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Research Article

Fermentation of Tomato juice by Probiotic Lactic acid bacteria

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ABSTRACT

Probiotic alternative using vegetables and fruits were very safe for consumption since these are not containing dairy allergens. These products are also safe for lactose intolerant consumers. Objective of this present research was to study the use of tomato juice as substrate for lactic acid bacteria (LAB), probiotic and to study the ability of tomato juice probiotics as functional food. This study was undertaken to determine the suitability of tomato juice as a raw material by three lactic acid bacteria, *L. acidophilus, L. plantarum* and *L. casei.* Tomato juice was inoculated with 24hr old culture and incubated at 37°C. The change in pH, acidity, sugar and viable counts during fermentation under controlled conditions were measured. As all the cultures survived at pH up to 5.5 but pH decreases from 0hr to 72hr of incubation. Similarly, the sugar content also decreases when observed at 0hr to 72hr incubation period. The results of reduced pH were well supported by the increase acidity in samples as observed from 0hr to 72hr. Finally the decrease in pH and sugar but increase in acidity and viable count support tomato juice as probiotic drink by lactic acid bacteria. The viability of *L. acidophilus, L. plantarum* and *L. casei* was promoted that with the utilization of sugar (inulin as a prebiotic). In the probiotics when sugar inulin was added as prebiotic; symbiotic formation take place and it was noticed that on the addition of prebiotic the generation time of probiotic decreases.

Key Words: Probiotic, Tomato Juice, Lactic acid bacteria

INTRODUCTION

Probiotic organisms are live microorganisms that are thought to be beneficial to the host organism. The World Health Organization defines probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics, but certain yeasts and bacilli may also be used. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements. Probiotics are also delivered in fecal transplants, in which stool from a healthy donor is delivered like a suppository to an infected patient [1].

Bifidobacteria were first isolated from a breast-fed infant by Henry Tissier who also worked at the Pasteur Institute. The isolated bacterium named *Bacillus bifidus* communis [2] was later renamed to the genus Bifidobacterium. The majority of probiotics recommended are the species of lactobacillus including *L. acidophilus, L. plantrum, L. casei, L. delbrueckii* etc [3]. Addition of probiotics to food provides several health benefits including reduction in the level of serum cholestrol, improvement of gastrointestinal function, enhancement of immune system and reduction in risk of colon cancer. For bacteria to exert any probiotic effect, they have to be able to survive both the stomach acids (pH as low as 1.5) and the bile acids (pH as low as 2). This is true of most lactobacilli. Secondly, the bacteria must arrive in the intestines in sufficient quantities to have an effect. Probiotic inhibition of pathogenic microbes in the intestinal tract may involve a variety of mechanisms, including competition for the use of nutrients, production of antimicrobial compounds, or competition for specific adhesion sites (competitive exclusion).

Tomato juice contains 93.1% moisture, 4.89%

carbohydrate, vitamins, and minerals, and is low in protein and fat [4]. Tomato juice is well recognized as one of the healthy beverages [5]. Currently, industrial probiotic food products mainly belong to dairy products such as yoghurt and fermented milk. Fruit juice is found as a healthy food product, and is currently consumed by a large global consumer population [6]. Fruits are healthy foods because they are rich in antioxidants, vitamins, dietary fiber and minerals. In addition, fruit do not contain any dairy allergens, such as casein and lactose [7]. They also demonstrated sensory "off-flavors" in probiotic fruit juices. Fruit juice was suggested to serve as good carrier of probiotics [8]. Keeping in mind, the importance of LAB, role of probiotics in today's life and drawbacks related to consumption of milk, this study was undertaken to check the acid tolerance ability of Probiotic *Lactobacillus* cultures, to fortify the ferment tomato juice with acid tolerant *Lactobacilli* and to observe the viability and biochemical activities of *Lactobacilli* during tomato juice fermentation.

MATERIALS AND METHODS

The *L. acidophilus* (LA) (MTCC 10307), *L. plantarum* (LP) (MTCC 9496), *L. casei* (LC) (MTCC 5381) were obtained from IMTECH (Institute of Microbial Technology, Chandigarh).

Purity and confirmation of cultures

Freeze-dried lactic cultures were activated in skim milk at 37°C/ 24hr and sub-cultured monthly. Before using for experiments, the cultures were activated by 2-3 transfers in MRS (Hi-media, Mumbai, India) broth at 37°C for 24hr.

Preparation of juice

The tomato juice was freshly prepared and filtered repeatedly so as to get a clear juice free of pulp. The quantity of juice collected was 400mL which was equally divided into four sterile flasks each labeled as LA, LP, LC and control flask.

Pasteurization of juice

Pasteurization of juice was carried out immediately after preparation of fresh juice. The juice was transferred into sterile flasks and heated in the water bath at 65°C for 30 minutes.

Inoculation of cultures into juice

Each flask containing 100mL of juice was inoculated using 1% culture of each type i.e. LA, LP and LC under aseptic conditions ensuring minimum contamination. No culture was added in the flasks labeled as control. The flasks were now incubated at 37°C.

Biochemical testing of juice

10ml of juices from the flasks was taken after every 24hr for biochemical testing viz., measurement of pH, % Lactic acid concentration, and estimation of sugars in terms of glucose concentration.

Measurement of pH

The pH of each juice sample inoculated with different bacteria viz., LA, LP and LC was measured after every 24hr using properly calibrated pH meter.

Measurement of % Lactic acid concentration

5mL of the sample (juice) was taken in a conical flask and diluted with equal quantity of distilled water. Three to four drops of Phenolphthalein were added to mark the end point. The base (0.1N NaOH) from the burette was added drop wise to the flask along with continuous mixing till the end point i.e. pink colour appeared. The readings were noted down. The above procedure was repeated thrice to obtain a concordant reading.

Estimation of sugars in the form of glucose concentration [9]

For the estimation of sugar in the juices, GOD/POD kit-based method was used. The kit was supplied by Avecon Healthcare Pvt. Ltd., Saha.

Generation Time

Combinations were made in MRS broth + LA, MRS broth + LP, MRS broth + LC, Tomato juice + LA, Tomato juice + LP, Tomato juice + LC, Tomato juice + LA + Prebiotic, Tomato juice + LP + Prebiotic, Tomato juice + LC + Prebiotic. All the combinations were incubated at 37°C. Aliquots at 0hr and 24hr were taken and diluted with 0.2% (w/v) EDTA (pH 12). The turbidity was then measured at 640 nm using uninoculated MRS diluted with EDTA as a blank.

$$\textbf{Growth rate } \mu = \frac{\ln D_2 - \ln D_1}{t_2 - t_1}$$

D= Cell Density

T= Time

$$\textbf{Generation time} = \frac{\ln_2}{\mu}$$

RESULTS AND DISCUSSION

Probiotic *Lactobacilli* are having a number of health benefits. They have anti-diarrheal, anti-pathogenic, anti-diabetic, anti-cholesterol and anti-cancerous activities etc. [10-15]. Fruit juices are rich source of antioxidants, vitamins, minerals, dietary fibers and many other beneficial nutrients. The fruit juices could serve as a good medium for cultivating probiotics [16-17]. Therefore, an attempt was made to prospect the fortification of fruit juices with probiotic *Lactobacilli*. The microscopic (Gram's Staining) examination of cultures were done and assessed for catalase test. All the *Lactobacilli* strains were found to be gram-positive, catalase-negative, rod-shaped, long and slender rods.

Since most of the fruit juices have low pH and high acid content, the culture to be fortified into the juices should be able to at least survive, if not grow in the juices. Moreover, in order to get established in the gut and be nominated as probiotic, a strain must be able to withstand the acidic conditions in the stomach or intestine. Therefore, all the three cultures were tested for their ability to tolerate varying concentration of acids (Table 1). All the cultures survived at pH 5.5 as well as 4.5 for upto 3hr; but at pH 3.5, only *L. acidophilus* survived for 3hr, while *L. plantarum* and *L. casei* could survive for 2hr only. At pH 2.5, *L. plantarum* and *L. casei* showed no growth; but *L. acidophilus* was able to tolerate pH 2.5 for up to 1hr. These results could indicate the possibility that these cultures shall be able to survive in fruit juices which contains pH around 4.0 to 4.5.

Therefore, all three cultures were selected for fortification or fermentation of tomato juice. The isolate *L. acidophilus* showed high acid tolerance which may enable it to successfully pass the low pH of the stomach and reach in the gut of the host needed for successful colonization and propagation for expression of its health promoting effects.

Many members of the society having problem of lactose intolerance, milk allergy or high cholesterol/ fat content are devoid of probiotics as most of the probiotic products available in the market are dairy-based. Therefore, fruit juices could be exploited as a suitable carrier or medium of probiotics for such people [15-16]. Fruits are extremely healthy having high content of antioxidant/ vitamin, minerals, dietary fibers and many other beneficial nutrients and moreover fruits are totally free from milk allergens or milk sugar [17] therefore, such fruit juices could prove to be an ideal candidate for the delivery of probiotics to a large segment of population who are allergic to milk products.

Therefore, in the present investigation, three isolates *L. acidophilus, L. plantarum* and *L. casei* were tested for their ability of utilizing tomato juice sugar and lactic acid production without any additional nutrient addition or pH adjustments.

When tomato juice was inoculated with cultures, a reduction in the sugar was observed in case of all the cultures (Fig.1). The initial sugar content of tomato juice was 87.95mg/dl, 86.85mg/dl, 85.0mg/dl of *L. acidophilus, L. plantarum* and *L. casei* at 0hr which was reduced to 82.46mg/dl, 81.64mg/dl and 77.85mg/dl at 24hr, 79.9mg/dl, 78.5mg/dl and 75.0mg/dl at 48hr, 79.0mg/dl, 77.65mg/dl and 74.52mg/dl at 72hr of incubation respectively.

The results of decreased pH also showed similar trend. The original pH was 4.5 of *L. acidophilus, L. plantarum* and *L. casei* at 0hr which reduced up to 4.3, 4.3 and 4.1 at 24 hr, 4.0, 4.1 and 3.9 at 48hr and 3.9, 4.0, 3.8 at 72hr respectively (Fig. 2). The decrease in the pH was concurrent with the decrease in the sugar content of tomato juice. The results of reduced pH were well supported by the similar trend in increased acidity in samples inoculated with *L. acidophilus, L. plantarum* and *L. casei* (Fig. 3). Isolates *L. acidophilus, L. plantarum* and *L. casei*, were able to increase the acidity from 0.43% at 0hr to 0.69%,0.61% and 0.51% at 24hr, 0.75%,0.62%,0.61% at 48 hr and 0.80%,0.70%,0.69% at 72hr of incubation respectively.

Besides, the three *Lactobacillus* isolates were also tested for their ability to survive during lactic acid fermentation of fruit juice (Table 2). The initial cell count in juice sample was $2.49x10^8$, $2.47x10^8$ and $2.47x10^8$ cfu/ml at 0hr for *L. acidophilus, L. plantarum* and *L. casei*, respectively. However, after 72hr incubation, the cell counts of *L. acidophilus* increased to $2.95x10^8$; while, in case of *L. plantarum*, the cell count observed was $2.89x10^8$, indicating that the two cultures were not only able to survive but also utilized and fermented the fruit sugar for their cell synthesis and metabolism. The cell counts for *L. casei* was $2.85x10^8$ after 72hr incubation indicating that growth was slower than that of *L. acidophilus* and *L. plantarum*.

Tomato juice is well recognized as one of the healthy beverages [18]. Growth of *L. acidophilus* was stimulated by addition of tomato juice to skimmed milk and resulted in higher viable counts, shorter generation time, and improved sugar utilization with more acid production and lower pH [19]. Probiotic fermentations of indigenous food mixtures containing tomato pulp using *L. casei* and *L. plantarum* showed a decrease of pH, increase of acidity, and improvement of the digestibility of starch and protein [20]. Yoon *et al.* also observed decrease in sugar and pH and increased acidity when tomato juice was inoculated and incubated with *L. acidophilus, L. plantarum, L. casei* and *L. delbrueckii* [21]. In present investigation, the two *Lactobacillus* isolates that is *L.*

plantarum and *L. acidophilus* were observed to be able to not only survive but utilize the fruit juices for their cell synthesis, as indicated by a decrease in fruit sugar and pH, and increase in acidity and bacterial counts. However, isolate *L. acidophilus* was found to consume the sugar at a faster rate than *L. plantarum*; although, the fall in sugar and pH and increase in acidity was faster during first 24hr and got little slower during the next 48hr which could be due to extremely low pH and high acidity achieved during the initial 24hr of fermentation. Moreover, there could be several other factors that could have affected the survival/ growth of cells, for e.g. accumulation of other metabolic end products such as lactic acid and other organic acids, diacetyl, acetylaldehyde and acetoin etc. which could reduce the viability of the cultures if accumulated in high amounts [22-25]. It has been reported that acid production ability by lactic acid bacteria, especially post-incubation (post-acidification), affects the cell viability of probiotic bacteria including *L. acidophilus* and *Bifidobacterium bifidum* [26-27].

Generation time

In an attempt to further improve the survival and growth of lactic cultures in the juice, a probiotic i.e. inulin was added to the tomato juice in an attempt to support/ promote the growth of this organism in the tomato juice samples.

In the growth kinetics for 24hr of incubation for *L. acidophilus*, *L. plantarum*, and *L. casei* in MRS broth, tomato juice and tomato juice containing inulin, it was observed that in case of *L. acidophilus* in MRS broth, tomato juice, tomato juice containing inulin the generation time (in min) was 46, 60, 55 respectively. In the case of *L. plantarum*, the generation time (in min) was 48, 60, 57 respectively for *L. casei*, it was found to be 49, 61 and 58 respectively (Fig. 4).

From the above data, it could be concluded that generation time was reduced when inulin was added as compared to tomato juice alone. LA, LP, LC utilized sugar for the cells synthesis and grow better in less time as compared to juice without inulin. Inulin nourishes the good bacteria in digestive tracks, improve nutrient absorption and enhance immune function in the clone [14]. Lactic acid bacteria were found capable of rapidly utilizing tomato juice for cell synthesis and lactic acid production. The combination of probiotic bacteria with nutrient-dense foods, such as dairy products, will have the added benefit of enhancing consumer nutrition. The tomato juice fermented with bifidobacteria can be developed as a potential pro-biotic product, and may benefit the consumers searching for an alternative beverage to replace fermented dairy products [28]. Mixture of malt extract and red fruit juices are a suitable substrate for the growth of lactic acid bacteria and production of functional beverage.[29]

In present investigation, the three *Lactobacillus* cultures that is *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus casei* were observed to able to not only survive but utilizing the fruit juice for their cell synthesis, as indicated by a decrease in fruit sugar, pH and increase in acidity. However, *L. acidophilus* was found to consume the sugar at a faster rate than *L. plantarum* and *L. casei*; although, the fall in sugar and pH and increase in acidity was faster during first 24hr and got little slower during the next 48hr which could be due to extremely low pH and high acidity achieved during the initial 24hr of fermentation. Moreover, there could be several other factors that could have affected the survival/ growth of cells, for e.g. metabolic end products such as lactic acid and other organic acids, diacetyl and acetylaldyhyde etc. which could reduce the viability of the cultures if accumulated in high amounts.

The incorporation of lactic acid bacteria into fruit juices with low pH may enhance the resistance of bacteria to subsequent stressful acidic conditions, such as those found in gastrointestinal tract.[30]

Since, in present study, all the cultures were found to be able to survive in the fermented juices with high acidity and low pH; therefore, it could be advocated that fruit juices could be exploited as a carrier/ medium for the fermentation and delivery of probiotic lactic acid bacteria, and these probiotic fortified fruit products could be used as a functional healthy beverage for the better health and nutrition of the people, especially for those who are allergic or intolerant or reluctant to milk based products. However, more extensive *in-vitro* and *in-vivo* studies shall be vital in order to authenticate the probiotic potential and safety of such cultures and fruit products based on these beneficial microbes before being endorsed for better health and nutrition of the society.

From the results of this study, it is concluded that the fermented tomato juice could be used as a raw material for lactic acid fermentation, and the product could serve as a health beverage for vegetarians and consumers who are allergic to dairy products.

Table 1
Acid tolerance of *Lactobacillus* isolates.

Culture	Time (hr)	pH			
		2.5	3.5	4.5	5.5
L. acidophilus	0	+	+	+	+
	1	+	+	+	+
	2	-	+	+	+
	3	-	+	+	+
L. plantarum	0	+	+	+	+
	1	-	+	+	+
	2	-	+	+	+
	3	-	-	+	+
L. casei	0	+	+	+	+
	1	-	+	+	+
	2	-	+	+	+
	3	-	-	+	+

Table 2
Viable counts *of Lactobacilli* during fermentation of tomato juice.

Culture	Viable Counts (x 10^7 cfu/ml)			
	0 hr	24 hr	48 hr	72 hr
L.acidophilus	2.49×10	2.85×10	2.99×10	2.95×10
L.plantarum	2.47×10	2.82×10	3×10	2.89×10
L.casei	2.47×10	2.80×10	2.95×10	2.85×10

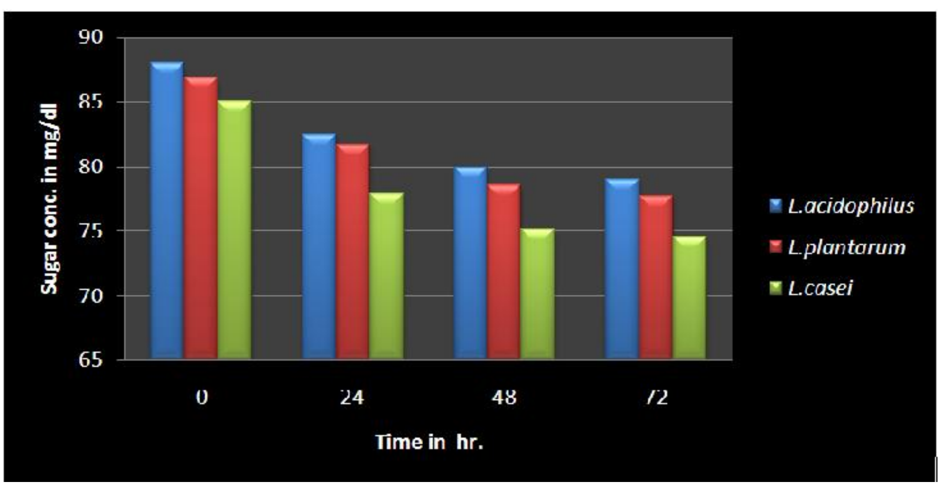

Fig. 1
Changes in sugar concentration during fermentation of tomato juice (values are mean of three replicates)

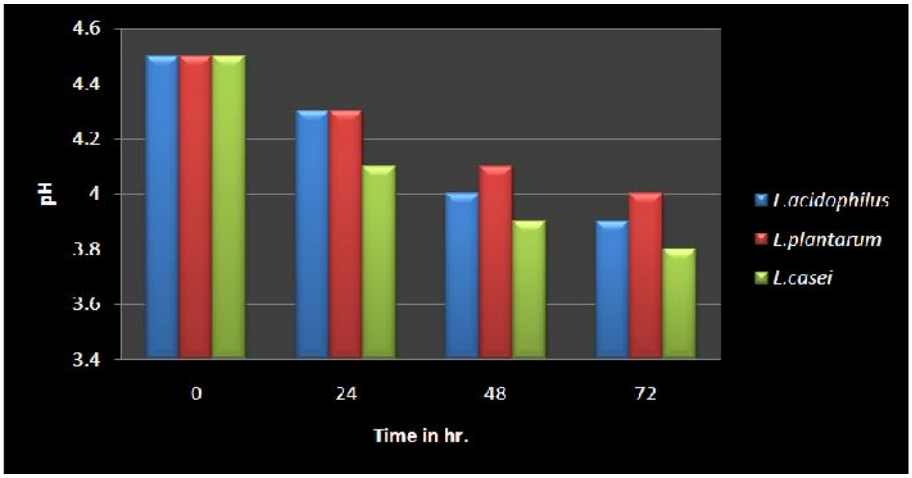

Fig. 2
Changes in pH during fermentation of tomato juice (values are mean of three replicates)

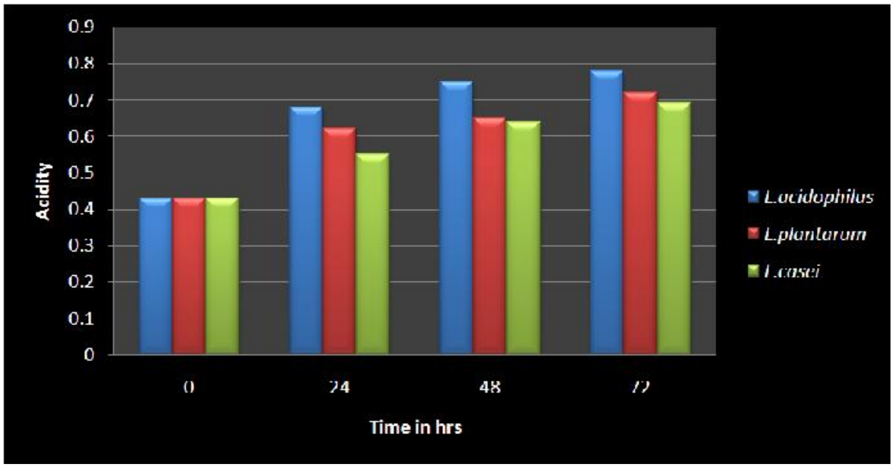

Fig. 3
Changes in acidity during fermentation of tomato juice (values are mean of three replicates)

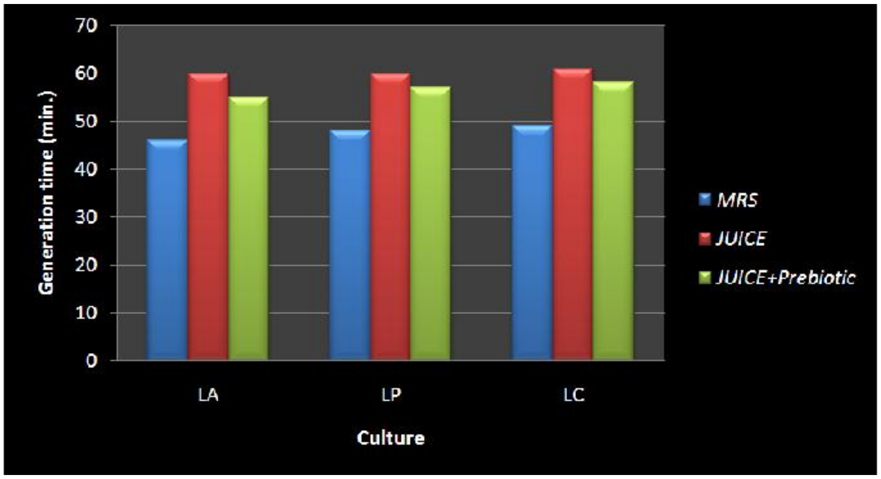

Fig. 4
Generation time (in min) of LA, LP, LC in MRS, juice, juice+probiotic (values are mean of three replicates)

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