

Antimicrobial Activity of *Lactobacillus plantarum* Strains Isolated from Fermented Olives Origin

Fazeli MR (Ph.D.)¹, Vaghari E (M.Sc.)^{2*}, Jamalifar H (M.Sc.)³,
Ebrahim Z (Student of Pharmacy)⁴, Samadi N (Ph.D.)⁵

1- Department of Microbiology, Drug and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

2- Department of Microbiology, Drug and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

3- Department of Microbiology, Drug and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

4- Department of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

5- Department of Pharmacy, Drug and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding author: Drug and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Tel: +989125504609, Fax: +98-21-66461178

E-mail: Vaghari_el@yahoo.com

Receive: 11 May 2009

Acceptance: 28 Jul. 2009

Abstract

Background: *Lactobacillus plantarum* isolated from fermented olives has been extensively studied with the aim of its use in starter cultures for olive or other production fermentations, but little is known about its antimicrobial resistance and its probiotic effects.

Objectives: The aim of this study was to determine the antimicrobial activity using different strains of *L. plantarum* isolated from fermented olives origin against *Salmonella typhimurium*.

Methods: Three strains of *L. plantarum* were isolated from fermented green olives (Lp1, Lp2, Lp3). They were grown in MRS broth at 37° C. Overnight culture of *S. typhimurium* was added to MRS broth containing 10⁸ CFU/mL of *L. plantarum*. Reduction of the viable cells was assayed on bismuth sulfite agar medium for 24h. Antimicrobial activities of *L. plantarum* cells against the test strain of *Salmonella* were also determined by measuring the diameter of growth inhibition zone in agar spot test.

Results: All of the *L. plantarum* strains could inhibit growth of *S. typhimurium*. Also visible inhibition zones were observed around the spotted cells of *L. plantarum* strains in the spot tests.

Conclusion: Results showed great inhibitory effect of the *L. plantarum* strains against *Salmonella* and were differed in their antimicrobial activities which could be due to the metabolite secreted by the *L. plantarum* strains specially type of bacteriocins.

Keywords: *Lactobacillus plantarum*, Olives origin, Antimicrobial activity, *Salmonella typhimurium*

Introduction

Probiotic are defined as live microbial food supplement that beneficially affect the host by improving the intestinal microbial balance. Lactic acid bacteria are regarded as a major group of probiotic bacteria, they are commercially used as starter cultures for the manufacture of probiotic foods [1].

The development and consumption of functional probiotic foods has been increasing along side awareness of their beneficial effects in promoting gut health as well as in disease prevention and therapy, and this has raised interest in health-promoting foods [2, 3]. The benefit derived from a regular intake of probiotic foods is also correlated to their ability to inhibit pathogens and protect human from gastrointestinal disease [4, 5].

Probiotic bacteria have been added to a variety of dairy-based products such as fermented milks and yogurts for their probiotic human health benefits [6]. Functional food industries are now focusing on new nondairy probiotics in individuals with lactose intolerance or with a diet lacking milk-derived products [7].

Fruits and vegetables are rich in functional food components such as minerals, vitamins, dietary fibers and antioxidants [8].

The olive phylloplane in particular the fruit surface, is suitable for the survival of microbial populations, in particular Lactic acid bacteria [9, 10], which are involved in developing the spontaneous are started lactic fermentation of table olives [11].

Lactobacillus plantarum isolated from fermented olives has been extensively studied with the aim of its use in starter cultures for olive or other vegetable fermentation [12] but little is known about its antagonistic action and its probiotic effects. The study was conducted to determine the antagonistic activity of *L. plantarum* isolated of fermented olives origin.

Materials and Methods

Culture of bacteria:

Strains of *L. plantarum* were isolated from fermented green olives (Lp1, Lp2, Lp3). They were grown in de mann, Rogosa and Sharp (MRS) broth (1.10661. merck) at 37° C for 24h and *Salmonella typhimurium* (PTCC1639) was obtained from the Microbial Stock Collection of the Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences.

Antimicrobial activity of *L. plantarum* against *S. typhimurium*

Fresh MRS broth containing 10⁸ CFU/mL of *L. plantarum* strains (Lp1, Lp2, Lp3) was used in challenge test against *S. typhimurium* MRS broth without *L. plantarum* was used as control.

To prepare a concentrated inoculum *S. typhimurium* bacterial suspension was centrifuged at 4000 rpm for 10 minutes and washed with PBS and re-suspended in the same buffer to obtain a cell density of 8.2×10⁸ CFU/mL which was further used to inoculate the MRS broth including *L. plantarum* strains (Lp1, Lp2, Lp3).

1 mL of the *S. typhimurium* suspension were added to flasks containing 100 mL of MRS broth including *L. plantarum* strains. The inoculated flasks were incubated at 37°C and viability of *S. typhimurium* was determined every 2h for 1d.

Inhibition assays

For detection of antimicrobial activity, an agar spotted (2-3 L) on the surface of MRS agar and incubated under anaerobic condition at 37°C for 24h to develop the spots. The agar plates were then overlaid with triptic soy agar (o.75%) medium containing *S. typhimurium*. The plates were incubated at 37°C. After 24h of incubation, zones of inhibition around the

central spots were measured [13]. The results were average of triplicate assays.

Results

All the strains of *L. plantarum* (Lp1, Lp2, Lp3) were able to grow in MRS broth and reached a cell density of 10^8 CFU/mL after 48h.

L. plantarum strains could inhibit growth of *S. typhimurium*. After 2h, the number of *S. typhimurium* reduced from 10^7 CFU/mL to 10^1 - 10^2 CFU/mL by different *L. plantarum* strains, but the number of control sample remained 10^7 CFU/mL (fig. 1).

Also, results of spot test method showed that *L. plantarum* strains were able to inhibit growth of the test strain of *Salmonella*. Visible inhibition zones about 20 mm in diameter were observed around the spotted cells of *L. plantarum* strains in the spot tests indicating great inhibitory effect of the *L. plantarum* strains against the tested strain of *Salmonella*.

The spectrum of antimicrobial activity was varied. One of the *L. plantarum* strains (Lp2) was the most potent inhibitory of *S. typhimurium* as was shown by the greatest zone of inhibition. The zone of inhibition around the spots of *L. plantarum* strains are used as a quantitative measure of inhibition capacity.

Discussion

Lactic acid bacteria have antimicrobial activity against most of the pathogens and synthesis bactericidal agent that vary in their spectrum of activity. The results of this survey indicated that all the *L. plantarum* strains used in test, could in fact exert their anti-pathogenic probiotic properties against a well known pathogen such as *S. typhimurium*. Result indicated that *L. plantarum* strains (originate of fermented olives) showed antimicrobial

activity against tested strain of *Salmonella*. *L. plantarum* isolated from fermented olives has been studied about its antibiotic resistance. Kacem et al. (2006), isolated that 11 strains of *L. plantarum* from fermented olives origin and tested for their in vitro antibiotics susceptibility, tolerance to bile, resistance to low pH values, acidifying activity, proteolytic activity, hemolytic activity, lactic acid and exopolysaccharide production. Collectively, the strains were susceptible to the most of antibiotics tested, showed the survivability at high bile salt concentration and resistance at pH 2. Most strains have showed fast or medium acidification activity with a good proteolytic activity [14].

Delgado et al. (2001), examined that *L. plantarum* strain LB17.2b, isolated from traditional table olive fermentation had been shown to produce thermo-stable antibacterial proteins. At pH: 6.5, the proteinaceous fraction obtained from culture supernatant was active against *Enterococcus faecalis* and against natural competitors of *L. plantarum* from olive fermentation brines. They also demonstrated the presence of another antimicrobial proteinaceous compound showing a wider inhibitory spectrum and produced during culture stationary phase [15].

Brashears et al. (1999), had shown the antagonistic effects of *L. lactis* on *S. typhimurium* 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 37°C for 24h and refrigeration temperature (6°C) for 5d. In experiments at 37°C the *L. lactis* completely inhibited *Salmonella* producing numbers that were not detectable after 24h of incubation. There were significant declines in the pH of both control and *L. lactis* inoculated samples, which suggested that

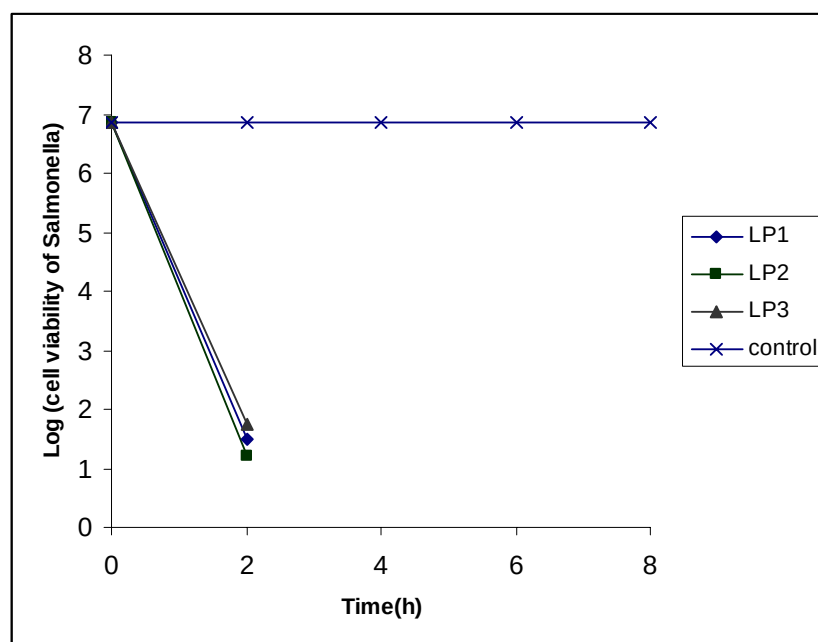


Fig. 1- The inhibitory effect of *L. plantarum* strains on *Salmonella typhimurium*

L. lactis did not inhibit *Salmonella* spp. at refrigeration temperature.

Also in this study *L. plantarum* strains completely could inhibited *Salmonella* producing numbers that were not detectable after 24h of incubation [16].

In the another study, Hedault et al. (1997), have found that spernatant of *L. casei* is able to prevent the invasion of Caco-2-cells by *S. typhimurium*. The mechanism of the antagonistic action of *L. casei* seems to be depend on an acidic environment, perhaps due to lactic acid itself or to a substance active at a low pH [17]. These results were similar to Silva et al. (1997) experiments, that they found

L. casei secretes into its cultures supernatant an antimicrobial substance which develops its activity in the pH range from 3-5 [18]. In conclusion, the *L. plantarum* strains have differ in their antagonistic activities against *Salmonella* which could be due to the metabolite secreted by the *L. plantarum* strains specially type of bacteriocins.

Acknowledgements

The authors wish to thank the deputy for Research and also Pharmaceutical Sciences research Center of the Tehran University of Medical Sciences for funding the current research work.

References

1. Fuller R. Probiotics in man and animals. *A Review Appl Bacteriol.* 1989; 66: 365 – 78.
2. Saarela M, Lähteenmäki L, Crittenden R, Salminen S and Mattila-Sandholm T. Gut bacteria and health foods -the European perspective. *Int. J. Food Microbiol.* 2002; 78: 99 – 117.
3. Cross ML. Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. *FEMS Immunol. Med. Microbiol.* 2002; 34: 245 - 53.
4. Drago L, Gismondo MR, Lombardi A, de Haën C and Gozzini L. Inhibition of in vitro

growth of enteropathogens by new *Lactobacillus* isolates of human intestinal origin. *FEMS Microbiol. Lett* 1997; 153: 455 - 63.

5. Marteau PR, de Vrese M, Cellier CJ, and Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics. *Am. J. Clin. Nutr* 2001; 73 (Suppl.): 430s - 46.

6. Siuta-cruce P and Goulet J. Improving probiotic survival rates. *Food Technol.* 2001; 55 (10): 36 - 42.

7. Charalampopoulos D, Wang R, Pandiella SS and Webb C. Application of cereals and cereal components in functional foods: a review. *Int. J. Food Microbiol.* 2002; 79: 131 - 41.

8. Luckow T and Delahunty C. Which juice is healthier? A consumer study of probiotic non-dairy juice drinks. Food Quality and Preference. Available from: www.sciencedirect.com.

9. Lavermicocca P, Gobetti M, Corsetti A and Caputo L. Characterization of lactic acid bacteria isolated from olive phylloplane and table olive brines. *Ital. J. Food Sci.* 1998; 10: 27 - 39.

10. Nychas GE, Panagou EZ, Parker ML, Waldron KW and Tassou C. Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. *Lett. Appl. Microbiol.* 2002; 34: 173 - 7.

11. Garrido Fernandez A, Fernandez Diez MJ and Adams MR. Physical and chemical characteristics of the olive fruit, In A. Garrido Fernandez et al. (ed.), Table olives. Chapman & Hall, London, United Kingdom. 1997, pp: 67 - 109.

12. Leal-Sánchez MV, Jiménez-Díaz R,

Maldonado-Barragán A, Garrido-Fernandez A., Ruiz-Barba JL. Optimization of bacteriocin production by batch fermentation of *Lactobacillus plantarum* LPCO10. *Applied and Environmental Microbiol.* 2002; 68: 4465 - 71.

13. Jacobsen CV, Rosenfeldt V, Hayford AE, Moller PL, Michaelsen KF, Paerregaard et al. 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.* 1999; 11: 4949 - 56.

14. Kacem M and Karam NE. In vitro preselection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin. *Inter. J. of Probiotics and Prebiotics* 2006; 1: 27 - 32.

15. Delgado A, Brito D, Fevereiro P, Feres C and Marques JF. Antimicrobial activity of *L. plantarum*, isolated from a traditional lactic acid fermentation of table olives. *EDP Sci.* 2001; 81: 203 - 15.

16. Brashears M and Durre W. Antagonistic action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherchia coli* O157: H7 during growth and refrigerated storage. *J. Food Prot.* 1999; 11: 1336 - 40.

17. Heudault S, Lievin V, Bernet-Camard MF, L. Servin A. Antagonistic activity exerted in vitro and Invivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. *Applied and Environmental Microbiol.* 1997; 2: 513 - 8.

18. Silva M, Jacobus NV, Deneke C, Gorbach SL. Antimicrobial substance from a human *Lactobacillus* strain. *Antimicrob Agent Chemother* 1997; 31: 1231 - 3.