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ORIGINAL RESEARCH

Investigation of Antagonistic Effect of Probiotic Food Supplement against Different Strains of Bacteria

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Abstract

Objective: Probiotics are an important part of the microbiota associated with many diseases. The production of food and food supplements containing probiotic products has increased considerably in recent years, due to their protective effect on health and antagonistic effect against some pathogenic microorganisms. However, it is unknown whether the content of these products has the expected effect. In this study was aimed to investigate the antagonistic effect of Probiotics & Prebiotics® commercial product.

Material-Method: The probiotic food supplement was obtained from Aym-Net®. Antagonistic effect of the product on 10 different test bacteria was investigated by the agar spot assay method. Also lyophilized probiotics were cultured in MRS broth medium and centrifuged at 3500 rpm for 25 minutes in order to obtain metabolites that the probiotic microorganisms provide their effectiveness. Effect of probiotic supernatant on test bacteria were determined to disk diffusion test by CLSI. Each test bacteria combined with the probiotic product incubated at 37 ^{0}C for 24 hours to determine the bacterial inhibition rate of product which analyzed using the Mann Whitney U test.

Results: Probiotic product inhibited growth of *L. monocytogenes* by (86 %), *Y. pseudotuberculosis* (82%), *S. aureus* (76%), *E. coli* (74%), *S. typhimurium* (73%), *P. aeruginosa* (69%) and *S. epidermidis* (67%) respectively. The lowest antagonistic effect was detected against *E. faecalis* 44%. While probiotic product provided significant growth inhibition on 8 strains ($p<0.05$), there were not statistically significant growth inhibition for *P. vulgaris* and *E. cloacae* ($p>0.05$).

Conclusion: It was concluded that antagonistic effect of probiotic food supplement on test bacteria. Therefore it may be beneficial to use in bacterial infections.

Keywords: Antimicrobial Effect, Food Supplement, *Lactobacillus*, Probiotics

INTRODUCTION

The gastrointestinal system hosts a microbial community called microbiota which is so important for health[1]. Studies show a correlation between microbiota changes with mental disorders, obesity, metabolic diseases, autoimmune diseases, allergies, irritable bowel syndrome (IBS), acute chronic bowel inflammation and gastroenteritis[2-4]. There could be a cycle in which various natural biological functions affect the composition of the microbiota or, due to developing health problems, the composition and function of the microbiota are affected and impaired, hence the emergence of other health problems. Therefore, the preservation of the natural structure of the microbiota is considered to be very important for human health[5]. Microbiota with dysbiosis, aside from the metabolic diseases it causes, directly leads to serious health problems such as constipation, diarrhea, other intestinal disorders and even colitis[6]. One of the important issues in preserving the natural structure of the microbiota is the presence of probiotics. Living microorganisms that live in the microbiota and are resistant to stomach acids and bile, which have significant beneficial effects on the host, are generally called probiotics[7]. Studies shows that probiotics have many important roles in the body: They play role in preventing some infections, preventing cancer, preventing allergies, boost to the immune system, and daily digestion[8, 9]. In addition, they colonize throughout the intestinal system and prevent pathogens from outside from settling into the intestine. Also probiotic bacterial proteins have an antagonistic effect on some pathogens[10, 11]. Therefore, it is frequently used as a supplementary food in the treatment of various infectious diseases. So returning to nature and naturalness appears in many areas worldwide[12]. Moreover, antibiotic resistance, which manifested itself as a serious crisis especially in recent years, and increased treatment costs have also led to an increase in the consumption of probiotic

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supplements[13]. However, not all probiotics work for all. Therefore, commercial probiotics have come into the agenda in recent years. This is because it is known which health outcomes are supported by identified probiotics. For instance a probiotic bacterium used for constipation does not work for digestive problems caused by antibiotics[14-16]. Probiotic market has grown rapidly in recent years. The efficacy of probiotics is specific to some strains and should not be generalized[17]. In this study, it was aimed to investigate the antagonistic effect of a commercial probiotic food supplement on some microorganisms.

MATERIALS AND METHODS

Probiotic food supplement

The probiotic food supplement used in this study was obtained from Aym-Net®. It contains mixed probiotic microorganism. Product content is given in Table 1.

Table 1. Content of Probiotics & Prebiotics®

Ingredients	Composition
Fibrous (from oat) (Avenasative L.)	700 mg
Lactobacillus acidophilus	$2x10^9$ CFU/gr
Saccharomyces boulardii:	$2x10^9$ CFU/gr
Bifidobacterium infantis	$1x10^9$ CFU/gr
Lactobacillus plantarum	$1x10^9$ CFU/gr
Bifidobacterium bifidum	$1x10^9$ CFU/gr
Bifidobacterium longum	$1x10^9$ CFU/gr
Lactobacillus paracasei	$5x10^8$ CFU/gr
Lactobacillus reuteri	$5x10^8$ CFU/gr
Lactobacillus bulgaricus	$5x10^8$ CFU/gr
Lactobacillus rhamnosus	$2,5x10^8$ CFU/gr
Lactobacillus brevis	$2,5x10^8$ CFU/gr

Test bacteria

Yersinia pseudotuberculosis ATCC 911, *Salmonella Salmonella enterica subsp. enterica typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* 12228, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *P. vulgaris* ATCC 29905, *Enterobacter cloacae* ATCC 13047 strains were used in the study and was obtained from the Microbiology Laboratory culture collection of Duzce University Traditional and Complementary Medicine Application and Research Center.

Agar spot assay

The capsule containing lyophilized probiotic microorganisms was dissolved in 10 ml of distilled water and kept at 37 ^{0}C for 1 hour. Afterwards, it was inoculated in MHA medium as a point with a sterile needle-tipped swab (Mueller Hinton Agar, Merck) and incubated at 37 ^{0}C for 24 hours. Test bacterial cultures prepared 24 hours ago in Mueller Hinton Broth (MHB, Merck) were mixed into 5ml soft agar media (MHB containing 0.7% agar) to contain $1x10^8$ cells and poured over probiotic culture. It was evaluated for the presence of inhibition zones after the incubating 24 h at 37 ^{0}C[7, 11]. All experiments were carried out three times.

Disk diffusion method

Supernatant of probiotics was obtained and inhibition zones formed on test bacteria were measured[18]. Test bacteria were prepared 24 h before the experiment on NA (Nutrient Agar, Merck). Then 2 -3 colonies were added in sterile physiological serum at a density of $1\text{-}1,5x10^8$ CFU / ml (McFarland 0.5) and inoculated on MHA. On the other hand of lyophilized probiotic food supplement dissolved in 10 ml distilled water and 1 ml of it inoculated into 9 ml of MRS broth (e man, Rogosa and Sharpe, Merck) incubated at 37 ^{0}C to 24 h. Bacterial supernatant after centrifuging the liquid medium containing the probiotic product for 25 minutes at 3500 rpm was absorbed on blank disks (Bioanalyase, blank disk 6mm). Gentamicin (Bioanalyase, CN 10µg disk) was used as positive control13. All experiment performed in triplicate in different days.

Growth inhibition rate

The inhibitory effect of the probiotic product on the growth of test bacteria was calculated separately in the form of a combination with probiotic product and each test bacterium. One hundred fifty µl of test bacteria culture (prepared $1x10^8$ CFU / ml) and 50 µl of probiotic food supplement were added to each well in 96 well plates. Each test bacterium and probiotic supplement were also put into the well alone. Accordingly, the growth turbidity of tested bacteria alone is considered 100%. The OD_{630} was recorded (Plate Reader, Biotek 800TS, USA) after the microplate was incubated at 37 ^{0}C for 24 h[19, 20]. All experiment performed in triplicate.

Statistical analysis

Whole experiments were performed in triplicate. All data have been given as mean (± SD) and SPSS 15.0 has been used to evaluate the data. Inhibition ratio of the probiotic product between control have been analyzed using the Mann Whitney U test.

RESULTS

Test product containing a mixture of probiotic bacteria did not form an inhibition zone in two different bacterial strains, while an inhibition zone was found in others. Accordingly, the highest inhibition was seen in *Y. pseudotuberculosis* and *L.*

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monocytogenes, while the lowest inhibition was seen in *E. faecalis*. The zones determined according to both methods are given in Tables 2 and 3.

Table 2. Antagonistic effect of product by agar spot assay

Test Bacteria	Inhibition Zone
Y. pseudotuberculosis	+
S. typhimurium	+
S. epidermidis	+
E. faecalis	+
P. aeruginosa	+
L. monocytogenes	+
E. coli	+
P. vulgaris	-
E. cloacae	-
S. aureus	+

Table 3. Zone diameters to the disk diffusion method

Test Bacteria	Zone Diameter (mm)± SD	
	Probiotic Product	Gentamicin
Y. pseudotuberculosis	18 (±1)	20
S. typhimurium	16 (±0)	20
S. epidermidis	13 (±0.57)	18 (±0.57)
E. faecalis	9 (±0,57)	20
P. aeruginosa	15 (±0)	20
L. monocytogenes	18 (±0.57)	25
E. coli	14(±1.15)	25
P. vulgaris	R	15
E. cloacae	R	20
S. aureus	14(±0.57)	20

R: Resistant, no inhibition zone.

The inhibition rate of probiotic food supplement on test bacteria is given in Figure 1. Probiotic product inhibited growth of *L. monocytogenes* by 86 %. Similarly, it was shown that 82% antagonistic effect against *Y. pseudotuberculosis* while it was determined against to *S. aureus (*76%), *E. coli (*74%), *S. typhimurium (*73%), *P. aeruginosa (*69%) and *S. epidermidis* (67%) respectively. The lowest antagonistic effect was detected against *E. faecalis* 44%.

DISCUSSION

Lifestyle and nutrition play an important role in the pathogen of infections. Hence, healthy nutrition and natural product preference is increasingly important in preventing disease. On the other hand, antimicrobial agent resistance caused by the consumption too much antibiotics and synthetic products leads to the need for natural products to protect against infectious diseases. In addition, probiotic food supplement intake is recommended in some cases, such as diarrhea caused by a decrease in probiotic bacteria in gut due to antibiotic consumption[13,21,22]. It is a known fact that probiotics are recommended for a number of indications in relation to this and such health[23]. They are often seen as part of the immune system and are reported to play an important role in preventing infectious diseases. It is frequently used in intestinal infections. *Lactobacillus* and *Bifidobacterium* species are also associated with obesity, anxiety, diabetes and brain function, as well as bowel disorders[24]. Therefore, commercial products containing probiotics are increasing day by day[25]. In particular, the lack of milk and dairy products as a source of probiotics suitable for consumption by some individuals due to lactose intolerance has led to an increase in alternative probiotic foods and food supplements[16].

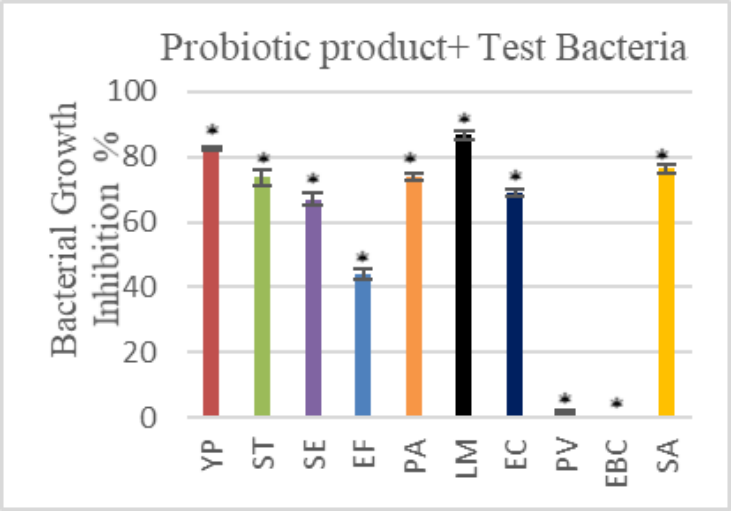

Figure 1. Growth inhibition of test bacteria. * p<0.05. Growth inhibition rates of test bacteria observed as a result of combined incubation of each test bacteria with probiotic food supplement were given. YP: *Y. pseudotuberculosis*, ST: *S. typhimurium*, SE: *S. epidermidis*, EF: *E. faecalis*, PA: *P. aeruginosa*, LM: *L. monocytogenes*, EC: *E. coli*, PV: *P. vulgaris*, EBC: *E. cloacae*, SA: *S. aureus*.

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Currently, food supplements containing dried probiotics have a fairly large market in this sense. This situation brings about some problems. Some of these problems are that products do not show the expected functional properties[23,26]. In this study, the content of commercial food supplements containing probiotic microorganisms was evaluated in total without being defined separately. Antagonistic effect of the product was tested in 10 different bacterial strains and the ratio of its possible inhibitory effect was investigated. Accordingly, it was observed that it formed an inhibition zone in 8 different bacterial strains. Edalati et al.[7] found that different species of *Leuconostoc*, *Lactobacillus* and *Weissella* formed an inhibition zone on *E. coli.* Iglesias et al.[27] reported that *L. acidophilus* has antagonistic effects on food-borne *Salmonella* and *L. monocytogenes* and that probiotics can be used to control the growth of pathogens in foods. Karimi et al.[13] investigated the effectiveness of lactobacilli on *E. coli*, a common agent of diarrhea, and reported that the highest inhibitory effect had *L. plantorum*. Corr et al.[28] have shown that various probiotics have a significant antagonistic effect in *Listeria* infections. In parallel, the highest antagonistic effect was determined against *L. monocytogenes* in this study. The Test product inhibited the growth of *L. monocytogenes* by 86%. Also it has been shown in studies that *S. boulardii* has antimicrobial activity against various intestinal pathogens[9]. Therefore, it can be said that this study has expected results. In another study, the inhibition zone diameters of pineapple juice containing *Pediococcus pentosaceus and L. rhamnosus* were detected on *Klebsiella* 10mm, *P.aureus* 11 mm, *Bacillus* spp. 13 mm, *E.coli* 14 mm, *Pseudomonas* 14 mm, similar to this study[16]. The zone diameters in this study were determined as 14 mm for *E. coli* and 15 mm for *P. aeruginosa*. But the inhibition ratio detected for *P. aeruginosa* (69%) was lower than the inhibition ratio of *E. coli* (74%). This may be due to the abduction of some antagonistic metabolites in obtaining the probiotic upper phase used in disc diffusion testing. Because the product tested contains many probiotic microorganisms. The effectiveness of the product containing multiple probiotic microorganisms was evaluated as a whole in this study. In general, there are many studies that probiotics contained in the test product show antagonistic effects on different strains of bacteria and yeast[29-31]. Studies are mostly on the antagonistic effect of the probiotic microorganism alone. Although the combination of probiotics is expected to be a synergistic effect in basically it is also possible that it will lead to a decrease in the effect[19]. Therefore, it has been evaluated totally in terms of whether the product is effective or not. In addition, it is a current problem that the content of probiotic products, which has increased frequently in recent years, must be controlled[26]. Therefore, it was important to demonstrate the effectiveness of commercial probiotic food supplements tested in this sense.

In his study, Valdez et al.[19] investigated the antagonistic effect of *B. infantis*, *B. lactis* and *B. longum* species on periodontal pathogens alone or in various combinations and reported an antagonistic effect in both cases. Probiotic microorganisms have an antagonistic effect on pathogens via various metabolites (bacteriocin etc.). Acid organics such as lactic acid, which produced by probiotics are highly effective in the Gram negative bacterial cell membrane[19,32,33]. In this study, antagonistic effect against gram negative bacteria was found to be higher. There are two bacterial strains (*P. vulgaris* and *E. cloacae*) that do not show growth inhibition ($p>0.05$). These bacteria probably showed resistance to the metabolites produced by probiotic microorganisms.

CONCLUSION

Evaluating the effectiveness of probiotic food supplements is a problem today. This study was investigated the antimicrobial efficacy of the commercial probiotic food supplement Probiotics & Prebiotics®.

It was concluded that antagonistic effect of probiotic food supplement was detected on a variety of test bacteria. Therefore, its use as a supplement in these bacterial infections can provide a beneficial effect.

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