

***In vitro* Antibacterial Activity of Carrot and Watermelon Juices Probioticated using Lactic Acid Bacteria against Intestinal Pathogens**

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Probioticated carrot and watermelon juices were produced using four different strains of lactic acid bacteria [*Lactobacillus plantarum* MTCC1407, *Lactobacillus rhamnosus* MTCC1408, *Lactobacillus casei* MTCC1423, *Lactobacillus lactis* MTCC911]. The watermelon and carrot juices were pasteurized for 30 minutes at 63°C and was inoculated with a 24h culture of individual lactobacilli and incubated at 37°C. Changes in pH, acidity, sugar content, and viable cell count during fermentation under controlled condition were measured. All of the lactobacilli were capable of growing in watermelon and carrot juices and reached a cell density of 10⁷ CFU/ml after 48h incubation at 37°C. Overnight cultures of *Clostridium perfringens*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Bacillus cereus* were added to probioticated juices and reduction of viable cells were assayed. The viable cell counts of the four-lactic acid bacteria in the fermented carrot and watermelon juices ranged from 10⁷ to 10⁹ CFU/ml and 10⁸-10⁹ CFU/ml respectively. Antimicrobial activities of the lactobacilli cells against the test strains were also determined by measuring the diameter of growth inhibition zones. Among all the four lactic acid bacteria *Lactobacillus casei* was the most potent inhibitor of all the tested intestinal pathogens such as *Salmonella typhimurium*, *Shigella dysenteriae*, *Bacillus cereus*, *Clostridium perfringens* and *Escherichia coli*.

Key Words: Probiotics, Lactobacilli, Watermelon, Carrot, Intestinal pathogens.

“Let food be thy medicine and medicine be thy food” the age-old quote by Hippocrates, is certainly the tenet of today. With the growing interest in self-care and integrative medicine coupled with our health embracing baby boomer population, recognition of the link between diet and health has never been stronger. As a result,

the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional food, is the small but rapidly expanding arena of probiotics-live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance (Suvarna and Boby 2005).

Lactic acid bacteria (LAB) are traditionally used as natural or selected starters in the preparation of various fermented foods. As they are involved in numerous food fermentations, it is assumed that most of the representatives of this group do not pose any healthy risk to man and are designated as generally recognized as safe (GRAS) organisms. The LAB, generally considered as

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'food grade' organisms are mostly acclaimed with various health-promoting traits. These LAB produce various antagonistic substances, which can inhibit pathogenic and spoilage microorganisms (Senesi *et al* 2001; Aditi Sourabh *et al.*, 2011).

A synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the friendly flora of the human intestine. A synbiotic product should be considered a functional food rather than some obscure chemistry formulation. In the symbiotic present scenario, food is no longer consumed for satisfaction of hunger alone but for promoting nutrition and health. The concept of functional foods has gained universal acceptance as a preventive and therapeutic approach to combat many disease that decrease the work productivity due to poor health (Sheela *et al.*, 2011).

Probiotics are widely used to treat gastrointestinal disorder. The belief in the beneficial effects of probiotics is based on the knowledge that the intestinal microflora can protect humans against infection and disturbance of this flora can increase susceptibility to infection. The gut flora consists of microorganisms that normally live in the digestive tract of humans. The average human body, consisting of about 10^{13} [10,000,000,000,000 or about ten trillion] cells, has about ten times that number of microorganism in the gut. Bacteria make up most of the flora in the colon and 60% of the dry mass of feces [Klaenhammer and Kullen 1999].

A continued interest is observed among dairy type probiotics food products, such as fermented meats, vegetables and fruits juices. A particular feature of probiotic cultures is that they regulate the balance of the gut bacterial population; presumably by competition for epithelium contact sites and nutrients and also by modulation of pH value. They may also induce synthesis of vitamins such as riboflavin. In addition, probiotic cultures are also suggested to stimulate the immune system. Functional food industries are now focusing on new non-dairy foods that can contribute to a regular consumption of probiotics in individuals with lactose intolerance. It has been suggested that fruit juice could serve as a good medium for cultivating probiotics [Mattila Sandholm *et al.*, 2002].

Fruit and vegetable juices could serve as a good medium for functional ingredients such

probiotics and prebiotics. These products are recognized as healthy foods and can be consumed frequently by a significant percentage of consumers. Not all lactic acid bacteria can survive in vegetable and fruit juices. Therefore, obtaining a fermented juice from these sources requires the use of specially selected cultures to ensure proper progress of the fermentation process and a guaranteed consistent product with beneficial organoleptic features [Brandt 2004].

Among the various fruits, Watermelons hold the honor of being among the most beloved of fruits all over the world. However, it is interesting to remember that the mean lycopene concentration of watermelon (4868 micrograms/100 g) is about 40% higher than the year-round mean for raw tomato (3025 micrograms/100 g), and watermelon ranks 5th among the major contributors of lycopene in the diet. Lycopene is a red pigment that occurs naturally in certain plant and algal tissues. In addition to giving watermelon and tomatoes their characteristic 'red' color, it is also believed to be a powerful antioxidant. Lycopene scavenges reactive oxygen species, which are aggressive chemicals always ready to react with cell components, causing oxidative damage and loss of proper cell function [Edwards, 2003].

Among the various vegetables, Carrots should be considered a goldmine of natural vitamins and nutrients. Among other raw vegetable juices, carrot juice is an absolute leader in the context of the variety of its therapeutic effects, the contents of useful elements and its compatibility with other juices or food. As we all know, no other vegetable contains as much beta-carotene as carrots. In our body, beta-carotene is converted into Vitamin A, which assists in improving our eyesight and the functioning of our immunity system, strengthening our bones and teeth, preventing possible problems with the functioning of the thyroid gland. Vitamin A also has positive effects on hair, nails and skin. Besides, this Vitamin is associated with good cleansing effects: it can clean our liver from fat and other unnecessary elements. However, for receiving maximum results for the liver, it is necessary to drink carrot juice on a regular basis. Drinking carrot juice is extremely beneficial for pregnant women and young children. Vitamins A and E are very important for normal development of the fetus. Since carrot juice has

good amounts of natural sugar, the majority of children like drinking it. Always remember that two glasses of fresh carrot juice a day can substantially improve the overall health of you and all the members of your family.

The present study deals with the probiotics, which have several health benefits and now a days it is mainly used to treat several intestinal disorders. So an attempt has been made in the present study to determine the suitability of carrot and watermelon juices as a raw material for the production of probiotic juice by four lactic acid bacteria *Lactobacillus plantarum* MTCC1407, *Lactobacillus rhamnosus* MTCC1408, *Lactobacillus casei* MTCC1423, and *Lactobacillus lactis* MTCC911 and the antagonist effect of probiotic juices was tested against the intestinal pathogens such as *Salmonella typhimurium*, *Clostridium perfringens*, *Escherichia coli*, *Shigella dysenteriae* and *Bacillus cereus*.

MATERIALS AND METHODS

Microorganism

Probiotic lactic acid bacteria [*Lactobacillus plantarum*1407, *Lactobacillus rhamnosus*1408, *Lactobacillus casei*1423, *Lactobacillus lactis* 911] were procured from MTCC [Microbial type culture collection] at Chandigarh, India. The test organism such as *Clostridium perfringens*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, and *Bacillus cereus* were obtained from Vaishnavi laboratory at Kumbakonam, Tamilnadu, India.

Culture of bacteria

The lactic acid bacteria were grown at 37°C for 48 hours in De Mann, Rogosa and Sharp broth [MRS] and were used as inoculum. Intestinal pathogens such as *Clostridium perfringens*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Bacillus cereus* were grown at for 24 hours in nutrient broth.

Probiotication of juices [carrot and watermelon]

Carrot and watermelon was purchased from a local vegetable market in mayiladuthurai. Juices were prepared from homogenized slices and were further pasteurized for 30 minutes at 63°C. Fermentation experiments were conducted in Erlenmeyer flask [250 ml] each containing 100 ml of

pasteurized juices were inoculated with 1 ml of 24 hours culture of lactic acid bacteria and were incubated at 37°C for 48 hours.

Effect of cold storage on cell viability in probiotic carrot and watermelon juices

After 72 hours of fermentation at 37°C, the fermented samples [25 ml] were stored at 4°C for four weeks. Samples were taken at weekly intervals, and viability of probiotic cultures in probiotic juices were determined and expressed as colony forming unit [CFU].

Chemical and microbiological analysis

Samples were taken at 24 hours intervals for chemical and microbiological analysis. pH was measured with a pH meter. Total acidity [percent lactic acid] was determined by titrating with 0.1M NaOH. Sugar content was analysed as glucose by the phenol sulphuric acid method [Dubois et al., 1956; Kyung Young Yoon et al., 2004]. Viable cell counts (CFU/ml) were determined by the standard plate method with Lactobacilli MRS medium after 48 hours incubation at 30°C

Inhibition assays

For detection of antimicrobial activity, an agar diffusion assay (well method) was used.

Statistical analysis

All experiments were carried out in triplicate, and each sample was analyzed in duplicate. The results are expressed as mean \pm S.D. the SPSS statistical computer package was used to analyze the experimental data. The values that have no common superscript are significantly different according to Duncan's multiple range tests. Any two means that are not marked by the same superscript [a and b or b and c] are significantly different. Any two means that are marked by the same superscript [a and a or b and b] are not significantly different.

RESULTS

The four lactic acid bacteria, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus lactis* used in this study were found capable of rapidly utilizing watermelon and carrot juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment and reached a cell density of 10^8 CFU/ml.

Fig .1. and Fig.2. shows the changes in

Table 1. Effect of cold storage on the cell viability of lactic acid cultures in fermented carrot juice

Time (Week)	Survival (CFU/ML)			
	<i>L. casei</i>	<i>L. plantarum</i>	<i>L. lactis</i>	<i>L. rhamnosus</i>
0	$4.2 \pm 2.7 \times 10^{8a}$	$2.4 \pm 0.5 \times 10^{9a}$	$2.3 \pm 1.9 \times 10^{8a}$	$2.4 \pm 0.4 \times 10^{8a}$
1	$3.8 \pm 1.2 \times 10^{8a}$	$2.1 \pm 1.7 \times 10^{9a}$	$2.2 \pm 0.7 \times 10^{8a}$	$2.2 \pm 0.1 \times 10^{8a}$
2	$2.5 \pm 0.5 \times 10^{8b}$	$2.0 \pm 0.5 \times 10^{9b}$	$2.1 \pm 0.8 \times 10^{8b}$	$1.8 \pm 0.3 \times 10^{8b}$
3	$1.6 \pm 1.0 \times 10^{8c}$	$1.9 \pm 0.7 \times 10^{7b}$	$2.2 \pm 0.1 \times 10^{8a}$	$1.2 \pm 1.0 \times 10^{8c}$
4	$2.8 \pm 0.7 \times 10^{8b}$	$1.3 \pm 0.3 \times 10^{8c}$	$1.8 \pm 0.5 \times 10^{8c}$	$1.1 \pm 0.4 \times 10^{8c}$

The experimental values (means and standard deviations for n=3) that have no common superscript are significantly different ($p < 0.05$) according Duncan's multiple test range. Any two means not marked by the same superscript (for example, a and b or b and c) are significantly different. Any two means not marked by the same superscript (for example, a and a or b and b) are not significantly different.

Table 2. Effect of cold storage on the cell viability of lactic acid cultures in fermented watermelon juice

Time (Week)	Survival (CFU/ML)			
	<i>L. casei</i>	<i>L. plantarum</i>	<i>L. lactis</i>	<i>L. rhamnosus</i>
0	$4.4 \pm 1.9 \times 10^{8a}$	$2.3 \pm 0.4 \times 10^{9a}$	$2.2 \pm 1.0 \times 10^{8a}$	$2.3 \pm 0.5 \times 10^{9a}$
1	$3.0 \pm 0.7 \times 10^{8a}$	$2.1 \pm 0.1 \times 10^{9b}$	$2.0 \pm 0.5 \times 10^{8a}$	$2.0 \pm 2.7 \times 10^{8a}$
2	$2.1 \pm 0.8 \times 10^{8b}$	$1.9 \pm 0.3 \times 10^{9b}$	$1.8 \pm 1.5 \times 10^{8b}$	$1.3 \pm 1.5 \times 10^{8c}$
3	$1.5 \pm 0.1 \times 10^{8c}$	$1.6 \pm 1.0 \times 10^{7b}$	$1.6 \pm 1.6 \times 10^{8c}$	$1.7 \pm 1.2 \times 10^{8b}$
4	$2.7 \pm 0.5 \times 10^{8b}$	$1.5 \pm 0.4 \times 10^{8c}$	$1.0 \pm 3.5 \times 10^{8c}$	$1.4 \pm 0.4 \times 10^{8c}$

The experimental values (means and standard deviations for n=3) that have no common superscript are significantly different ($p < 0.05$) according Duncan's multiple test range. Any two means not marked by the same superscript (for example, a and b or b and c) are significantly different. Any two means not marked by the same superscript (for example, a and a or b and b) are not significantly different.

Table 3. Inhibition assay of probioticated juices against intestinal pathogens by well diffusion method

Organisms	Zone of inhibition in (mm) (Concentrated 10µl of probioticated juices)									
	<i>Escherichia coli</i>		<i>Salmonella typhimurium</i>		<i>Shigella dysenteriae</i>		<i>Clostridium perfringens</i>		<i>Bacillus cereus</i>	
	Carrot juice	Water Melon juice	Carrot juice	Water Melon juice	Carrot juice	Water Melon juice	Carrot juice	Water Melon juice	Carrot juice	Water Melon juice
<i>L. casei</i>	28	30	26	26	30	30	22	20	20	22
<i>L. rhamnosus</i>	11	13	17	13	16	14	11	11	20	18
<i>L. lactis</i>	18	15	15	17	12	16	14	16	18	16
<i>L. plantarum</i>	15	11	12	14	11	12	13	14	15	12

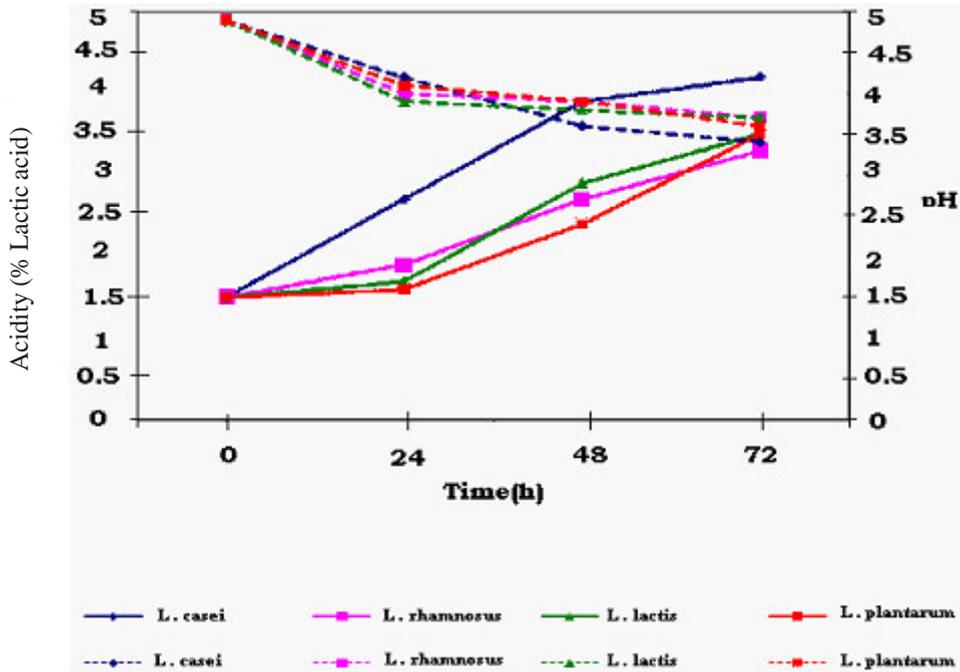


Fig. 1. Changes in pH and acidity during lactic acid fermentation of watermelon juice

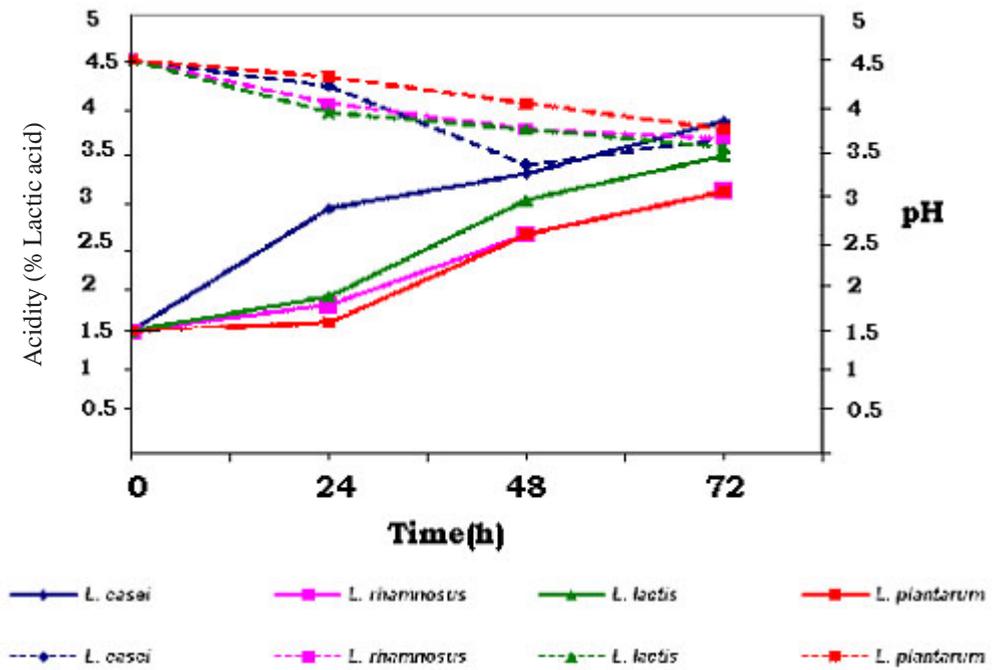


Fig. 2. Changes in pH and acidity during lactic acid fermentation of carrot juice

pH and acidity during fermentation of watermelon and carrot juices. Although watermelon and carrot juices had an initial pH value of 4.9 and 4.5, the four lactic acid bacteria actively fermented the watermelon and carrot juices and lowered the pH to as low as 3.4 and 3.5 and increased the acidity from 1.5% and 1.6% to 4.3% and 3.9% respectively, after 72 hours of fermentation. In both juices *Lactobacillus casei* showed a more rapid drop in pH and increased the acidity than the other three lactic acid bacteria.

Changes in the sugar content during watermelon and carrot juice fermentation are given in Fig. 3. and Fig. 4. The lactic acid cultures rapidly fermented watermelon and carrot juices reduced the level of sugar. *Lactobacillus casei* consumed the sugar at a much faster rate than the other three lactic acid bacteria. For example *Lactobacillus casei* reduced the sugar level from an initial value of 30.4mg/ml to 23.2mg/ml, 19.0mg/ml and 17.3mg/ml respectively after 24, 48, 72 hours fermentation.

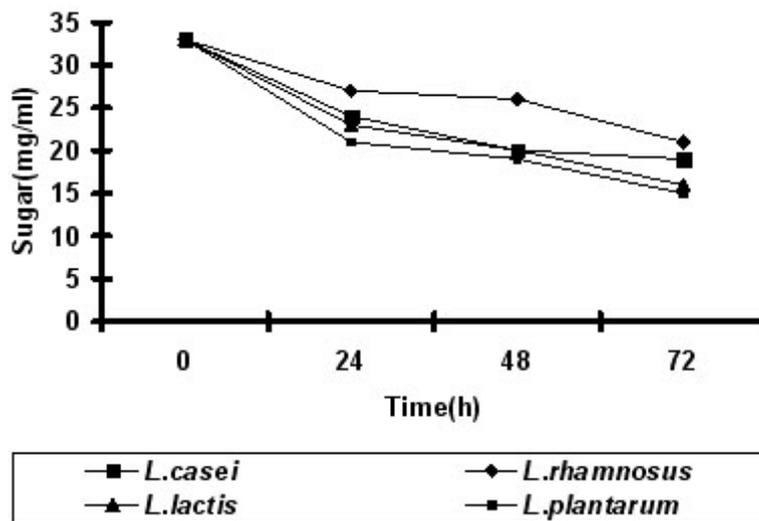


Fig 3. Changes in sugar content during lactic acid fermentation of watermelon juice

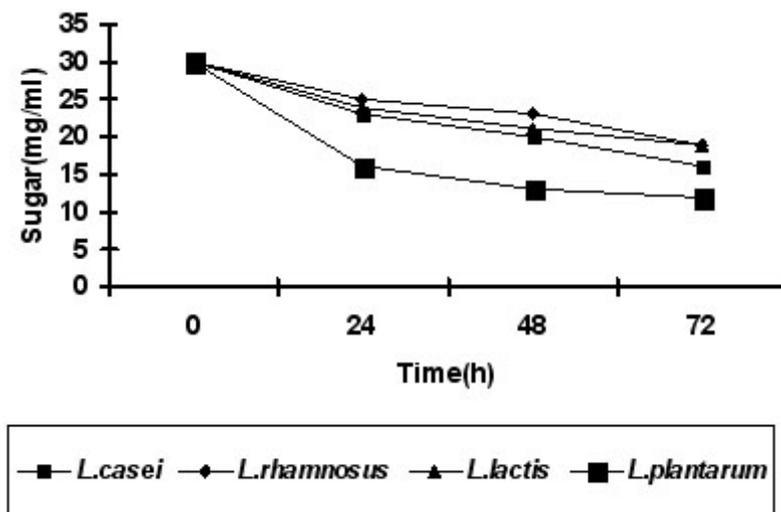


Fig 4. Changes in sugar content during lactic acid fermentation of carrot juice

The four lactic acid bacteria grew rapidly in watermelon and carrot juices and reached a viable cell population of greater than 1.0×10^7 cells/ml after 48 hours fermentation at 30°C. Table 1. and Table 2. shows the effect of cold storage on the cell viability of four lactic acid bacteria in fermented juices. The results were tabulated according to Duncan's multiple test range. The viable cell counts of *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus lactis* were higher than 10^7 cells/ml even after 4th week. *Lactobacillus casei* was more viable during cold storage up to the 4th week and reached a cell count of 2.9×10^8 cells at 1st week and 2.6×10^8 cells at 4th week in carrot juice and 2.1×10^8 and 1.86×10^8 in watermelon juice. After 5th week, the viable cell counts of four lactic acid bacteria were decreased slightly during cold storage.

Visible inhibition zones in mm were observed around the cells of four lactic acid bacteria by well diffusion method are shown in Table 3. indicating great inhibitory effect of the lactic acid bacteria against the tested intestinal pathogens. *Lactobacillus casei* was the most potent inhibitor of all the tested intestinal pathogens and formed a zone of about 30mm, 26mm, 30mm, 20mm and 22mm against *Salmonella typhimurium*, *Shigella dysenteriae*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* in probioticated watermelon juice and 28mm, 26mm, 30mm, 22mm, and 20mm in probioticated carrot juice respectively.

DISCUSSION

Sindhu and Khetarpaul, (2001) reported that probiotic fermentations of indigenous food mixtures containing tomato pulp using *Lactobacillus casei* and *Lactobacillus plantarum* showed a decrease of pH, increase of acidity, and improvement of the digestibility's of starch and protein. This investigation showed that watermelon and carrot juices had an initial pH value of 4.9 and 4.5, the four lactic acid bacteria actively fermented the watermelon and carrot juices and lowered the pH to as low as 3.4 and 3.5 respectively and increased the acidity from 1.5% and 1.4% to 4.2% and 3.8% respectively, after 24 hours of fermentation. In both juices *Lactobacillus casei* showed a more rapid drop in pH and increased the

acidity than the other three lactic acid bacteria. Shah *et al.*, (1995) reported that acid production ability by lactic acid bacteria, especially (post-acidification), affected the cell viability of probiotic bacteria including *L. acidophilus* and *Bifidobacterium bifidum*.

Shah, (2001) reported that, it is important to have a significant number of viable lactic acid bacteria present in the probiotic products for maximum health benefits ($\geq 10^6$ CFU/ml). Gobbetti *et al.*, (1998) reported that, the *Bifidobacteria* and *L. rhamnosus* strains showed a good survival rate until 30 days ($\geq 10^6$ CFU/ml); afterwards the population remained unvaried for *L. rhamnosus* strains, while a slight decline was observed for *Bifidobacteria*. In this present study the four lactic acid bacteria grew rapidly in watermelon and carrot juices and reached a cell viable cell population of greater than 1.0×10^7 cells/ml after 48 hours fermentation at 30°C.

Holcomb *et al.*, (1991) studied viability of *L. acidophilus* and *Bifidobacterium bifidum* in soft serve frozen yoghurt. Modler *et al.*, (1990) studied survival of *Bifidobacterium* in ice cream over 70 d of frozen storage and found approximately 90% survival of these bacteria during the storage period. The effects of cold storage on the cell viability of four lactic acid bacteria in fermented juices were observed. The viable cell counts of *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus lactis* were higher than 10^7 cells/ml even after 4th week.

Brashears *et al.*, (1999) had examined the antagonistic effects of *Lactobacillus lactis* on *Salmonella typhimurium*. The results of this survey indicated that all the lactic acid bacteria present in probioticated watermelon and carrot juices could in fact exert their anti-pathogenic properties against the tested intestinal pathogen such as *Salmonella typhimurium*, *Shigella dysenteriae*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*. *Lactobacillus casei* was the most potent inhibitor of all the tested intestinal pathogens and formed a zone of about 30mm, 26mm, 30mm, 20mm and 22mm against *Salmonella typhimurium*, *Shigella dysenteriae*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* in probioticated watermelon juice and 28mm, 26mm, 30mm, 22mm, and 20mm in probioticated carrot juice respectively. The

antagonistic effect of *Lactobacillus casei* GG against *Salmonella typhimurium* has been also investigated by Houdault *et al.*, (1997).

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