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## Antibacterial Effect of Autochthonous *Lactobacillus* Strains Isolated from Traditional Yogurts

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**Abstract:** It is obvious that some of resident gastrointestinal bacterial flora represented by *Lactobacilli* have protective role in pathogenic infections. There are many examples of probiotic effect of Lactic Acid bacteria on enteropathogens. Lactic acid bacteria are derived from the intestinal microbiota of healthy humans or dairy products. These bacteria interact with the diet and the host, contributing to protection against intestinal pathogens through colonization resistance and providing nutritional and colonic health benefits via their metabolic activities. In this study we isolated strains of *Lactobacilli* from Iranian traditional yogurts and identified by biochemical tests. We tested antibacterial activity of strains against *Escherichia coli* and *salmonella typhi* by spot test method. Then we assayed zone of pathogenic bacteria. Also, we determined death kinetic of pathogenic bacteria. Most of *Lactobacilli* strains had potential activity against the enteropathogenic bacteria of *E. coli* and *Salmonella*. This antagonistic effect against *E. coli* was more than *Salmonella*. *Lactobacillus Casei* showed the most preventive effect. Activity of probiotics in prevention and treatment of infections by *E. coli* and *salmonella* are effective.

**Key words:** Probiotic, pathogen, *E. coli*, salmonella

### INTRODUCTION

Residing in the human gastrointestinal tract is a large and complex microbial ecosystem that develops through infancy and childhood to form a diverse, but relatively stable community in adults (Vaughan *et al.*, 2002).

These autochthonous bacteria interact with the diet and the host, contributing to protection against intestinal pathogens through colonization resistance and providing nutritional and colonic health benefits via their metabolic activities (Isolauri *et al.*, 2002; Guarner and Malagelada, 2003).

It has become clear that these bacteria also interact with the host's immune system and are essential for the maturation and homeostasis of a healthy immune system (Schiffirin and Blum, 2002).

Recognition of the importance of the intestinal microbiota to health has led to increasing interest in manipulating the composition and activity of the microbiota to improve both human and animal health.

Examples of bacteria demonstrated to have beneficial effects include *Lactobacillus*, *Bifidobacterium*, *Escherichia coli* Nissle 1917, *Clostridium butyricum*, *Streptococcus salivarius thermophilus* and a non pathogenic yeast *Saccharomyces boulardii* (Sartor, 2004; Donohue *et al.*, 1998).

Mechanisms by which probiotics exert their therapeutic effects include (Guarner and Malagelada, 2003) modulation of barrier function (Sartor, 2004), mucosal trophic action (Guarner and Shaafsma, 1998), inhibition

of pathogenic bacteria (Donohue *et al.*, 1998), blockade of epithelial attachment and invasion by pathogenic bacteria (Sartor, 2004), modulation of intestinal cytokine production (Fioramonti *et al.*, 2003), anti-inflammatory properties (Farina *et al.*, 2001), enhancement of digestion and absorption of food (Donohue *et al.*, 1998; Sartor, 2005).

Probiotics are defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (J-Boyle *et al.*, 2006).

It is believed by many that the ideal probiotic should remain viable at the level of the intestine and should adhere to the intestinal epithelium to confer a significant health benefit. Some evidence supports the importance of viability in human studies, with viable bacteria having greater immunologic effects than nonviable bacteria and killed bacteria being associated with adverse effects in some instances (Aila *et al.*, 1995; Irjavainen *et al.*, 2003). Some of the best characterized probiotics have also been shown to adhere strongly to intestinal epithelium in both *in vitro* and *in vivo* studies (Alander *et al.*, 1999). Probiotics must be resistant to gastric acid digestion and to bile salts to reach the intestine intact and they should be nonpathogenic.

Most probiotics are strains of *Bifidobacterium* or *Lactobacillus* species. Some are derived from the intestinal microbiota of healthy humans and others are nonhuman strains used in the fermentation of dairy products. Species from other bacterial genera such as

*Streptococcus*, *Bacillus* and *Enterococcus* have also been used as probiotics, but there are concerns surrounding the safety of such probiotics because these genera contain many pathogenic species, particularly *Enterococcus* (J-Boyle *et al.*, 2006).

Nonbacterial microorganisms such as yeasts from the genus *Saccharomyces* have also been used as probiotics for many years.

The probiotic effects of this organism include the treatment of various types of diarrhea, alleviation of Crohn's disease and balancing of intestinal micro flora through the growth modulation of bacteria present in the gastrointestinal tract (Ernet *et al.*, 1994; Fuller, 1991; Kaila *et al.*, 1992; Lidbeck *et al.* 1992).

Acute infectious gastroenteritis remains the most common cause of diarrhea world wide and is a leading cause of death in childhood. Despite improvements in public health and economic wealth, the incidence of intestinal infections remains high in the developed world and continues to be an important clinical problem with relevant morbidity (Casburn-Jones and Farthing, 2004).

The risk of diarrheal disease is increased in selected groups including young infants, immune deficient individuals (HIV, cancer, chemotherapy, malnutrition) and people with a high exposure to pathogens (informal settlements travellers, contaminated food and medications buffering gastric acid), etc. (Lilly and Stillwell, 1965). The use of probiotic microorganisms for the prevention or therapy of gastrointestinal disorders is the most usual application of probiotics because most health effects attributed to them are related directly or indirectly (i.e., mediated by the immune system) to the gastrointestinal tract (Vaughan *et al.*, 2002).

In this study we identified *Lactobacilli* species by yogurt samples and investigated their antipathogenic activity.

## MATERIALS AND METHODS

In this study 194 yogurt samples were collected from Iranian Ghashghaie and Bakhtiari tribes.

The samples had been cultured in MRS broth Medium and had been incubated in anaerobic condition with 5%  $\text{CO}_2$  and 37°C within 48 h. The grown samples were sub cultured in MRS agar for several times to get pure colony in anaerobic condition with 5%  $\text{CO}_2$  and 37°C for 48 h.

In each step Gram stain had been carried out for bacteria until getting pure colonies.

The pure colonies were cultured in TSI (triple-sugar-iron) agar Medium to study sugar fermentation and were cultured in SIM Medium for investigation of motility and production of  $\text{H}_2\text{S}$ .

MRS broth Medium was prepared to culture bacteria without sugar. Then each of the carbohydrates was added into separated Medium in every tube to study

fermentation. The bacteria were inoculated into these Mediums and were incubated in 37°C for 4 days. *Lactobacillus casei* was used as a positive control in all steps.

Two pathogens, *E. coli* O: 157 H: 7 and *Salmonella typhi* grew in Caso agar Medium and were incubated in 37°C for 24 hr to study antipathogenic activity.

A suspension was prepared in concentration of 0.5 McFarland by adding of 2-3 colonies from grown bacteria to 10 mL sterile distilled water. Then 2 mL of this suspension was added to semi solid Caso agar Medium.

Antibacterial activity was studied by spot test method, *Lactobacillus* strains were cultured in MRS agar in spot form at the middle of plate and were incubated in anaerobic condition with 5%  $\text{CO}_2$  and 37°C within 48 h.

The pathogenic bacteria in semisolid Caso agar were distributed on the surface of grown *Lactobacilli* and were incubated in 37°C for 24 h. Then the inhibition effect was studied by assaying of each bacterial zone. The pathogenic bacterium without *Lactobacillus* was as a negative control and the *Lactobacillus* without pathogenic bacteria was as a positive control.

*Lactobacilli* strains were cultured in MRS broth Medium, centrifuged in 12000 rpm for 7 min to determine pathogenic bacteria death kinetic. Then a suspension was prepared in 0.5 McFarland concentrations from pathogenic bacteria that had grown in Caso agar. One ml of this suspension was added to Caso broth Medium in three separated parts. *Lactobacilli* species in concentration of 2, 5 and 10% were added to these suspensions at the same time. Then OD (optical density) of pathogenic bacteria was measured each h. There was one control with pathogen and without *Lactobacilli*.

## RESULTS

102 species of *Lactobacilli* were isolated from 194 yogurt samples. All of them were *Lactobacillus* in Gram Stain. The yeast samples were omitted from this study. *Lactobacilli* species didn't have any motivation in SIM Medium and production of  $\text{H}_2\text{S}$ . All of *Lactobacilli* fermented three types of sugar in TSI Medium.

There were used twelve types of sugar (Esculin, Maltose, Lactose, Melibiose, Raffinose, Salicin, Trehalose, Galactose, Mannitol, Cellobiose, Arabinose, and Mannose) to identify *Lactobacilli* strains. Some *Lactobacillus* strains such as *L.casei*, *L.gasseri*, *L.acidophilus*, *L.salivarius*, *L.delbrueckii* and *L.plantarum* were identified.

Fifty nine *Lactobacillus* species had antagonistic effect against *E. coli* and *salmonella typhi morium*. The mean diameter of zone for *E.coli* was 40.4 mm and for *salmonella* was 26 mm. The highest antagonistic effect was shown by *L.casei*. The inhibition activity of *Lactobacilli* on *E.coli* is shown in Fig. 1. The zone for *E.coli* was 30 mm up to 45 mm in diameter. The activity

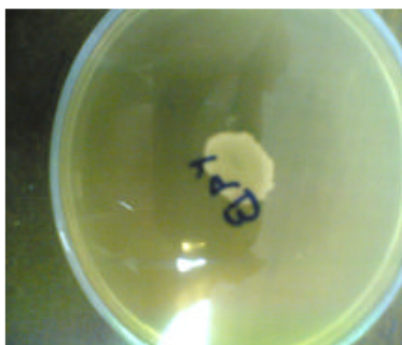


Fig. 1: Zone of Lactobacillus for *E. coli*



Fig. 2: Zone of Lactobacillus for salmonella

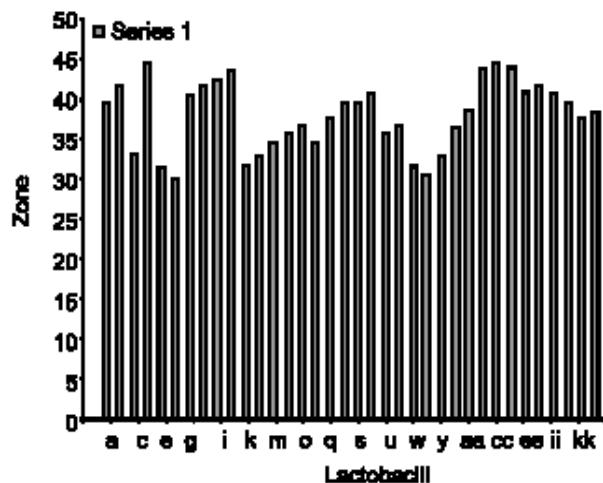


Fig. 3: Zone of bakhtiari Lactobacilli on *E. coli*

of Lactobacilli by Bakhtiari tribe yogurts on *E. coli* is shown in Fig. 3. The activity of Lactobacilli by Ghashghaie tribe yogurt against *E. coli* is shown in Fig. 4.

The inhibition effect of Lactobacilli on *Salmonella* is shown in Fig. 2. Inhibition zone for *Salmonella* was 18 mm up to 32 mm in diameter. The activity of Lactobacilli

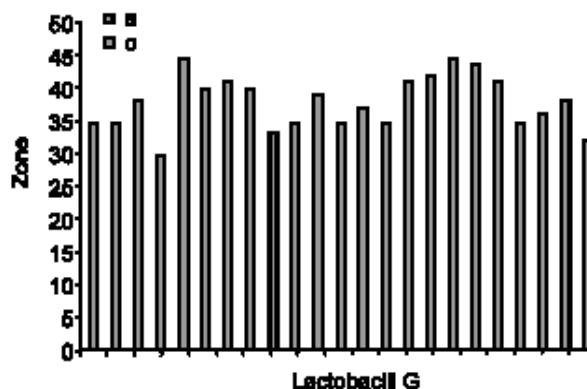


Fig. 4: Zone of Ghashghaie Lactobacilli on *E. coli*

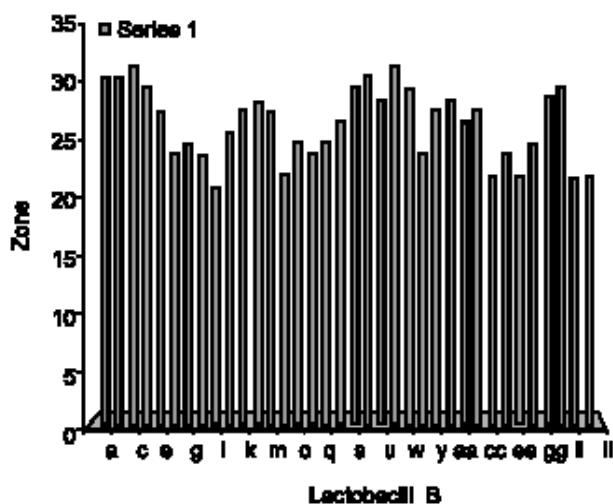


Fig. 5: Zone of bakhtiari Lactobacilli on *Salmonella*

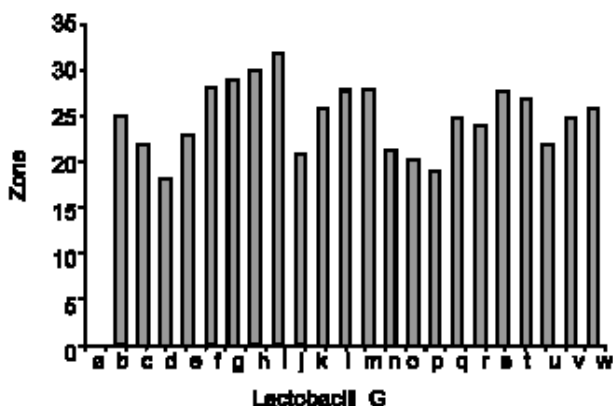


Fig. 6: Zone of ghashghaie Lactobacilli on salmonella

by Bakhtiari tribe yogurt against *Salmonella* is shown in Fig. 5. The activity of Lactobacilli by Ghashghaie tribe yogurt on *Salmonella* is shown in Fig. 6.

## DISCUSSION

The use of probiotic microorganisms for prevention or therapy of gastrointestinal disorders is an obvious measure and perhaps the most usual application of probiotics, because most health effects attributed to them are related directly or indirectly (i.e., mediated by the immune system) to the gastrointestinal tract. Many strains of probiotic microorganisms have shown inhibition of growth and metabolic activity as well as the adhesion of enteropathogenic bacteria (*Salmonella*, *Shigella*, enterotoxigenic *E.coli* and *Vibrio cholerae*) to intestinal cells to modulate (temporarily) the intestinal micro flora and to have immunostimulatory or -regulatory properties (Coconnier and *et al.*, 1997; Gopal *et al.*, 2001; De Vrese and Marteau, 2007).

*Lactobacillus acidophilus* culture has repeatedly demonstrated effectiveness at reducing *E. coli* O: 157 H: 7 in feedlot cattle up to 50% (LeJeune, 2007).

The antagonistic activity of *Lactobacilli* and *Bifidobacteria* against facultative anaerobic Gram-negative target bacteria; uropathogenic *E. coli*, *S. enterica ssp. Enterica* and *Sh. sonnei* was tested by co-cultivation experiments using micro aerobic or anaerobic conditions. *E. coli* was highly suppressed by *L.rhamnosus* GG and both of *Bifidobacteria* strains, but no significant activity was found against cystitic *E.coli*.

The effective probiotics against *S.enterica ssp. enterica* were *L.paracasei* 8700:2, *L.plantarum* 299v and *L.fermentum* ME-3 showing high activity in micro aerobic milieu. *L.fermentum* ME-3 and both *Bifidobacteria* expressed high activity against *Sh.sonnei* in anaerobic milieu. However, the highest antagonistic activity was expressed by *L.rhamnosus* GG, *L.paracasei* 8700:2 and *L.plantarum* 299v. The inhibitory activity of probiotic bacteria against strict anaerobic *C.difficile* strain was low (6–8 mm) expressed by both *Bifidobacteria* strains and *L.paracasei* 8700:2.

It was demonstrated the enhancement for eradication of *Salmonella* in chronic carriers due to administration of *L.acidophilus*. Some placebo-controlled double-blind studies have shown the positive effect of *L.rhamnosus* GG by reducing the incidence of travellers' diarrhea (Hutt *et al.*, 2006).

Perusal of the data pertaining to the antibacterial activity of *L. acidophilus* cultures against some common intestinal pathogenic organisms indicated that all the cultures of *L. acidophilus* were active against the tested intestinal pathogenic organisms. The zones of inhibition of pathogenic organisms tested were ranging from 10.5 -16.25 mm in diameter (Padmanabha *et al.*, 2006).

But in this study the inhibition zone of *E. coli* O: 157 H: 7 was 30-45 mm in diameter and the inhibition zone of *Salmonella typhi* was 18-32 mm in diameter. In comparison with other studies, *Lactobacilli* of Iranian tribes (Bakhtiari and Ghashghaie) had good

antagonistic effect on *E.coli* and *Salmonella*. These preventive activities are shown in Fig. 1, 2, 3, 4, 5 and 6. The effect of *Lactobacillus casei* (Yakult) on the growth of enterotoxigenic *E. coli* at different contact time was determined by Godiosa *et al.* (1993). At zero contact time, there was  $32 \times 10^3$  colony forming units/mL (CFU/mL) with significant reduction to  $9.7 \times 10^3$  CFU/mL at 5 minutes contact time. At 5 minute intervals, there was progressive reduction in colony count to  $0.8 \times 10^3$  CFU/mL at 60 min contact time (Godiosa, Consignado, Adrian, Peña, Antoni and Jacalne, 1993).

In this study we assayed death kinetic of pathogens. 5% concentration of *Lactobacillus* decreased OD of both *E.coli* and *Salmonella* within 2 and 3 h after contact time. At zero time, OD of *E.coli* was 0.04 but it showed reduction to 0.03 after 3 h (Fig. 7, 8).

OD of *Salmonella* was 0.067 in zero time, but it showed reduction to 0.051 during 3 h after contact with *Lactobacillus*. In 10% concentration of *Lactobacillus*, OD of *E. coli* decreased from 0.005 to 0 during 2 h after contact time and OD of *Salmonella* decreased from 0.011-0.005 3 h after contact time (Fig. 9, 10).

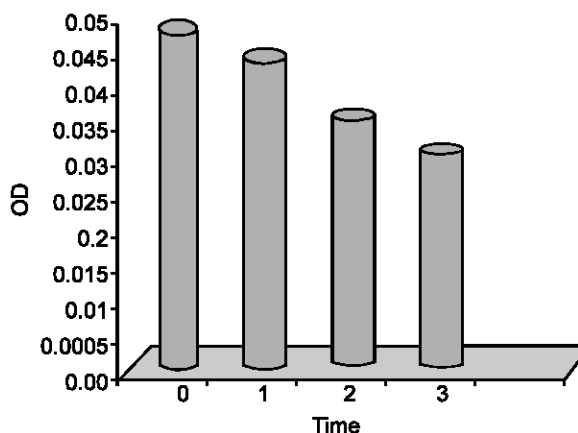


Fig. 7: Effect of 5 mL *lactobacillus* on OD of *E.coli*

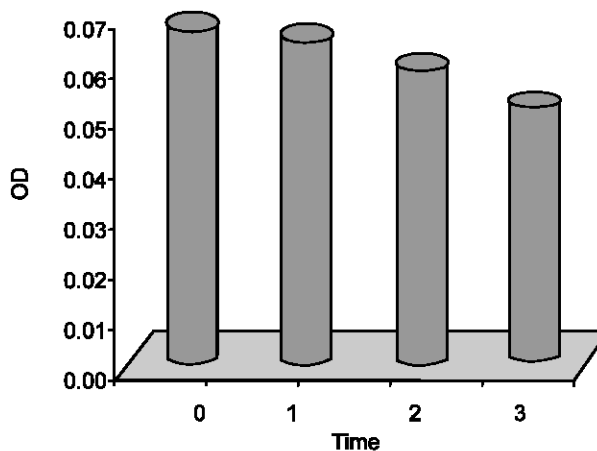


Fig. 8: Effect of 5 mL *lactobacillus* on OD of *Salmonella*

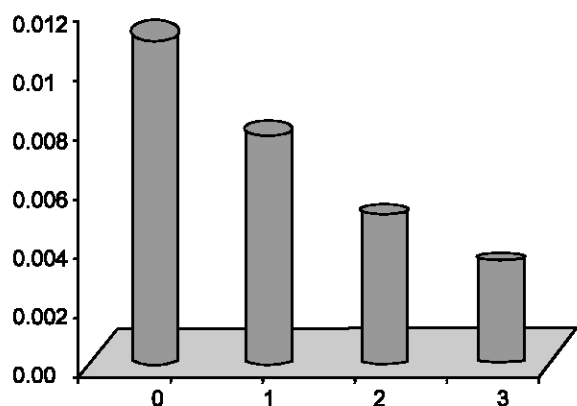


Fig. 9: Effect of 10 mL *Lactobacillus* on OD of *Salmonella*

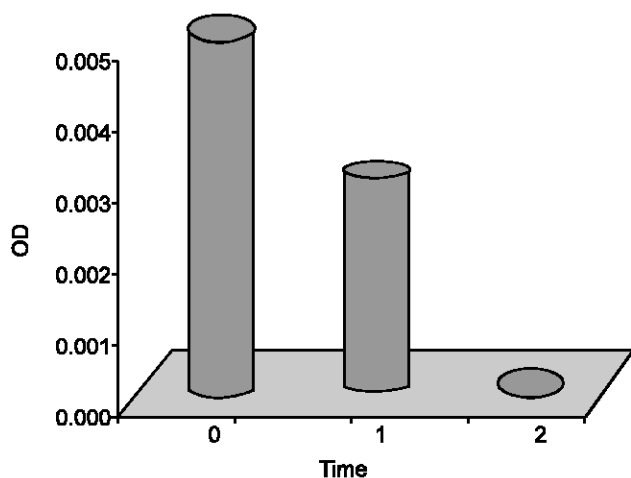


Fig. 10: Effect of 10 mL *lactobacillus* on OD of *E. coli*

The *Lactobacillus* species of Iranian tribes (Bakhtiari and ghashghaie) hadn't been studied. In this study we got significant results of antibacterial activity in comparison with others. In this study the inhibition zone for *E.coli* and *salmonella* were assayed and compared with the others.

These studies have been continued on probiotic bacteria. Specially probiotic of Iranian tribes should be studied for their antibacterial effect on another pathogen and they should be identified by molecular method. In conclusion, the activity of probiotics in prevention and treatment of infections by *E.coli* and *salmonella* are effective.

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