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Probiotication of Tomato Juice by Lactic Acid Bacteria

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This study was undertaken to determine the suitability of tomato juice as a raw material for production of probiotic juice by four lactic acid bacteria (*Latobacillus acidophilus* LA39, *Lactobacillus plantarum* C3, *Lactobacillus casei* A4, and *Lactobacillus delbrueckii* D7). Tomato juice was inoculated with a 24-h-old culture and incubated at 30°C. Changes in pH, acidity, sugar content, and viable cell counts during fermentation under controlled conditions were measured. The lactic acid cultures reduced the pH to 4.1 or below and increased the acidity to 0.65% or higher, and the viable cell counts (CFU) reached nearly 1.0 to 9.0×10^9/ml after 72 h fermentation. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 CFU/ml after 4 weeks of cold storage at 4°C. Probiotic tomato juice could serve as a health beverage for vegetarians or consumers who are allergic to dairy products.

Key words: probiotics, tomato juice, lactic acid bacteria

The relationship between certain foods and health benefits has been investigated for many years. Development of foods that promote health and well-being is one of the key research priorities of food industry (Klaenhammer and Kullen 1999). This trend has favored consumption of foods enriched with physiologically active components such as prebiotics, probiotics, vitamins, minerals, dietary fiber, fish oils, and plant sterol (Betoret *et al.*, 2003). Probiotics are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance (Fuller, 1989). The majority of probiotics recommended are the species of *Lactobacillus* including *L. acidophilus, L. plantarum, L. casei* and *Streptococcus lactis*, etc (Sindhu and Khetarpaul, 2001). Probiotication is one of the methods used to produce fermented functional foods. Addition of probiotics to food provides 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 (Berner and ODonnell, 1998; Saarela, et al., 2002; McNaught and MacFie, 2001; Rafter, 2003). For health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^6 cfu/g of product (Shah, 2001).

Currently, probiotic products are usually marketed in the form of fermented milk and yogurt. However, lactose intolerance and the cholesterol content are two drawbacks related to their consumption. It has been suggested that fruit juice could serve as a good medium for cultivating probiotics (Mattila-Sandholm, *et al.*, 2002). Fruits and vegetables are healthy foods, because they are rich in antioxidants, vitamins, dietary fibers and minerals. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow & Delahunty, 2004).

Tomato juice contains 93.1% moisture, 4.89% carbohydrate, vitamins, and minerals, and is low in protein and fat (Abdel-Rahman, 1982). Tomato juice is well recognized as one of the healthy beverages (Suzuki *et al.*, 2002).

The objective of this study was to determine the suitability of tomato juice for production of probiotic juice by lactic acid bacteria.

Materials and Methods

Strains and cultures

Probiotic lactic acid bacteria (*Lactobacillus acidophilus* LA39, *Lactobacillus casei* A4, *Lactobacillus delbrueckii* D7, and *Lactobacillus plantarum C3*) were obtained from the New York State Agricultural Experiment Station Culture Collection, Geneva, New York. The cultures were grown at 30°C for 24 h in MRS broth and were used as an inoculum. Commercial tomato juice (Campbell Soup Company, NJ) was purchased from a local store and kept at 4°C prior to use.

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Fermentation of probiotic tomato juice

Fermentation experiments were conducted in test tubes (25×200 mm), each containing 40 ml of pasteurized tomato juice. All samples were inoculated with a 24 h culture (>10^5 CFU/ml) and incubated at 30°C for 72 h.

Effect of cold storage on cell viability in probiotic tomato juice

After 72 h of fermentation at 30°C, the fermented samples (25 ml) were stored at 4°C for 4 weeks. Samples were taken at weekly intervals, and viability of probiotic cultures in probiotic tomato juice was determined and expressed as colony forming unit (CFU).

Chemicial and microbiological analyses

Samples were taken at 24 h intervals for chemical and microbiological analysis. pH was measured with a pH meter. Total acidity, expressed as percent lactic acid, was determined by titrating with 0.02 N NaOH to pH 8.2. Sugar content was analyzed as glucose by the phenol sulfuric acid method of Dubois *et al.* (1956). Viable cell counts (CFU/ml) were determined by the standard plate method with Lactobacilli MRS medium after 48 h incubation at 30°C.

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 SAS statistical computer package was used to analyze the experimental data (SAS Institute, USA). The values that have no common superscript are significantly different ($p<0.05$) according to Duncan's multiple range test. Any two means not marked by the same superscript (for example, a and b or b and c) are significantly different. Any two means marked by the same superscript (for example, a and a or b and b) are not significantly different.

Results and Discussion

The four lactic acid bacteria, *L. acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii.*, used in this study were found capable of rapidly utilizing tomato juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment. Babu *et al.* (1992) reported that addition of tomato juice to skimmed milk stimulated the growth of *L. acidophilus and* resulted in higher viable counts, shorter generation time, and improved sugar utilization with more acid production and lower pH. It was reported that 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 digestibilities of starch and protein (Sindhu and Khetarpaul, 2001).

Fig. 1 shows the changes in pH and acidity during tomato juice fermentation by *L. acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii*. Although the tomato juice had an initial pH value of 4.1, the four lactic acid cultures actively fermented the tomato juice and lowered the pH to as low as 3.5 after 72 h fermentation. Especially, *L. plantarum* showed a more rapid drop in pH than the other three lactic acid cultures examined. As shown in Fig. 1, *L. plantarum* produced significantly more acid during tomato 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.*

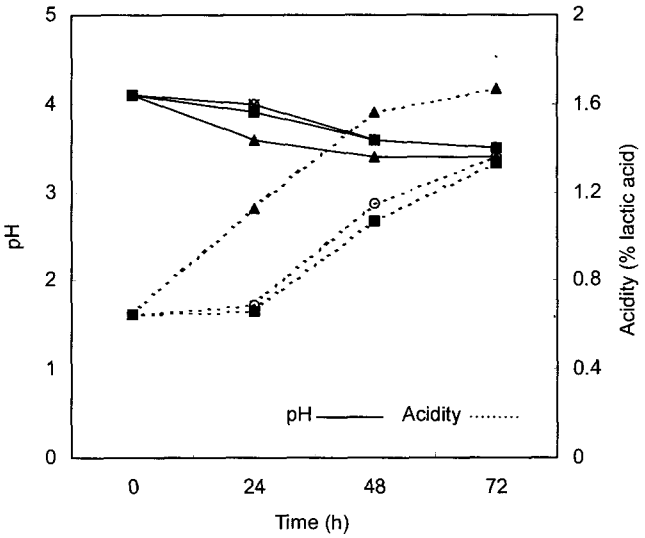

Fig. 1. Changes in pH and acidity during lactic acid fermentation of tomato juice fermentation (■, *L. acidophilus*; ○, *L. casei*; ▲, *L. plantarum*; ×, *L. delbrueckii*).

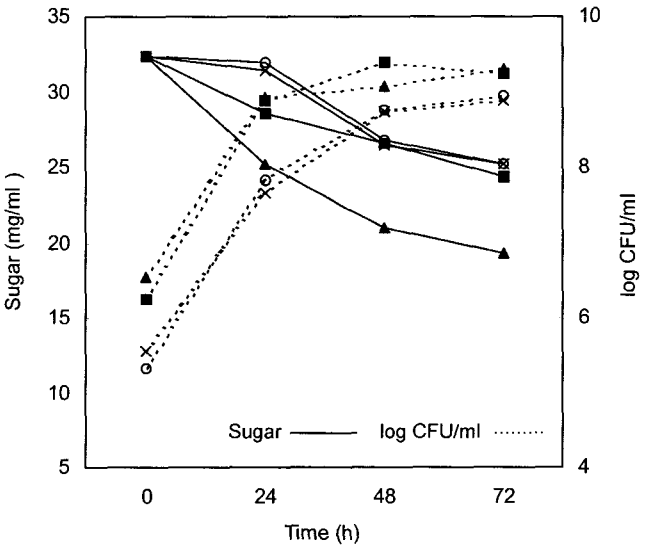

Fig. 2. Changes in sugar content and vaiable cell count during lactic acid fermentation of tomato juice (■, *L. acidophilus*; ○, *L. casei*; ▲, *L. plantarum*; ×, *L. delbrueckii*).

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

Time (week)	Survival (CFU/ml)			
	L. acidophillus	*L. casei*	*L. plantarum*	*L. delbrueckii*
0	$1.7 \pm 0.4 \times 10^{9a}$	$9.0 \pm 1.9 \times 10^{8a}$	$2.0 \pm 0.5 \times 10^{9a}$	$7.7 \pm 1.0 \times 10^{8a}$
1	$1.4 \pm 0.1 \times 10^{9a}$	$5.8 \pm 0.7 \times 10^{8b}$	$8.4 \pm 2.7 \times 10^{8b}$	$6.9 \pm 0.5 \times 10^{8a}$
2	$1.5 \pm 0.3 \times 10^{9a}$	$1.8 \pm 0.8 \times 10^{8c}$	$4.6 \pm 1.6 \times 10^{7c}$	$6.2 \pm 1.5 \times 10^{8a}$
3	$2.4 \pm 1.0 \times 10^{9b}$	$1.4 \pm 0.1 \times 10^{8c}$	$1.1 \pm 0.5 \times 10^{7c}$	$6.8 \pm 1.6 \times 10^{8a}$
4	$1.4 \pm 0.4 \times 10^{9a}$	$1.7 \pm 0.5 \times 10^{8c}$	$3.4 \pm 1.0 \times 10^{6c}$	$8.1 \pm 3.5 \times 10^{8a}$

The experimental values (means and standard deviations for n=3) that have no common superscript are significantly different ($p<0.05$) according to 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 marked by the same superscript (for example, a and a or b and b) are not significantly different.

acidophilus and Bifidobacterium *bifidum* (Ishibashi and Shimamura, 1993; Shah *et al.*, 1995).

Changes in sugar content and viable cell count during tomato juice fermentation by *L. acidophilus*, *L. plantarum*, *L. casei* and *L. delbrueckii.* are given in Fig. 2. The lactic acid cultures rapidly fermented tomato 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 32.4 mg/mL to 25.2, 21.0, and 19.3 mg/mL after 24, 48, and 72 h fermentation, respectively. As illustrated in Fig. 2, the four lactic acid cultures grew rapidly in tomato juice and reached a viable cell population of greater than 1.0×10^8/ml after 48 h 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 tomato juice.

Table 1 shows the effect of cold storage on the cell viability of four lactic acid cultures in fermented tomato juice. The viable cell counts of *L. acidophilus*, *L. plantarum*, *L. casei*, and *L. delbrueckii* were higher than 10^6/ml even after 4 weeks of cold storage at 4°C. Especially, the viable cell counts of *L. acidophilus* and *L. delbrueckii* did not decrease during cold storage and remained at 1.4×10^9 and 8.1×10^8 /ml, respectively, after 4 weeks of cold storage. The viable cell counts of *L. casei* and *L. plantaarum* 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 week 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 (Shah, 2001). 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 fermentaion such as lactic acid, diacetyl, and acetaldehyde could be associated with the loss of viability of added probiotic bacteria (Post, 1996). Lactic acid starters are reported to produce bacteriocin against probiotic bacteria and vice versa (Dave and Shah, 1997). 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 (Hood and Zottola, 1988; Shah and Jelen, 1990). In this study, most of the four lactic acid bacteria survived in the fermented tomato juice with high acidity and low pH. The results of this research suggest that the fermented tomato juice might serve as a probiotic beverage for vegetarians or consumers allergic to dairy products.

Conclusion

Four lactic acid bacteria (*Latobacillus acidophilus* LA39, *Lactobacillus plantarum* C3, *Lactobacillus casei* A4, and *Lactobacillus delbrueckii* D7) were found capable of rapidly utilizing tomato juice for cell synthesis and lactic acid production without pH adjustment. They reduced the pH to as low as 3.5 and increased the acidity to as high as 1.67%, and the viable cell counts (CFU) reached 10^8/ml after fermentation of 72 h at 30°C. Most of the 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 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.

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References

Abdel-Rahman, and Y. Abdel-Hamd. 1982. Nutritional value of some canned tomato juice and concentrates. Food Chemistry 9,

303-306

Babu, V., B.K. Mital, and S.K. Grag. 1992. Effect of tomato juice addition on the growth and activity of *Lacobacillus acidophilus*. *Int. J. Food Microbiol.* 17(1), 67-70

Berner, L. and J. ODonnell. 1998. Functional foods and health claims legislation: Applications to dairy foods. *Int. Dairy J.* 8, 355-362.

Betoret, N., L. Puente, M.J. Díaz, M.J. Pagán, M.J. García, M.L. Gras, J. Martínez-Monzó, and P. Fito. 2003. Development of probiotic-enriched dried fruits by vacuum impregnation. *J. Food Eng.* 56, 273-277.

Dave, R.I. and N.P. Shah. 1997. Viability of yogurt and probiotic bacteria in yogurt made from commercial starter culture. *Int. Dairy J.* 7, 31-41

Dubios, M., K.A. Gilles, J.K. Hamilton, D.A. Roberts, and F. Smith. 1956. Colormetric methods for determination of sugars and related substances. *Anal. Chem.* 28, 350-356

Fuller, R. 1989. Probiotics in man and animals. *J. of Appl. Bacteriol.* 66, 365-378

Hood, S.K. and M.L. Zottola. 1998. Effect of low pH on the ability of *Lactobacillus acidophilus* to suvive and adhere to human intestinal cell. *J. Food Sci.* 53, 1514-1516

Ishibashi, N. and S. Shimamura. 1993. Bifidobacteria: Research and development in Japan. *Food Technol.* 47, 126-136

Klaenhammer, T.R. and M.J. Kullen. 1999. Selection and design of probiotics. *Int. J. of Food Microbiol.* 50,45-57.

Luckow, T. and C. Delahunty. 2004. Which juice is healthier? A consumer study of probiotic non-dairy juice drinks. *Food Qual. Prefer.* Available online at www.sciencedirect.com

Mattila-Sandholm, T., P. Myllärinen, R. Crittenden, G. Mogensen, R. Fondén, and M. Saarela. 2002. Technological challenges for future probiotic foods. *Int. Dairy J.* 12, 173-182

McNaught, C.E. and J. MacFie. 2001. Probiotics in clinical practice: a critical review of the evidence. *Nutr. Res.* 21, 343-353.

Post, R.C. 1996. Regulatory perspective of the USDA on the use of antimicribials and inhibition in foods. *J. Food Prot.* Supplement, 78-81

Rafter, J. 2003. Probiotics and colon cancer. Best Prac. *Res. Cli. Gastroentrol.* 17(5), 849-859

Saarela, M., L. Lähteenäki, R. Crittenden, S. Salminen, and T. Mattila-Sandholm. 2002. Gut bacteria and health foods-the European perspective. *Int. J. of Food Microbiol.* 78, 99-117.

Shah, N.P. 2001. Functional Foods from probiotics and prebiotics. *Food Technol.* 55(11), 46-53.

Shah, N.P. and P. Jelen. 1990. Survival of lactic acid bacteria and their lactases under acidic conditions. *J. of Food Sci.* 55, 506-509.

Shah, N.P., W.E.V. Lankaputhra, M. Britz, and W.S.A. Kyle. 1995. Survival of L. acidophilus and Bifidobacterium bifidum in commercial yogurt during refrigerated storage. *Int. Dairy J.* 5, 515-521.

Sindhu, S.C. and N. Khetarpaul. 2001. Probiotic fermentation of indigenous food mixture: Effect on antinutrients and digestibility of starch and protein. *J. of Food Comp. Anal.* 14, 601-609.

Suzuki, T., K. Tomita-Yokotani, H. Tsubura, S. Yoshida, I. Kusakabe, K. Yamada, Y. Miki, and K. Hasegawa. 2002. Plant growth-promoting oligosaccharides produced from tomato waste. *Bioresource Technol.* 81, 91-96.