in the fermented mango juice. The previous reports have proved that metabolism of carbohydrates by *Lactobacillus* varies from strain to strain and depends on the substrate and even on the fermentation time [5, 24]. Wang *et al.* [25] have also suggested that the glucose is a very good carbon and energy source for *Lactobacilli* and *Bifidobacteria*.

**pH and acidity during mango juice fermentation**

Fig. 3 and Fig. 4 show the changes in pH and acidity during mango juice fermentation by *L. acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii*, respectively. Although the mango juice had an initial pH value of 4.5, the four lactic acid cultures actively fermented the mango juice and lowered the pH to as low as 3.2 after 72 h of fermentation (Fig. 3). Especially, *L. plantarum* showed a more rapid drop in pH than the other three lactic acid cultures examined. As shown in Fig. 4, *L. plantarum* produced significantly more acid during mango juice fermentation than the other three cultures examined. It was reported that acid production ability by lactic acid bacteria, especially post-incubation (post-acidification), affected the cell viability of probiotic bacteria including *L. acidophilus* and *Bifidobacterium bifidum* [6, 20]. As depicted in the aforementioned results, the majority of the changes observed in microbial population, pH, acidity, sugar consumption, and lactic acid metabolism, happened in the period between 30 h to 48 h of the fermentation. Further extension of the fermentation process (from 48 h to 72 h) did not result in significant changes and similar results were reported by Mousavi *et al.* [11] and Yoon *et al.* [28].



**Fig. 3. pH decrease during mango juice fermentation by *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, and *L. casei* at 30°C with initial pH of 4.5.**



**Fig. 4. Acid production during mango juice fermentation by *L. plantarum*, *L. delbrueckii*, *L. acidophilus*, and *L. casei* at 30°C with initial pH of 4.5.**

**Effect of cold storage on cell viability of lactic acid bacteria**

The effect of cold storage on the cell viability of four lactic

**Table 2. Effect of cold storage on the viability of lactic acid bacteria cultures in fermented mango juice.**

Storage time (weeks)	Viable cells (CFU/ml)			
	<i>L. acidophilus</i>	<i>L. delbrueckii</i>	<i>L. plantarum</i>	<i>L. casei</i>
0	$1.72 \pm 0.2 \times 10^9$	$2.0 \pm 0.2 \times 10^9$	$2.2 \pm 0.2 \times 10^9$	$1.5 \pm 0.2 \times 10^9$
1	$1.5 \pm 0.1 \times 10^9$	$1.0 \pm 0.1 \times 10^9$	$1.5 \pm 1.6 \times 10^9$	$2.2 \pm 0.1 \times 10^8$
2	$1.0 \pm 0.1 \times 10^8$	$7.3 \pm 0.2 \times 10^7$	$1.2 \pm 0.1 \times 10^8$	$5.8 \pm 0.7 \times 10^6$
3	$5.2 \pm 0.4 \times 10^7$	$4 \pm 0.2 \times 10^6$	$1.6 \pm 0.6 \times 10^7$	$1.8 \pm 1.0 \times 10^6$
4	$1.5 \pm 1.0 \times 10^7$	$1.6 \pm 0.4 \times 10^6$	$1.4 \pm 0.4 \times 10^7$	$1.0 \pm 0.3 \times 10^6$

acid cultures in fermented mango juice was presented in Table 2. The viable cell counts of *L. acidophilus*, *L. plantarum*, *L. casei*, and *L. delbrueckii* were higher than  $1.0 \times 10^6$  CFU/ml even after 4 weeks of cold storage at 4°C. Especially, the viable cell counts of *L. delbrueckii* and *L. casei* decreased gradually during cold storage and remained at  $1.5 \times 10^6$  CFU/ml and  $6.5 \times 10^6$  CFU/ml, respectively, after 4 weeks of cold storage. The viable cell counts of *L. acidophilus* and *L. plantarum* decreased slightly during cold storage, but the cell viability of the two lactic acid bacteria remained at a considerably high level ( $>10^6$  CFU/ml) after 4 weeks of cold storage. It is important to have a significant number of viable lactic acid bacteria present in the probiotic products for maximum health benefits [11, 21]. Several factors could affect the cell viability of lactic acid cultures in probiotic food products. Probiotic cultures are commonly used in the dairy industry, and some products produced during lactic acid fermentation such as lactic acid, diacetyl, and acetaldehyde could be associated with the loss of viability of added probiotic bacteria [12]. Probiotic lactic acid starters have been suggested to produce bacteriocin against probiotic bacteria and vice versa [13, 24].

In general, the cell viability depends on the strains used, interaction between species present, culture condition, oxygen content, final acidity of the product, and the concentration of lactic acid and acetic acid. The main factors for loss of viability of probiotic organisms have been attributed to the decrease in the pH of the medium and accumulation of organic acid as a result of growth and fermentation [4, 18]. In this study, all the four lactic acid bacteria used were survived in the fermented mango juice with high acidity and low pH without addition of any nutrients. The results of this research suggest that the fermented mango juice might serve as a probiotic beverage for vegetarians or consumers allergic to dairy products.

In conclusion, four lactic acid bacteria such as *L. casei*, *L. delbrueckii*, *L. plantarum*, and *L. acidophilus* were found capable of rapidly utilizing mango juice for cell synthesis and lactic acid production without pH adjustment. They reduced the pH to as low as 3.2 and increased the acidity to as high as 1.72%, and the viable cell counts reached to  $1.0 \times 10^9$  CFU/ml after fermentation of 72 h at 30°C. All the four lactic acid bacteria survived under the low pH and high acidity conditions during 4 weeks of cold storage at 4°C. From the results of this study, it can be concluded that the mango juice could be used as one of the non-dairy raw

material for lactic acid bacteria and the product could serve as a probiotic/health drink/beverage for vegetarians and fruit juice lovers and consumers who are allergic to dairy products. Further studies have to be focused on the characterization and evaluation of functional properties of the probiotic mango juice.

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## 국문 초록

### 유산균 발효에 의한 프로바이오틱 망고 주스의 생산

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본 연구에서는 유산균 발효를 통하여 프로바이오틱 망고 주스를 생산하고자 하였으며, 이를 위하여 미호기성 조건으로 30°C에서 72시간 동안 망고 주스 발효를 실시하였다. 유산균 발효가 진행되는 동안 유산균수, pH, 산도, 환원당 및 유기산을 분석하였으며, 프로바이오틱 망고 주스를 4°C에서 4주간 저장하면서 유산균의 생존도를 측정하였다. 유산균 발효 72시간 후 pH는 초기 4.5에서 3.2로 감소하였으며, 환원당 농도는 초기 12%(w/v)에서 5.8%(w/v)로 감소하였다. 본 연구에 사용한 다른 유산균들과 비교하여 *Lactobacillus plantarum*의 경우 환원당을 가장 빠르게 대사하였으며 망고 주스의 pH를 가장 빠르게 감소시켰다. 프로바이오틱 망고 주스를 4°C에서 4주간 저장하는 동안 유산균수는  $1.0 \times 10^7$  CFU/ml를 유지하였다. 이상과 같은 결과로부터 망고 주스는 프로바이오틱 음료의 생산에 적합하다는 것을 알 수 있었다.