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Original Article

Antimicrobial Effects of Different Synbiotic Compounds against Pathogenic Bacteria Isolated from Beef, Mutton, and Chicken

Hassanzadeh, P[1 *], Nouri Gharajalar, S[2], Mohammadzadeh, S[1]

1. Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

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Corresponding Author: p.hassanzadeh6@yahoo.com

Abstract

Today, there has been a growing interest in synbiotic usage in the food industry to solve the problems related to food contaminations. The present study aimed to evaluate the antibacterial effects of nine symbiotic compounds on bacteria isolated from different meat types. Pathogenic bacteria were isolated from 60 different meat samples. Then, the antibacterial effects of nine synbiotic components were assessed against isolated bacteria using well diffusion and radial streak methods. In addition, minimum inhibitory and minimum bactericidal concentrations of each synbiotic formulation were determined. The highest antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus* was for synbiotic compounds consisting of *Streptococcus salivarius*, raffinose, inulin, and trehalose, respectively. Furthermore, the highest antibacterial efficacies against *Escherichia coli* and *Salmonella* were for synbiotic formulations consisting of *Bacillus cereus* and inulin, raffinose, and trehalose, respectively. In conclusion, synbiotic formulations containing *S. salivarius* and *B. cereus* may be an alternative approach to preventing food-borne pathogens.

Keywords: Synbiotic, Antimicrobial property, Beef, Mutton, Chicken

1. Introduction

Meat is an essential source of nutrients such as proteins, vitamins, lipids, and minerals. As it has high nutrient content, it is a suitable culture media for the growth of many species of microorganisms (e.g., bacteria and fungi) (1). Food-borne diseases remain a significant concern in developing countries caused by *Escherichia coli*, *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Campylobacter jejuni*. In addition, it has been reported that antibiotic resistance is prevalent among food-borne pathogens (2). The application of naturally produced antimicrobials without any side effects on human health to stop the bacterial spread in food is an ideal way to solve the problems related to food contamination (3). Probiotics like *Lactic acid* bacteria are usually recognized as live microorganisms or their end products, which, when used in sufficient amounts, beneficially affect the host by modulating the intestinal immune system (4). They contain bacteria belonging to the genera *Lactobacillus*, *Bifidobacteriums*, *Leuconostoc*, *Pediococcus*, *Propioni bacterium*, and *Bacillus*. *Streptococcus Salivarius* subsp thermophiles is also a probiotic microorganism (5).The antimicrobial properties of probiotics are related to producing organic acids, such as lactic and acetic acid, hydrogen peroxide, antimicrobial enzymes, inhibitory compounds, and reducing the availability of the essential nutrients for pathogens in their living environment (6). Prebiotics are non-digestible organic food component fibers that positively affect the host by promoting

intestinal health and providing substrates for confident intestinal bacteria like lactobacilli and bifidobacteria, which have positive effects on the host's health to ferment. Coupling the application of probiotics with prebiotics is known as synbiotic which is believed to be more beneficial in terms of gut health and function (7). The present study aimed to investigate *in vitro* effects of synbiotic combinations of *L. acidophilus*, *B. cereus*, and *S. salivarius* with inulin, trehalose, and raffinose upon bacterial species isolated from beef, mutton, and chicken meet.

2. Materials and Methods

2.1. Sample Collection

Between December 2018 till April 2019, 60 retail raw meat samples were collected from Urmia city of Iran, including beef (n=20), mutton (n=20), and chicken (n=20). All samples were collected within 8 h post-slaughter and were kept below 4 ℃ during transportation to the lab (2).

2.2. Sample Preparation

Twenty-five grams of each sample was weighed and transferred to sterile flasks with 100 ml of phosphate buffer saline. After homogenizing by the meat grinder, the samples were stored for further analysis (2).

2.3. Isolation and Identification of Pathogens

Homogenized meat samples were transferred to nutrient agar, mannitol salt agar, MacConkey agar, Eosin- Methylene blue agar, selenite F broth, and listeria selective agar (Merck, Germany). Standards biochemical methods such as gram staining, oxidase, catalase, citrate, sulfide indole motility, triple sugar iron, DNase tests, and urea hydrolysis were used for bacterial identification (1). Then, pathogenic isolates were stored at -80 ºC in 15% (w/w) glycerol (8). *Escherichia coli* ATTC 15224, *Staphylococcus aureus* ATTC 12600, *Listeria monocytogenes* ATTC 2374, and *Salmonella enterica* ATTC 14028 were used as indicator organisms.

2.4. Probiotics, prebiotics, and their synbiotic combinations

The *L. acidophilus* ATCC 4356 were inoculated in 5 ml Man Rogosa Sharpe (MRS) broth (Sigma-Aldrich, USA) containing the carbohydrate substrate of inulin, trehalose, or raffinose (1%w/w; Sigma-Aldrich, USA) and incubated for 24 h at 37 ºC under microaerobic conditions with turbidity equivalent to 0.5 McFarland (1.5×10^8 CFU/ml) (9). *Bacillus cereus* var. toyoi (Toyocerin®, Asahi Vet. S.A., Barcelona, Spain, 200 mg/kg, containing 2×10^5/g *Bacillus cereus* var. *toyoi* spore) was grown in 5 ml nutrient broth with 1% w/w of inulin, trehalose, or raffinose. Then, incubated for 24 h at 37 ºC under aerobic conditions until reaching 1.5×10^8 CFU/ ml (10). Finally, *S. salivarius* k12 (OralBiotic® Lozenges, Canada) was inoculated into mitis-salivarius broth (Sigma-Aldrich, USA) supplemented with inulin, trehalose, or raffinose and incubated at 37 ºC under anaerobic conditions to give approximately 1.5×10^8 cfu/ml (11).

2.5. Preparing Supernatant of Probiotic Bacteria

Bacterial cells were removed by centrifugation at 2000 g for 10 min, and pH was adjusted for each culture supernatant to 7.0 using (1M NaOH) and sterilized by filtration using 0.22 µm Millipore filters (Sigma-Aldrich, USA).

2.6. Well Diffusion Agar

In this method, culture was done on Muller Hinton agar (MHA) plates with a sterile swab from a suspension of *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes* in TSB broth medium (0.5 McFarland). Wells of 10 mm in diameter were cut into agar plates with a sterile Pasteur pipette, and 100µl of supernatants of probiotic bacteria (*L. acidophilus* ATCC 4356 growing on MRS, *Bacillus cereus* var. toyoi growing on nutrient broth, and *S. salivarius* k12 growing on mitis-salivarius broth each supplemented with 1% w/w of prebiotics) was placed into each well. All the plates were incubated for 24 h at 37 ºC, and antimicrobial activity was measured as growth-free inhibition zones around the wells (mm) (12).

2.7. Radial Streak Method

Nutrient agar, MRS agar, and MSA agar plates were cultured with 0.5 McFarland of *B. cereus*, *L. acidophilus*, and *S. salivarius* suspensions by covering a circular area in the center of the plate. After

incubation of 48 h at 37 ºC, the plates were seeded with *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes* (0.5 McFarland) by radial lines of inoculum from the border to the center of the petri dish. After 24 h of incubation at 37 ºC, the growth inhibitory activity was measured by subtracting the circle diameter (cm) of the probiotic spreading zone from the inhibition zone diameter detected (8).

2.8. Assessment of Minimum Inhibitory Concentration (MIC)

The MIC of formulations was determined using the broth microdilution method in 96 well plates. After adding 100µl Muller Hinton broth to each well, 100µl of each probiotic culture supernatant was added to the first well, and then serial dilutions were made (100, 50, 25, 12.5, and 6.25µg/ml). Then, 100µl of each indicator strain (1.5×10^8 CFU/ml) in Muller Hinton broth was added to each well. After overnight incubation at 37 ºC, the plates were read using a microplate reader at the wavelength of 600 nm, and the highest dilution in which no growths was determined as the MIC (11).

2.9. Assessment of Minimum Bactericidal Concentration (MBC)

For MBC testing, aliquots (20µl) of broth from wells containing no growth were plated onto MHA plates and again incubated overnight at 37 ºC. The MBCs were detected as the lowest concentration of the cell-free supernatant at which bacterial growth was not seen (11).

Statistical analysis was performed using the SPSS statistical program (version 25). Data were presented as mean ± SE, analysis of variance (ANOVA) was used to compare the data, and ($P\leq0.05$) was considered statistically significant.

3. Results

Figure 1 presented data for the microbial analysis of different meat samples.
All 60 meat samples (beef =20, mutton =20, and chicken =20) had microbial contaminations. Seventy percent of mutton samples were contaminated with *S. aureus* and *L. monocytogenes*. The contamination percentage with *E. coli* and *Salmonella* among mutton samples was 20% and 30%, respectively. When chicken samples were analyzed for microbial quality, they were contaminated with different kinds of bacteria, namely, *L. monocytogenes* (20%), *S. aureus* (50%), *E. coli* (20%), and *Salmonella* (40%). Among beef samples, *L. monocytogenes* was detected in 90% of samples, which was followed by *S. aureus* (50%), *E. coli* (50%), and *Salmonella* (10%).

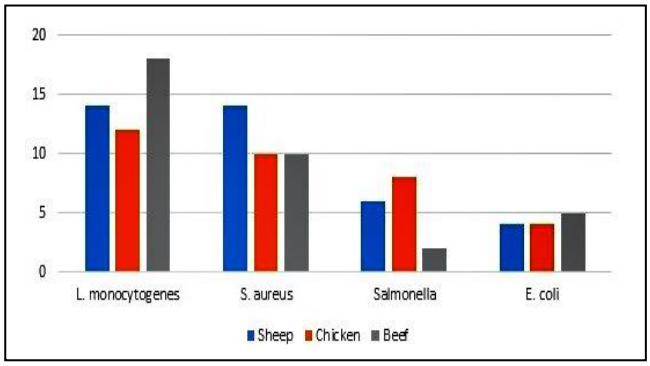

Figure 1. Frequency of food-borne bacteria isolated from beef (n=20), mutton (n=20), and chicken (n=20)

3.1. Antimicrobial Activity by Agar Well Diffusion and Radial Streak Methods

The antimicrobial effect of the supernatant of tested probiotics against *L. monocytogenes* and *S. aureus* isolated from beef, mutton, and chicken samples using well diffusion assay is shown in tables 1, 2, and 3. The diameter of inhibition zones was different among tested probiotics. The widest diameter of inhibition zones was related to *S. salivarius*+raffinose, *S. salivarius*+inulin, and *S. salivarius*+trehalose, respectively. The results of the study on antimicrobial activity of indicated probiotics supernatants against *L. monocytogenes* and *S. aureus* using the radial streak method on different meat samples were the same as well diffusion method (tables 4, 5, and 6). In *Salmonella* and *E. coli*, well diffusion and radial streak methods results indicated that the highest inhibitory effects were for *B. cereus*+inulin, *B. cereus*+raffinose, and *B. cereus*+trehalose, respectively (tables 1-6).

Table 1. Mean±std. Error antimicrobial effects of synbiotic compounds against food-borne pathogens isolated from chicken (well diffusion method)

	Diameter of inhibition zone (mm)								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
L. monocytogenes	1 ± 1.0^{ghi}	3.8 ± 1.7^{i}	4.1 ± 2.0^{i}	2.1 ± 2.1^{ghi}	5 ± 2.3^{i}	8.5 ± 2.8	11.8 ± 4.5^{ad}	10.8 ± 3.2^{ad}	17.1 ± 1.9^{abcde}
S. aureus	5.6 ± 2.3^{ghi}	2 ± 2.3^{ghi}	2.4 ± 1.4^{ghi}	6.2 ± 2.8^{ghi}	11 ± 3.0^{gi}	6.2 ± 1.5^{ghi}	24.8 ± 2.1^{abcdef}	21.4 ± 1.1^{abcdf}	$29.6\pm7.6a^{bcdef}$
Salmonella	3.5 ± 3.5^{def}	4.5 ± 2.5^{df}	2 ± 2.0^{def}	25 ± 5^{abcgi}	22.5 ± 2.9^{abci}	25 ± 2.8^{abcgi}	4.5 ± 2.7^{df}	7.2 ± 2.8	0^{def}
E. coli	5 ± 3.3^{d}	0^{d}	2 ± 2.0^{d}	27.5 ± 2.5^{abcghi}	24 ± 2.9	24 ± 2.4	1.7 ± 1.7^{d}	5.5 ± 3.4^{d}	3.2 ± 1.8^{d}

$P\leq0.05$, 1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose. a= significant differences with group 1, b= significant differences with group 2, c= significant differences with group 3, d= significant differences with group 4, e= significant differences with group 5, f= significant differences with group 6, g= significant differences with group 7, h= significant differences with group 8, i= significant differences with group 9

Table 2. Mean±std. Error antimicrobial effects of synbiotic compounds against food-borne pathogens isolated from beef (well diffusion method)

	Diameter of inhibition zone (mm)								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
L. monocytogenes	0^{ghi}	0^{ghi}	0^{ghi}	11.3 ± 1.0	9.6 ± 0.5	9.7 ± 0.6	14.4 ± 1.7^{abc}	14.4 ± 3.8^{abc}	17.1 ± 3.5^{abc}
S. aureus	0^{ghi}	0^{ghi}	0^{ghi}	6.2 ± 2.5^{i}	5.8 ± 2.3^{i}	8 ± 2.3^{i}	17.2 ± 5.6^{abc}	15.8 ± 2.3^{abc}	22.6 ± 1.6^{abcdef}
Salmonella	6.5 ± 0.5^{cdef}	8.5 ± 0.5^{cdf}	$0^{abdefgi}$	33.5 ± 1.5^{abcefgi}	20 ± 1.0^{abcdfgi}	26.5 ± 0.5^{abcdegi}	6.5 ± 0.5^{cdef}	8 ± 1^{def}	6.5 ± 0.5^{cdef}
E. coli	7.6 ± 3.4^{def}	8.8 ± 3.2^{def}	9.2 ± 3.1^{def}	22.4 ± 3.4^{abcghi}	22.4 ± 2.4^{abcghi}	22.4 ± 3.1^{abcghi}	1 ± 1.0^{def}	0^{def}	0^{def}

$P\leq0.05$, 1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose. a= significant differences with group 1, b= significant differences with group 2, c= significant differences with group 3, d= significant differences with group 4, e= significant differences with group 5, f= significant differences with group 6, g= significant differences with group 7, h= significant differences with group 8, i= significant differences with group 9

Table 3. Mean±std. Error antimicrobial effects of synbiotic compounds against food-borne pathogens isolated from mutton (well diffusion method)

	Diameter of inhibition zone (mm)								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
L. monocytogenes	4 ± 1.6^{gi}	5.8 ± 1.6^{i}	4.2 ± 1.1^{gi}	4.8 ± 1.8^{gi}	10 ± 1.9	9.8 ± 1.9	21.5 ± 2.4^{acd}	11.8 ± 2.8	25.5 ± 1.2^{acd}
S. aureus	3.5 ± 1.7^{ghi}	1.5 ± 1.0^{ghi}	1.5 ± 1.0^{ghi}	6 ± 1.6^{i}	5.2 ± 2.3^{i}	10.4 ± 2.5	21.2 ± 2.3^{abc}	20.7 ± 1.4^{abc}	22.4 ± 0.9^{abcde}
Salmonella	5.6 ± 2.8^{def}	2 ± 2.0^{def}	2 ± 2.0^{def}	34 ± 2.6^{abcghi}	29 ± 5.8^{abchi}	30 ± 4.7^{abchi}	10.6 ± 1.2^{d}	3 ± 3.0^{def}	1.6 ± 1.6^{Def}
E. coli	0^{def}	0^{def}	0^{def}	30 ± 0^{abcghi}	20 ± 0^{abcghi}	28 ± 2^{abcghi}	0^{def}	0^{def}	0^{def}

$P\leq0.05$ 1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose. a= significant differences with group 1, b= significant differences with group 2, c= significant differences with group 3, d= significant differences with group 4, e= significant differences with group 5, f= significant differences with group 6, g= significant differences with group 7, h= significant differences with group 8, i= significant differences with group 9

Table 4. Mean±std. Error antimicrobial effects of synbiotic compounds against food-borne pathogens isolated from chicken (Radial streak method)

	Diameter of inhibition zone (mm)								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
L. monocytogenes	5 ± 5.0^{ci}	0^{cghi}	30 ± 0^{abef}	11 ± 6.0^{cgi}	5 ± 5.0^{cgi}	5 ± 5.0^{cgi}	25 ± 5.0^{abdefi}	9.5 ± 3.6^{bi}	38 ± 8.0^{abdefg}
S. aureus	7.4 ± 1.6^{ghi}	0.6 ± 0.6^{ghi}	6.4 ± 2.2^{ghi}	0^{ghi}	1.2 ± 0.4^{ghi}	1.2 ± 0.4^{ghi}	34 ± 2.4^{abcef}	29.2 ± 0.8^{abcef}	39 ± 1.0^{abcef}
Salmonella	7.5 ± 7.5^{def}	2.5 ± 0.5^{def}	7.5 ± 7.5^{def}	40 ± 0^{abchi}	32.5 ± 4.7^{abchi}	37.5 ± 2.5^{abchi}	7.5 ± 7.5^{def}	2.7 ± 0.7^{def}	0^{def}
E. coli	22.5 ± 7.5^{bgh}	0^{ad}	30 ± 0^{bghi}	27.5 ± 2.5^{bghi}	20 ± 7.0	22.5 ± 02.5	0^{acd}	5 ± 0.5^{acd}	2 ± 2.0^{cd}

$P\leq0.05$, 1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose. a= significant differences with group 1, b= significant differences with group 2, c= significant differences with group 3, d= significant differences with group 4, e= significant differences with group 5, f= significant differences with group 6, g= significant differences with group 7, h= significant differences with group 8, i= significant differences with group 9

Table 5. Mean±std. Error antimicrobial effects of synbiotic compounds against food-borne Pathogens isolated from beef (Radial streak method)

	Diameter of inhibition zone (mm)								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
L. monocytogenes	0^{ghi}	0^{ghi}	0^{ghi}	0.3 ± 0.3^{i}	3.3 ± 3.3	3.3 ± 3.3	13.3 ± 5.2^{abc}	13.3 ± 5.1^{abc}	15.5 ± 5.0^{abcd}
S. aureus	0^{ghi}	0^{ghi}	0^{ghi}	1.6 ± 0.6^{ghi}	4 ± 4.0^{gi}	1 ± 1.0^{ghi}	28 ± 2.0^{abcdefi}	18 ± 7.3^{abcdefi}	34 ± 2.4^{abcdefhi}
Salmonella	4.5 ± 0.5^{bcdef}	11.5 ± 1.5^{acdefh}	$0^{bdefghi}$	37 ± 1.0^{bcfghi}	22.5 ± 0.5^{abcdghi}	27 ± 1.0^{abcdghi}	7.5 ± 1.5^{cdef}	5.5 ± 0.7^{bcdef}	7 ± 1.0^{cdef}
E. coli	6 ± 6^{def}	6 ± 6^{def}	0.8 ± 0.4^{def}	36 ± 2.4^{abcghi}	28 ± 2.0^{abcghi}	30 ± 0^{abcghi}	0.4 ± 0.4^{def}	0.8 ± 0.4^{def}	0.4 ± 0.4^{def}

$P\leq0.05$, 1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose. a= significant differences with group 1, b= significant differences with group 2, c= significant differences with group 3, d= significant differences with group 4, e= significant differences with group 5, f= significant differences with group 6, g= significant differences with group 7, h= significant differences with group 8, i= significant differences with group 9

Table 6. Mean±std. Error antimicrobial effects of synbiotic compounds against food-borne pathogens isolated from mutton (Radial streak method)

	Diameter of inhibition zone (mm)								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
L. monocytogenes	21.4 ± 5.5^{cfg}	12.8 ± 6.0^{i}	0^{aghi}	13.5 ± 15.8	8.5 ± 5.5^{ghi}	3 ± 2.0^{aghi}	28.5 ± 1.4^{cefg}	24.2 ± 4.2^{cfg}	31.7 ± 5.2^{bcdefg}
S. aureus	21.7 ± 5.3	30 ± 0^{e}	17.4 ± 5.9^{i}	17.1 ± 6.0^{i}	8.5 ± 5.5^{gi}	21.4 ± 5.5	29.8 ± 0.7^{abce}	15.2 ± 5.4^{abch}	34.2 ± 1.7^{abcdeg}
Salmonella	20 ± 10^{d}	10 ± 10^{def}	30 ± 0^{hi}	46 ± 1.6^{abghi}	33.3 ± 3.3^{bhi}	36.6 ± 3.3^{bhi}	20 ± 10^{d}	0^{cdef}	3.6 ± 3.1^{cdef}
E. coli	0^{bcdefg}	30 ± 0^{aehi}	30 ± 0^{aehi}	32.5 ± 2.5^{aehi}	17.5 ± 2.5^{abcdfghi}	30 ± 0^{aehi}	30 ± 0^{aehi}	0^{bcdef}	0^{bcdef}

$P\leq0.05$, 1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S.alivarius*+trehalose, 9= *S. salivarius*+raffinose. a= significant differences with group 1, b= significant differences with group 2, c= significant differences with group 3, d= significant differences with group 4, e= significant differences with group 5, f= significant differences with group 6, g= significant differences with group 7, h= significant differences with group 8, i= significant differences with group 9

3.2. Determination of MIC and MBC

Tables 7-9 show MICs and MBCs of different synbiotic formulations against bacterial strains isolated from different meat samples. In *L. monocytogenes* and *S. aureus* of sheep origin, the highest inhibitory efficacies were for *S. salivarius*+raffinose (12.5), *S. salivarius*+inulin (12.5), and *S. salivarius*+trehalose (25), respectively. In sheep originating *Salmonella* isolates, the highest inhibitory effects were for *B. cereus*+inulin (12.5), *B. cereus*+raffinose (12.5), and *B. cereus*+trehalose (25), and in *E. coli* of the same origin were for *B. cereus*+inulin (6.25), *B. cereus*+raffinose (12.5), and *B. cereus*+trehalose (25). Moreover, the highest inhibitory efficacies of studied formulations against *L. monocytogenes* and *S. aureus* with the chicken origin, were related to *S. salivarius*+raffinose (12.5), *S. salivarius*+inulin (12.5), and *S. salivarius*+trehalose (25) and in chicken originated *Salmonella* were for *B. cereus*+inulin (12.5), *B. cereus*+raffinose (25), and *B. cereus*+trehalose (25). Finally, in *E. coli* of chicken origin, were for *B. cereus*+inulin (6.25), *B. cereus*+raffinose (6.25), and *B. cereus*+trehalose (12.5). About *L. monocytogenes* and *S. aureus* with cow origin, the highest inhibitory effects were belonging to *S. salivarius*+raffinose (12.5), *S. salivarius*+Inulin (12.5), and *S. salivarius*+trehalose (25) and in cow originated *Salmonella*, were for *B. cereus*+inulin (6.25), *B. cereus*+raffinose (12.25), and *B. cereus*+trehalose (25). In *E. coli* of the same origin, the highest inhibitory properties were for *B. cereus*+inulin (12.5), *B. cereus*+raffinose (12.25), and *B. cereus*+trehalose (25).

Table 7. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of synbiotic formulations against food-borne pathogens isolated from mutton

Bacteria	1		2		3		4		5		6		7		8		9	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
L. monocytogenes	50	-	25	50	25	-	100	100	25	-	25	25	12.5	12.5	25	25	12.5	12.5
S. aureus	25	-	100	100	100	100	100	-	100	-	25	100	12.5	12.5	25	25	12.5	12.5
Salmonella	50	-	100	100	50	50	12.5	25	25	25	12.5	25	50	-	50	-	50	-
E. coli	50	-	50	50	50	-	6.25	6.25	25	25	12.5	25	50	-	50	-	-	-

1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose

Table 8. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of synbiotic formulations against food-borne pathogens isolated from chicken

Bacteria	1		2		3		4		5		6		7		8		9	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
L. monocytogenes	50	-	50	100	50	100	100	100	50	100	50	50	12.5	25	25	25	12.5	25
S. aureus	25	-	50	100	25	50	100	100	100	100	100	-	12.5	25	25	25	12.5	25
Salmonella	50	-	50	-	-	-	12.5	25	25	25	25	25	100	100	100	100	-	-
E. coli	50	-	50	-	50	-	6.25	125	12.5	25	6.25	12.5	50	100	100	-	100	100

1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose

Table 9. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of synbiotic formulations against food-borne pathogens isolated from beef

Bacteria	1		2		3		4		5		6		7		8		9	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
L. monocytogenes	-	-	-	-	-	-	100	100	50	100	100	-	12.5	25	25	25	12.5	12.5
S. aureus	-	-	-	-	-	-	50	-	50	50	100	-	12.5	25	25	25	12.5	12.5
Salmonella	50	-	50	100	-	-	6.25	6.25	25	25	12.5	25	100	-	100	-	100	100
E. coli	50	-	50	-	50	-	12.5	125	25	25	12.5	12.5	50	50	-	-	-	-

1= *L. acidophilus*+inulin, 2= *L. acidophilus*+trehalose, 3= *L. acidophilus*+raffinose, 4= *B. cereus*+inulin, 5= *B. cereus*+trehalose, 6= *B. cereus*+raffinose, 7= *S. salivarius*+inulin, 8= *S. salivarius*+trehalose, 9= *S. salivarius*+raffinose.

The MBC values obtained for extracts against *L. monocytogenes* and *S. aureus* of sheep origin resulted in the highest bactericidal efficacies for *S. salivarius*+raffinose (12.5), *S. salivarius*+inulin (12.5), and *S. salivarius*+trehalose (25). In sheep originated *Salmonella* were for *B. cereus*+inulin (25), *B. cereus*+raffinose (25), and *B. cereus*+trehalose (25) and in *E. coli* of the same origin were related to *B. cereus*+inulin (6.25), *B. cereus*+raffinose (25), and *B. cereus*+trehalose (25). Among chicken *L. monocytogenes* and *S. aureus* isolates, the highest bactericidal activity was observed in *S. salivarius*+raffinose (25), *S. salivarius*+inulin (25), and *S. salivarius*+trehalose (25), in chicken *Salmonella* strains, were for *B. cereus*+inulin (25), *B. cereus*+raffinose (25), and *B. cereus*+trehalose (25), and in chicken *E. coli* isolates were for *B. cereus*+Inulin (12.5), *B. cereus*+raffinose (12.5), and *B. cereus*+trehalose (25). Among *L. monocytogenes* and *S. aureus* of cow origin, the highest bactericidal properties were related to *S. salivarius*+raffinose (12.5), *S. salivarius*+inulin (25), and *S. salivarius*+trehalose (25), in cow originate *Salmonella* were for *B. cereus*+inulin (6.25), *B. cereus*+raffinose (25), and *B. cereus*+trehalose (25). Finally, in cow originated *E. coli* were for *B. cereus*+inulin (12.5), *B. cereus*+raffinose (12.5), and *B. cereus*+trehalose (25).

4. Discussion

Enteric infections are the fifth leading cause of death worldwide. About 70% of these infections are food-borne. Antibiotics are usually the choice drug for preventing and treating such infections, and contaminated raw meat is an essential source of food-borne diseases. Additionally, the extent of meat contamination is highly related to the standard of hygiene (1, 2). Nowadays, the application of probiotics, prebiotics, and synbiotics has been increased as an alternative strategy in fighting against food-borne pathogens (13). The present study aimed to access the antibacterial properties of synbiotic compounds containing *L. acidophilus*, *B. cereus*, and *S. salivarius* as probiotics and inulin, trehalose, and raffinose as prebiotics against pathogenic bacteria (e.g., *Salmonella*, *E. coli*, *L. monocytogenes*, and *S. aureus*) isolated from beef, mutton, and chicken meat. IrohaI., UgboE. (2) investigated the bacterial contamination of raw meat, including beef, chicken, and chevron. Among isolated bacteria, the most frequent isolates were *E. coli*, *K. pneumoniae*, *S. typhi*, *S. dysenteriae*, *P. aeruginosa*, and *S. aureus*, respectively (2). In another study by Acharya, Poudel (1), pathogenic bacteria were isolated from meat processing units. The isolated pathogenic microorganisms were *Vibrio spp*, *Salmonella spp*, *Shigella spp*, *Proteus spp*, and *Staphylococcus*. As in our study, the highest prevalence was found in *L. monocytogenes* and *S. aureus*, respectively. A similar result was reported by Acharya, Poudel (1), (2). However, *Salmonella* and *E. coli* had the lowest occurrence in our studied samples, which is different from Acharya and Iroha's findings. *Lactobacillus* is one of the critical groups of probiotic microorganisms. *L. acidophilus*, *L. casei*, *L. reuteri*, *L. rhamnosus*, and *L. plantarum* are commonly used probiotics in functional

foods (14). Tharmaraj and Shah (3) studied the antimicrobial properties of some probiotic formulations against selected pathogenic and spoilage bacteria in cheese-based dips. The highest antimicrobial effects were for *L. rhamnosus, L. acidophilus, L. casei,* and *L. paracasei,* respectively. The most potent impact of all probiotics was against *B. cereus,* and the weakest was against *E. coli.* Our isolates showed nearly similar antagonistic activity against *B. cereus* and *E. coli* (3). In 2013, the antibacterial activity of *L. acidophilus* strains was characterized against *E. coli* and *S. aureus*. The metabolites of *L. acidophilus* showed antimicrobial properties against two tested pathogens (15). The results of all these studies are in accordance with our findings on mutton and chicken isolates. Although in our research, synbiotic compounds containing *L. acidophilus* exhibited antimicrobial activity against studied bacteria of mutton and chicken origins, they have no antibacterial effects on *L. monocytogenes* and *S. aureus* of cow origin. However, their inhibitory properties were weak compared to other formulations.

Bacillus spp have been used for food production and preservation for many years. Their ability to produce secretory proteins, enzymes, antimicrobial compounds, and carotenoids, tolerate a hostile environment of the gastrointestinal tract, and stability during food processing and storage make them suitable candidates for health elevating formulations (16). Jadamus, Vahjen (17) evaluated the growth behavior of *B. cereus* var. toyoi in the gastrointestinal tract of chickens and piglets. This bacteria germinated rapidly in both animal species, which is essential for its probiotic effects. Here we also used *B. cereus* var. toyoi against pathogenic bacteria isolated from different meat types. In addition, in the study of Altmeyer, Kroger (18), *B. cereus* var. toyoi, was investigated for its positive effect on pigs. The present assessment reveals that synbiotics formulations containing *B. cereus* showed the highest antagonistic activity against *E. coli* and *Salmonella* strains of beef, mutton, and chicken origin.

S. salivarius is an oral streptococcal species with no disease relationships in healthy humans (19). According to a study by Wescombe, Upton (20), although *S. salivarius* TOVE-R could decrease dental caries in animal models, it showed weak bacteriocin activity *in vitro*. In 2013, Burton, Drummond (19) investigated the effects of probiotic *S. salivarius* strain M18 on indices of dental health. The M18-treated children exhibited reduced cariogenic bacteria indicating the antibacterial properties of the M18 probiotic. In addition, *S. salivarius* K12 can prevent different upper respiratory tract infections, including streptococcal sore throat, otitis media, and halitosis (4, 21-23). Furthermore, Fantinato, Camargo (24) studied *S. salivarius* strains for potential application as a probiotic for producing bacteriocin against *S. pyogenes*. The bacteriocin test showed that 133 strains could prevent *S. pyogenes* (24). In this study, synbiotic compounds containing *S. salivarius* were the most effective mixtures against *L. monocytogenes* and *S. aureus* with beef, mutton, and chicken origin. In conclusion, synbiotic formulations exhibit promise as alternatives for antibiotics as pressure to omit growth-promoting antibiotic use increases.

Authors' Contribution

The idea of the project: S. N. G. and P. H.
Interpretation of data: S. N. G., P. H. and S. M.
Preparing the manuscript: S. N. G. and P. H.
Critical revision of the manuscript for the development of the protocol and abstracting the data: S. N. G. and P. H.

Ethics

All procedures were approved by the ethics committees of the University of Tabriz, Tabriz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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