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Antibacterial Effects of Some Fermented Commercial and Homemade Dairy Products and 0.9% Lactic Acid against Selected Foodborne Pathogens

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ABSTRACT

In this study, the antibacterial effects of homemade yoghurt, commercial yoghurt, commercial kefir and probiotic yoghurt on viability of *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) was investigated by using disc diffusion method. Antibaterial effects of the samples were tested at 24, 48 h and the 7 day intervals. Homemade yoghurt showed the maximum antibacterial effect against the pathogens. It was determined that the most sensitive pathogenic bacteria to dairy products were *Salmonella typhimurium* whereas the least sensitive pathogen was *Pseudomonas aeruginosa*. Comparing to the antimicrobial effect of 0.9% lactic acid which was used as positive control, our results concluded that the bacteria found in fermented dairy products have an antibacterial activity and this activity is not caused by only lactic acid.

Key words: Probiotic, yoghurt, kefir, antimicrobial activity

INTRODUCTION

Probiotics are known as some beneficial yeasts and bacteria, especially lactic acid bacteria, Fermented dairy products (yoghurt, kefir etc.) have played an important role in human nutrition for centuries, when administered in adequate amounts, confer a health benefit on the host. Early in the 20th century, the Nobel-winner Russian scientist, Elie Metchnikoff (1845-1916) laid the foundation for the studies in this field by attributing the longevity of Bulgarian peasants who consume a lot of fermented dairy products (Hung *et al*., 2008; Sanders, 2003).

Yoghurt is a dairy product consumed extensively in Turkey. *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *S. salivarius* subsp. *thermophilus* cultures are used in its production. It has been revealed that the antibacterial effect of bacteria is used in yoghurt production against various food pathogens. It has been reported that antibacterial effect of yoghurt results from the lactic acid produced by cultures (Kılıç, 1990; Gülmez *et al*., 2003). In addition to the positive impact of yoghurt on the pathogenic microorganisms, there are also some studies on the ability of reducing the negative effects of antibiotics on intestinal flora (Coşkun, 2006).

Kefir is an acidic milk product in which some bacteria and yeasts can live in harmony and weak alcoholic fermentation occurs. Bacteria and yeasts have a symbiotic relationship in kefir and it has been proved that compounds they produce have an antibacterial effect on pathogenic bacteria such as *Salmonella*, *Helicobacter*, *Shigella*, *Staphylococcus* and *E. coli* (Ulusoy *et al*., 2007; Raja *et al*., 2009). For this reason, it has been thought that kefir can be used alternatively for therapeutic purposes in food borne pathogens infections (Schneedorf and Anfiteatro, 2004).

Although probiotic microorganisms are widely known as lactic acid bacteria, some yeasts and bacteria that do not produce lactic acid has been accepted as probiotic bacteria (Young and Huffman, 2003; Senok *et al*., 2005). Because they inhibit the growth of other microorganisms in the environment, lactic acid bacteria are used in the production of safe food in terms of pathogenicity. Moreover, low pH, other organic acids, bacteriocins, hydrogen peroxide (H_2O_2), ethanol, low oxidation-reduction potential are among the other inhibiting factors (Turantaş, 1998). Bacteriocins produced by lactic acid bacteria are proteins showing bactericidal effects. Although the control mechanisms in the synthesis of bacteriocins are unknown, these are thought to be synthesized as mechanisms to continue the life under the conditions of ecological stress (Aymerich *et al*., 1996). It is thought that bacteriocins change the potential of membranes by corrupting K^+ ion and ATP and cause cells fail to balance the intracellular pH of cell (Sezer and Guven, 2009).

The objective of the this study is aimed to determined the antibacterial activity in yoghurt (traditional and commercial), kefir (commercial), probiotic yoghurt, (biogard) and lactic acid (0.9%) on growth inhibition of *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

MATERIALS AND METHODS

In this study, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 obtained from Dicle University, Faculty of Medicine, Department of Microbiology at April 2007 were used.

In the study, homemade and commercial yoghurt, commercial probiotic yoghurt (biogard) and commercial kefir (probiotic) were used. Probiotic yoghurt (Biogard, Bioghurt Biogarde GmbH and Co. KG, Freising, Germany) used in this study contained, *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Streptococcus thermophilus* strains.

The pH of the fermented milk products was determined using an indicator strip (Universal indicator-Merck). A portion of the samples of the fermented milk product was taken under aseptic conditions and its filtrate was drained in the sterile bandage. The obtained filtrate was labeled separately and transferred into two glass tubes (Tube 1, 2) and tube 1 was used for pH measurement using the indictor strip. The filtrate in the tube 2 was absorbed by into the sterile blank discs (Oxoid) (100 μL disc^{-1}). Procedures were repeated for each fermented milk product. Fermented milk products (kefir 0.9%, yoghurt 0.6%) contain various concentrations of lactic acid. In our study, the antibacterial effects of lactic acid and other milk products were compared. An average percentage of lactic acid in kefir was used in this study. Lactic acid (90% pure) was diluted to a 0.9% lactic acid solution by adding sterile distilled water and media were adjusted to pH of 4.0 with dilute NaOH in order for them to have the same level of pH as the filtrate of the other milk products have.

Ceftriaxone Sodium (Cephalosporin group half synthetic, broad-spectrum antibiotic) was used as the positive control group in the study. Antibiotic solution was diluted with sterile distilled water to 10 μg mL^{-1} concentration. The antibiotic solution was absorbed by into the sterile blank discs (50 μL disc^{-1}) (Bonade *et al*., 2001).

Pure cultures containing *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were activated in nutrient broth at 35°C for 24 h. One loopful of bacteria from the activated pathogen suspensions was taken into a 2 mL normal saline and its turbidity was adjusted to 0.5 McFarland density (Gürgün and Halkman, 1990). Probable bacterial density was adjusted to 10^8 mL^{-1} according to spectrophotometric method. BPLS (Brilliant-green Phenol-red Lactose Sucrose) Agar medium (Merck) for *Salmonella typhimurium*, TBX (Tryptone Bile X-glucuronide) Agar medium (Merck) for *Escherichia coli*, Plate Count Agar medium (Merck) for *Pseudomonas aeruginosa* and Baird-Parker Agar Base medium (Merck) for *Staphylococcus aureus* were used.

Inoculation process was conducted by homogeneously applying the 0.1 mL sample extracted from pathogenic suspensions which approximately contained 10^8 mL^{-1} bacteria on the medium surface. Discs contained the antibiotic or lactic acid as control and discs contained the filtrate of probiotic products were placed on the surface of agar medium in Petri dishes in which bacteria had been inoculated under aseptic conditions. These Petri plates were incubated at 37°C. The inhibition zones forming around the discs were measured as mm/diameter at the 24, 48 h and on the 7 day intervals.

The data were analyzed by One-way ANOVA. Duncan's new multiple range test was used to compare means values of 5 replicates at $p<0.05$ using SPSS 13.0 (SPSS Inc. Chicago, USA).

RESULTS

As it is seen in the Table 1, all of the fermented products had acidic pH and their pH values were between 3.96 and 4.52.

Among fermented milk products used in our study, maximum inhibition zone (24.0 mm) against *E. coli* was observed in homemade yoghurt, followed by commercial yoghurt (18.4 mm), kefir (16.7 mm) and yoghurt biogard (14.1 mm) (Table 2). A statistically significant difference was determined among all groups ($p<0.05$). But, inhibition zone disappeared in kefir and biogard after 48 h and in homemade yoghurt after 7 day. Antibacterial effect was determined against *E. coli* only in commercial yoghurt on the 7 day with inhibition zones of 14.4 mm (Table 2).

The highest antibacterial effect against *S. aureus* was determined in the homemade yoghurt (28.6 mm) followed by commercial yoghurt, biogard and kefir after 24 h. It has been determined that antibacterial activity of biogard disappeared after 48 h and that of kefir disappeared after 7 day. The best antibacterial effect (30.4 mm) against *S. aureus* was seen in homemade yoghurt after 48 h of incubation (Table 2).

It has been determined that the fermented milk product which had the best antibacterial effect against *Salmonella typhimurium* at the 24 h was the homemade yoghurt. When the inhibition zones of all groups were examined at the 24 h and on the 7 day, it was understood that the antibacterial efficiencies of all were gradually increasing. When the other pathogenic bacteria were examined, gradually increasing effect was observed only in *Salmonella typhimurium*. The best antibacterial activity against pathogens was identified in homemade yoghurt at 7 day with inhibition zones of 36.7 mm. It was also found out that this effect was better than antibiotic (Ceftriaxone Sodium) disc was used as control group and the difference between homemade yoghurt and Ceftriaxone Sodium was statistically significant ($p<0.05$) (Table 2).

Among the milk products used in the study, the best antibacterial activity (24 h) against *P. aeruginosa* has been determined in commercial yoghurt group with inhibition zones of 20.4 mm. But, no inhibition zone has been found in kefir groups against to *P. aeruginosa* at all analysis days.

Table 1: pH values of fermented dairy products

	pH±SD	
Fermented dairy products	Whole product	Product filtrate
1Homemade yoghurt	3.96±0.11	4.08±0.21
Commercial kefir	4.36±0.16	4.30±0.07
Commercial biogard	4.30±0.10	4.52±0.19
Commercial yoghurt	3.90±0.18	4.12±0.31

SD = Standard deviation, n = 3

Table 2: Antibacterial activity of fermented dairy products and 0.9% lactic acid on foodborne pathogens

	Inhibition zone (mm±SD)		
Groups	24 h	48 h	7 day
***Escherichia coli* ATCC 25922**			
Homemade yoghurt	21.9±0.46^{b}	24.0±0.23^{b}	ND
Commercial yoghurt	18.2±0.23^{c}	18.4±0.34^{c}	14.4±0.38^{b}
Commercial biogard	14.1±0.35^{e}	ND	ND
Commercial kefir	16.7±0.79^{d}	ND	ND
Ceftriaxone	38.4±0.16^{a}	39.9±0.26^{a}	36.0±0.37^{a}
Lactic acid	ND	ND	ND
***Staphylococcus aureus* ATCC 25923**			
Homemade yoghurt	28.6±0.79^{b}	30.4±0.79^{b}	14.4±0.29^{c}
Commercial yoghurt	23.0±0.67^{c}	24.2±0.36^{c}	16.2±0.71^{b}
Commercial biogard	19.9±0.79^{d}	ND	ND
Commercial kefir	14.0±0.11^{e}	22.1±0.17^{d}	ND
Ceftriaxone	32.3±0.25^{a}	38.2±0.18^{a}	36.4±0.25^{a}
Lactic acid	ND	ND	ND
***Salmonella typhimurium* ATCC 14028**			
Homemade yoghurt	31.9±0.49^{b}	38.6±1.77^{a}	36.7±0.38^{a}
Commercial yoghurt	24.0±0.40^{c}	28.4±0.23^{c}	32.5±0.43^{d}
Commercial biogard	17.2±0.52^{d}	22.3±0.28^{d}	29.9±0.30^{d}
Commercial kefir	14.0±0.34^{e}	20.5±0.39^{e}	35.3±0.67^{c}
Ceftriaxone	35.7±0.37^{a}	35.0±0.33^{b}	35.9±0.51^{b}
Lactic acid	14.2±0.23^{e}	22.6±1.01^{d}	20.5±0.65^{f}
***Pseudomonas aeruginosa* ATCC 27853**			
Homemade yoghurt	16.3±0.30^{d}	16.5±0.37^{c}	14.3±0.33^{b}
Commercial yoghurt	20.4±0.40^{c}	18.7±0.68^{b}	14.3±0.25^{b}
Commercial biogard	14.4±0.28^{e}	ND	ND
Commercial kefir	ND	ND	ND
Ceftriaxone	40.6±0.44^{a}	38.5±0.34^{a}	30.5±0.45^{a}
Lactic acid	22.5±0.36^{b}	ND	ND

Mean values with different superscripts in the same column are significantly different ($p<0.05$), n = 5, SD: Standard deviation, ND: Not determined (no zone of inhibition)

However, in biogard yoghurt, while there was inhibition zone at the 24 h, it disappeared completely after 48 h of incubation. Antibacterial activity both in homemade and commercial yoghurt was the same after 7 day of incubation with inhibition zones of 14.3 mm (Table 2).

When we examined 0.9% lactic acid group, antibacterial activity was not determined against *E. coli* and *S. aureus*. It was observed that antibacterial activity occurred against *P. aeruginosa* at the 24 h, it disappeared at 48 h. It was determined that the best antibacterial activity occurred against *S. typhimurium* (Table 2). All of the pathogens were found to be sensitive to Ceftriaxone Sodium which was used as positive control group, with inhibition zones of 39.9, 38.2, 35.9 and 40.6 mm against *E. coli, S. aureus, S. typhimurium* and *P. aeruginosa*, respectively (Table 2).

DISCUSSION

In our study, the *in vitro* antibacterial effects of homemade yoghurt, commercial yoghurt, commercial kefir, commercial probiotic yoghurt on foodborne pathogens including *Salmonella typhimurium* (ATCC 14028), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were measured by using the disc diffusion method. Ceftriaxone, a broad-spectrum antibiotic, was used as control. In addition, it was investigated whether anti-bacterial activities of fermented dairy products were resulted from lactic acid only. For this reason, 0.9% lactic acid solution was used.

The highest antibacterial effect against foodborne pathogens used in this study was seen in homemade yoghurt. The antibacterial activity of homemade yoghurt, which is effective against all pathogens tested, decreased in incubation period increased to day 7. The best results with a 35.7 mm zone were seen against *S. typhimurium*. It was also determined that less susceptible pathogenic bacteria to homemade yoghurt were *P. aeruginosa* and *E. coli* (Table 2). Kotz *et al.* (1990) reported that yoghurt filtrate has *in vitro* antibacterial effect against three different *E. coli* strains. In this study, when inhibition zones are compared, typically, homemade yoghurt resulted in larger inhibition zones than commercial yoghurt. This may be because of making yoghurt using different strains and contamination of homemade yoghurt with different lactic acid bacteria during yoghurt making.

In this study, it was found that the antibacterial effect of lactic acid was effective only on *S. typhimurium* with 19.1 mm inhibition zone. Rubin *et al.* (1982) investigated the effect of lactic acid on *S. typhimurium* and it was claimed that the effect of lactic acid stemmed not only from lowering the pH of medium but also from intracellular activity by entering into cell. Similarly, the result of our study about *S. typhimurium* is thought to be due to the metabolical changes caused by lactic acid after entering into the bacterial cell.

It has been found that the most sensitive pathogen to the antibacterial activity of commercial kefir is *S. typhimurium*. Kefir showing limited effect on *S. aureus* and *E. coli,* did not create an inhibition zone against *P. aeruginosa*. In a previous study, the antibacterial activity of kefir on 7 bacterial species and 1 fungus was examined with disc diffusion method and it has been reported that the most sensitive pathogen was *S. pyogenes* and that *S. aureus, S. salivarus, S. typhimurium, C. albicans, L. monocytogenes* were less sensitive pathogens and *P. aeruginosa* and *E. coli* were the least sensitive (Rodrigues *et al.*, 2005). While similar results have been obtained from our study, it is also thought that no effect on *P. aeruginosa* was due to differences of pathogen bacteria and strains in commercial kefir. In another study on kefir, it has been stated that kefir does not show antibacterial activity against *E. coli* O157: H7 and *E. coli* strains in neutral pH but when the environment is acidic development of both pathogens is inhibited (Fernando *et al.*, 2006).

In commercial probiotic yoghurt sample prepared using biogard culture, a significantly larger zone size was seen against *S. typhimurium*. But it has been found that biogard yoghurt has lower antibacterial activity than homemade yoghurt and commercial yoghurt against all pathogens studied. The zones that commercial biogard formed against *S. aureus, E. coli* and *P. aeruginosa* was not found significantly different ($p>0.05$).

In this study, it was observed that the studied probiotic source for fermented dairy products created antibacterial effects, but not to same extents statistically, against foodborne pathogens while these effects vary according to the pathogen type and time. In some studies which were carried out with various lactic acid bacteria, it was reported that antibacterial effect was obtained against pathogens (Rodrigues *et al.,* 2005; Batdorj *et al.,* 2007). Furthermore, in another study carried out in the same way, it was indicated that the antibacterial effect created by probiotic bacteria was proved on genus *Salmonella, Coliform, Staphylococcus* and *Pseudomonas* (Pullusani *et al.*, 2006).

Abdel-Bar *et al.* (1987) reported that *L. bulgaricus* strain formed inhibition zones of 29.5 mm and 34.67 against *S. aureus* and *P. fragi*, respectively. In another study carried on the antibacterial effects of dairy products on the market, it was demonstrated that the antibacterial activities of the products varied and the commercial yoghurt was bactericidal for *S. aureus* and *P. aeruginosa;* inhibitor for *S. typhi*; inactive against *E. coli* and *Candida albicans* (Eduardo *et al.,* 2003). Differences in antibacterial effects of fermented dairy products were also observed in our study. In our study, It is determined that inhibition zone decreased after 7 days in some cases, this manner may be a result of antagonistic effect between probiotic and pathogen bacteria.

As a result, the antibacterial effects of probiotic source fermented dairy products against common foodborne pathogens showed different results according to depending on the type of fermented dairy product used, species of pathogen bacteria and time. When the results obtained from the lactic acid which was used as control are taken into consideration, it was demonstrated that the antibacterial effect does not depend solely on the lactic acid contained in the fermented dairy products. This effect is also thought to be caused by other metabolites (H_2O_2 and bacteriocins) created by the probiotic bacteria. These antibacterial effects between probiotic and pathogen bacteria may explain quorum sensing mechanisms. It is suggested that the probiotic bacteria or/and metabolites in food can be used as an antibacterial agent against to food-borne pathogens alone or in various combinations. Additional studies are needed to understand underlying mechanisms.

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