Iranian J Publ Health, Vol. 36, No.4, 2007, pp.70-73

Original Article

Antagonistic Action of Watermelon Juice Probioticated Using Different Strains of Lactobacilli against *Salmonella typhimurium*

*MR Fazeli [1], N Amirmozafari [2], *R Golbooi nejad [3], H Jamalifar [4]*

[1] *Dept. of Drug and Food Control, Faculty of Pharmacy, Medical Sciences/University of Tehran, Iran*
[2] *Dept. of Microbiology, Iran University of Medical Sciences, Hemmat Subway, Tehran, Iran*
[3] *Islamic Azad University, Sciences and Research unit, Ashrafi Esfehani Boulevard, Hesarak, Tehran, Iran*
[4] *Dept. of Drug and Food Control, Faculty of Pharmacy, Medical Sciences/University of Tehran, Tehran, Iran*

(Received 9 Mar 2007; accepted 21 Oct 2007)

Abstract

Background: The aim of this study was to compare the antimicrobial activity of watermelon juice probioticated using different strains of lactobacilli against *Salmonella typhi*murium.

Methods: Probioticated watermelon juice was produced using four strains of lactobacilli *(Lactobacillus casei, L. acidophillus, L. fementum* and *L. plantarum*). The watermelon juice was pasteurized for 30 minutes at 63º C and was inoculated with a 24 h culture of individual lactobacilli and incubated at 37º C. All of the lactobacilli were capable of growing in watermelon juice and reached a cell density of 10^8 CFU/ml after 48 h incubation at 37º C. Overnight culture of *S. typhi*murium was added to probioticated watermelon juice and reduction of the viable cells were assayed, on bismuth sulfite agar medium for 24 h. Antimicrobial activities of the lactobacilli cells against the test strain of *Salmonella* were also determined by measuring the diameter of growth inhibition zone in agar spot test.

Rsults: All of the lactobacilli could inhibit growth of *S. typhimurium* with *L. casei* being the most potent. *S. typhimurium* was totally eradicated in probioticated watermelon juice after 2-6 h.

Conclusion: The probioticated watermelon juices could differ in their antagonistic activities against *Salmonella* which could be due to the metabolite secreted by the lactic acid bacteria specially type of organic acids.

Keywords: *Probiotics,* Lactobacillus, Salmonella typhimurium, *Watermelon*

Introduction

Probiotics are defined as live microbial food supplements that beneficially affect the host by improving its intestinal balance (1). In recent years there is a great interest for viable microorganisms that promote or support a beneficial balance of autochthonous microbial population of gastrointestinal tract. Probiotics may be consumed in different forms, comprising foods, mainly in fermented state and pharmaceutical products, mainly as capsules or in microencapsulated forms (2). A continued interest is observed among the dairy type probiotic food products, such as fermented meats and vegetable and fruit 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 (3, 4).

Watermelon juice is considered as a healthy drink which is rich in lycopene, minerals and vitamins such as A, B and C. Regular consumption of watermelon juice can increase blood concentration of lycopene and beta-carotene (5). Studies suggest that these potent antioxidants may have protective effects against heart disease and certain cancers, such as prostate, bladder, and cervical cancer (6). The beneficial nutrients values of watermelon juice could still be increased by

***Corresponding author:** Tel: +98 912 1597732, E-mail:r_golbooi@yahoo.com

probiotication of the juice using different probiotic lactobacilli (7).

The objective of the present study was to determine whether different lactic acid bacteria present in the probioticated watermelon juice could eradicate *Salmonella typhimurium*.

Materials and Methods

Watermelon was purchased from a local vegetable market in Tehran, Iran. Juice was prepared from homogenized skinless slices and was further pasteurized for 30 min at 63º C.

Lactobacillus casei, L. fermentum, L. acidophillus and *L. plantarum* were all sourdough isolates and were from stock of the Deportment of Drug and Food Control Faculty of Pharmacy Medical Sciences/University of Tehran. The *S. typhimurium* used in this study was a reference type (PTCC1639).

Culture of bacteria The Lactic acid bacteria were grown at 37º C for 18 h in de Mann, Rogosa and Sharp (MRS) broth (1.10661.merck). *S. typhmuriumi* was grown for 18 h at 37º C in Trypticase soy broth (TSB) medium (1.05458. Merck).

Probiotication of watermelon juice Erlenmeyer flasks (250 ml) each containing 200 ml of pasteurized watermelon juice were inoculated with 2 ml of 18 h culture of lactobacilli (>10^5) CFU/ml and were further incubated at 37º C for 48 h. Enumeration of the cells was done by plating serial dilutions from bacterial suspensions on MRS agar (Merck) plates. Plates were incubated at 37º C in a 5% CO_2 atmosphere for 48 h (8, 9).

Challenging* S. typhimurium *with lactic acid bacteria in probioticated watermelon juice:

Probioticated watermelon juice containing 10^8 CFU/ml of individual lactobacilli strains was used in challenge tests against *S. typhimurium*. Watermelon juice with no lactobacilli was used as control. To prepare a concentrated inoculum *S. typhimurium* bacterial suspension was centrifuged at 4000×g for 10 minutes at 11° C and washed with PBS and resuspended in the same buffer to obtain a cell density of 4.6×10^8 CFU/ mL which was further used to inoculate the probioticated watermelon juices. Alliquotes of one ml of the *S. typhimurium* suspension were added to flasks containing 100 ml of probioticated as well as plain watermelon juice. The inoculated flasks were incubated at 37º C and viability of *S. typhimurim* was determined every 2 h for 1 d.

Inhibition assays For detection of antimicrobial activity, an agar spot test was used. Test cultures for lactobacilli were spotted (2-3 µL) on the surface of MRS agar and incubated under anaerobic condition for 24 h at 37º C to develop the spots. The agar plates were then overlaid with triptic soy agar (0.75%) medium containing *S. typhmurimi*. The plates were incubated at 37º C. After 24 h of incubation. Zones of inhibition around the central spots were measured (10).

Statistical analysis The defined dots for every four lactobacilli strains in fig.1 is the mean of three tests done in three different days. The numbers are the mean of triplicates.

Results

All the strains of lactic acid bacteria were able to grow in watermelon juice and reached a cell density of 10^8 CFU/ml after 48 h. Significant drop in the pH of the probioticated watermelon was observed decreasing the initial pH of the juice from 5.5 to 4. Visible inhibition zones about 20 mm in diameter were observed around the spotted cells of lactobacilli in the spot tests indicating great inhibitory effect of the lactic acid bacteria against the tested strain of *Salmonella*.

Lactobacillus casei was the most potent inhibitor of *S. typhimurium* as was shown by the greatest zone of inhibition. All the probioticated watermelon juices were able to significantly reduced the number of *S. typhimurium* (5-6 log reduction) after 2 h except the *L. acidophilus* juice which only caused to 0.5 log reduction of *S. typhimurium* but showed the a effect after 6 h of incubation.

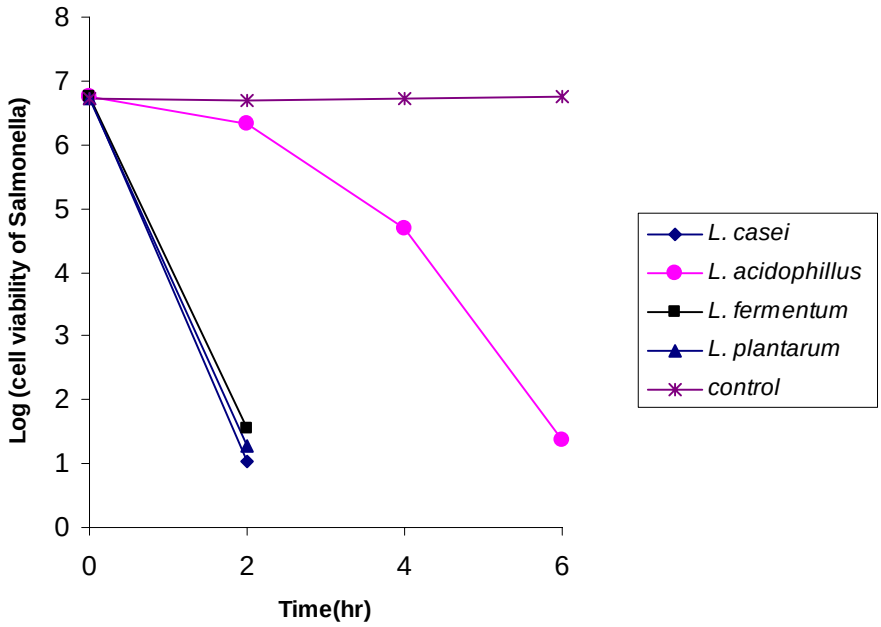

Fig. 1: The inhibitory effect of lactobacilli strains on *Salmonella typhimurium*

Discussion

The results of this survey indicated that all the lactic acid bacteria present in probioticated watermelon juices could in fact exert their anti-pathogenic probiotic properties against a well known pathogen such as *S. typhimurium*. Brashears et al. (11) had examined the antagonistic effects of *L. lactis* on *S. typhi*murium. Cells of *L. lactis* were added to trypticase soy broth that contained cells of *Salmonella* spp. The inhibition of *S. typhimurium* was examined during growth at 37º C for 24 h and refrigeration temperature (6º C) for 5 d. In experiments at 37º C the *L. lactis* completely inhibited *Salmonella* producing numbers that were not detectable after 24 h of incubation. There were significant increases in numbers of the *Salmonella* in the control samples containing no *L. lactis*. There were significant declines in the pH of the both control and *L. lactis* inoculated samples. Numbers of *Salmonella* spp. incubated at 6º C did not decline significantly for control or inoculated samples, which suggested that *L. lactis* did not inhibit *Salmonella* spp. at refrigeration temperature.

The antagonistic effect of *L. casei* GG against *S. typhimurium* has been also investigated by Hedault et al. (12). They have found that supernatant of *L. casei* is able to prevent the invasion of Caco-2-cells by *S. typhimurium*. Since the neutralized *L. casei* supernatant has no inhibitory effect, the activity of the *L. casei* supernatant could be related to the pH, however their experiments showed that pH alone had no effect, since MRS broth at pH 4 had only a slight effect on the invasion rate of *S. typhiurium*, The mechanism of the antagonistic action of *L. casei* seems to be dependent on an acidic environment, perhaps due to lactic acid itself or to a substance active at a low pH. Altogether, their results were in accordance with those of Silva et al. (13), showing that *L. casei* secrets into its cultures supernatant an antimicrobial substance which develops its activity in the pH range from 3-5.

In conclusion the probioticated watermelon juices could differ in their antagonistic activities against *Salmonella* which could be due to the metabolite secreted by the lactic acid bacteria specially type of organic acids.

Acknowledgements

The authors wish to thank The Deputy for Research and also Pharmaceutical Sciences Research Centre of the Tehran University of Medical Sciences for funding the current research work.
The authors declare that they have no Conflict of Interests.

References

1. Fuller R (1989). Probiotics in man and animals. *A Review Appl Bacteriol*, 66:365-78 .
2. Salminen S, Deighton MA, Benno Y, Gorbach SL (1998). Lactic acid bacteria in health and disease. *Microbiology and functional Aspacts*, 2nd ed, marcel Dekker, New York, pp.211-53.
3. Gill HS (1998). Stimulation of the immune system by lactic cultures. *Int Dairy*, 8: 535-44.
4. Goktepe I (2005). Introduction to prebiotics and probiotics. In: *Probiotic in food safety and human health*; edited by Ipek Goktepe. Ed., Juneja VK. 1st ed Taylor & Francis Group, LLC. New York, p.4.
5. Edwards AJ, Vinyard BT, Wiley ER, Brown ED, Collins JK**,** Perkins-Veazie PA, et al. (2003). Consumption of Watermelon Juice Increases Plasma Concentrations of Lycopene and ß-Carotene in Humans. *Nutr*, 133:1043-50.
6. De Roos N, Katan MB (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *Am J Clin Nutr*, 71:405-11.
7. Moura LN, Neumann E, Vieira LQ, Nicoli JR (2001). Protection by *Lactobacillus acidophillus* UFV-H2B20 against experimental oral infection with *Salmonella enterica* subsp. *Enterica* ser. *Typhimurium* in gnotobiotic and conventional mice. *Brazilian Journal of Microbiology*, 32:66-9.
8. Yoon KY, Woodams E, Hang YD (2006). Production of probiotic cabbage juice by lactic acid bacteria. *J Biortech*, 97(12): 1427-30.
9. Yoon KY, Woodams E, Hang YD (2004). Probioticaton of tomato juice by lactic acid bacteria. *J Microbiol*, 4:315-18.
10. Jacobsen CV, Rosenfeldt V, Hayford AE, Moller PL, Michaelsen KF, Paerregaard et al. (1999). Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by In vitro techniques and evaluation of the colonization ability of five selected strains in humans. *J Appl Microbiol*, 11:4949-56.
11. Brashears M, Durre W (1999). Antagonistic action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherchia coli* O157:H7 during growth and refrigerated storage. *J Food Prot*, 11:1336-40.
12. Houdault S, Lievin V, Bernet-Camard MF, L.Servin A (1997). Antagonistic activity exerted in vitro and Invivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. *Applied and Environmental Microbiology*, 2:513-18.
13. Silva M, Jacobus NV, Deneke C, Gorbach SL (1987). Antimicrobial substance from a human *Lactobacillus* strain. *Antimicrob Agent Chemother*, 31:1231-33.