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, Drag and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
2- Department of Microbiology, Drag and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
3- Department of Microbiology, Drag 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, Drag and Food Control Department, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
*Corresponding author: Drag 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 10^8 CFU/mL of *L. plantarum*. Reduction of the viable cells was assayed on bismuth sulfit 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**

[DOR: 20.1001.1.2717204.2009.8.31.2.7]

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^{o} 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 10^8 CFU/mL of *L. plantarum* strains (Lp1, Lp2, Lp3) was used in challenge test against *S. typhymurium* 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 8.2×10^8 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^{o}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^{o}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^{o}C. After 24h of incubation, zones of inhibition around the

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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 diammeter 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].

Delgardo 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 faecalic 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

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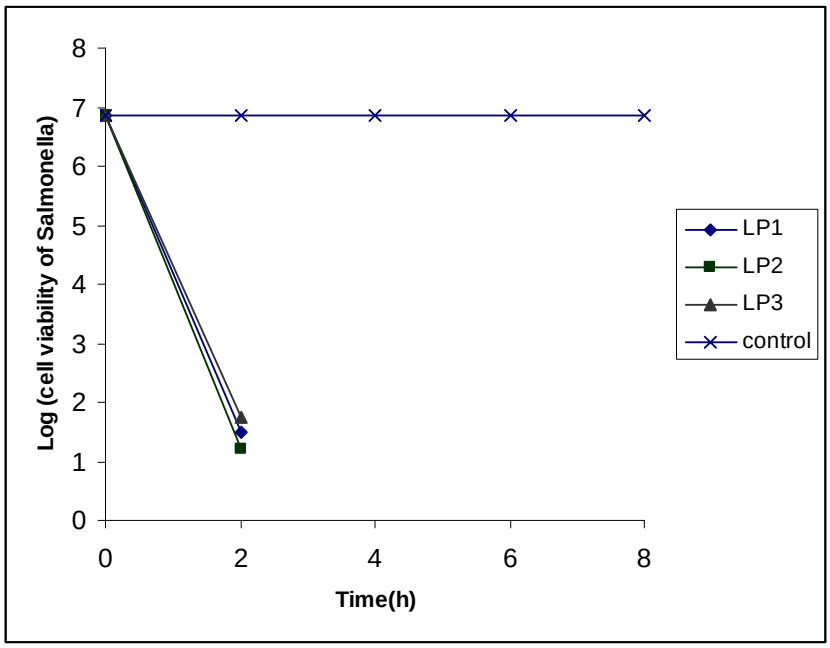

Fig. 1- The inhibitory effect of *L. plantarum* strains on *Salmonella typhimurium*

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

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* secrets 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 authers 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.

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[DOR: 20.1001.1.2717204.2009.8.31.2.7]

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[DOR: 20.1001.1.2717204.2009.8.31.2.7]