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Inhibitory Effects of *Bifidobacterium infantis* 15697 Filtrates on the Growth of *Salmonella* Choleraesuis and *Escherichia coli*

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Abstract

A study was conducted to investigate the mode of action of *Bifidobacterium longum* subsp. *infantis* ATCC 15697 culture (*B. infantis*) filtrates for inhibitory capabilities against *Salmonella* Choleraesuis and *Escherichia coli*. Filtrates were also tested for bacteriocins by examining acidic or proteinaceous properties of the inhibitory component. Additionally, *B. infantis* culture filtrates were used to treat raw turkey thigh meat to examine inhibition of pathogens in a food medium. When *B. infantis* filtrates were compared to a blend of acetic and lactic acids (at the same level produced by the bacteria, 3:2 ratio), the acid blend achieved a 40% lower inhibition rate against *S.* Choleraesuis and *E. coli* than the filtrate. Growing *B. infantis* on de Man-Rogosa-Sharpe agar (MRS) plus cysteine produced a filtrate with twice as much inhibitory action (measured as zones of inhibition) as filtrates prepared from cultures grown on Tripicase Peptone Yeast Extract (TPY) media. Bacterial inhibition was observed for filtrates applied to turkey thigh meat; however, this inhibition was the same as that of the acid blend. Results demonstrate that while the primary mode of action for *B. infantis* against other bacteria is acetic and lactic acids, another synergistic mechanism (possibly a bacteriocin) is involved.

Keywords

Bifidobacterium, *B. infantis*, Probiotics, Bacteriocins

1. Introduction

Probiotics are becoming more popular as consumers see them as an easy way to improve their health and well-being [1]. The growing consumer demand for probiotics is reflected in the global market, which reached nearly $100 billion USD in

2024, and is estimated to exceed $374 billion by 2034 [2]. Major health benefits suggested to be associated with probiotics include, but are not limited to antimicrobial, antiviral, anticarcinogenic, and effects on obesity and metabolism, cardiovascular health, coronary disease, and the immune system [1]. According to the National Institute of Health (NIH), probiotics should be defined by their genus, species and strain designation. *Bifidobacterium* and *Lactobacillus* species are the most common microorganisms used in probiotics [3] [4]; however, specific strains from *Saccharomyces* (yeast), *Streptococcus*, *Enterococcus*, *Escherichia* and *Bacillus* genera are also found in some probiotics [3] [4]. *Bifidobacterium* strains have been recognized for playing a critical role in establishing and maintaining healthy gastrointestinal microbiota [5] [6]. High levels of *Bifidobacterium* have been found in fecal material collected from breastfed infants, and although the presence of Bifidobacterium decreases in the gastrointestinal tract during adulthood, high levels during infancy have been linked to overall health later in life [6].

Bifidobacteria species produce acetic and lactic acids from the fermentation of sugars [7]. These acids are produced in a 3:2 ratio of acetic (and acetic acid) and lactic (and lactic acid) from every 2 mol of glucose [8], which lowers the pH and results in an antimicrobial effect [7]. Bifidobacteria have been of interest due to this antimicrobial effect, but researchers have been unable to confirm the mode of action. According to Khalighi and coworkers [9], the mode of action by which probiotics accomplish their beneficial effects may arise from competition for adhesion sites in the gastrointestinal tract, production of antimicrobials (acids or bacteriocins), host immune system stimulation and/or competition with pathogens for nutrients.

Bifidobacterium infantis naturally inhabits the mouth and digestive tract, and it is specifically known for its ability to treat irritable bowel syndrome and gastrointestinal distress [10]. Meghrous *et al.* reported that *Bifidobacterium infantis* (ATCC 15697) could produce an inhibitory substance, which was most likely a bacteriocin [11]. These researchers cultured *Bifidobacterium infantis* in Tripticase Peptone Yeast medium (TPY) and the culture supernatant was shown to be pH stable, heat stable, and destroyed by proteolytic enzymes [10]. Meghrous *et al.* also showed that *B. infantis* 15697 inhibited growth of other gram-positive species, such as *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus acidophilus* [10] [11]. Other species of *Bifidobacterium* were found to inhibit *Clostridium* spp. and *Lactococcus* spp. Ibrahim and Bezkorovainy concluded that the inhibitory substances produced by *B. infantis* (ATCC 15697) were not bacteriocins but were only the acetic and lactic acids that are produced during the growth of the organisms [8]. A wild-type human isolate of *E. coli* was unable to survive when cultured with *B. infantis* at a pH of 5.0 and below. After the pH of the spent TPY broth was altered to a pH of 7.0, *E. coli* was able to reestablish growth, whereas before pH alteration, there was little survival of *E. coli*. Upon analysis of the inhibitory capabilities of a 3:2 ratio of acetic acid and lactic acid, it was found that this combination of acids inhibited *E. coli* to approximately the same extent as the spent TPY broth from *B.*

infantis [8]. Makras and De Vuyst examined the ability of several strains of *Bifidobacterium* to inhibit *Salmonella typhimurium* SL 1344 and *E. coli* C1845 and reported that the contribution of bacteriocins for inhibiting *Salmonellae* and *E. coli* was negligible [12]. Inturri *et al.* found that *Bifidobacterium longum* BB536 inhibited the adhesion of *E. coli* EC3960, *E. coli* EC4219, *Salmonella enteritidis* SEN6 and *Salmonella typhi* STN12 to the human intestinal cell line HT-29 and these researchers suggested the inhibition was due to a protein (bacteriocin) or non-protein substance (acetic acid) [13].

The current research objective was to determine if the inhibitory substance produced by culture supernatants of *B. infantis* 15697 was proteinaceous or acidic in nature. Heat stability and pH testing were performed, along with a variety of tests to determine the acidic or proteinaceous nature of the inhibition. *Bifidobacterium infantis* culture filtrates were then added to turkey thigh meat to determine if this antimicrobial activity could be beneficial for bacterial inhibition in food products.

2. Materials and Methods

2.1. Microorganisms

Bifidobacterium longum infantis (ATCC 15697) was obtained in lyophilized form from the American Type Culture Collection (ATCC, Rockville, MD), grown in Lactobacilli Man, Rogosa and Sharpe (MRS) broth (Difco Laboratories, Detroit, MI) + 0.05% cysteine (MRSc) and in Tripticase-peptone-yeast extract medium (TBY). This organism was grown in test tubes under anaerobic conditions, with 0.4 LPM CO_2 present, at 37˚C, and propagated twice for 24 hours. Freezer stocks were made from these cultures using MRSc or TPY medium with 20% glycerol and stored at −80˚C.

The following four bacterial strains were used as indicators. *Salmonella* Choleraesuis serotype *typhi* (ATCC 12179), obtained in lyophilized form from the American Type Culture Collection, was grown in Tripticase Soy Broth (TSB) (Difco Laboratories) and propagated twice for 24 hours at 37˚C. *Escherichia coli* (ATCC 25922) was also grown in TSB and propagated for 24 hours at 37˚C. Both cultures were maintained as freezer stocks at −80˚C in TSB plus 20% glycerol. *Lactococcus lactis* subsp. *lactis* (ATCC 11454) and *Streptococcus salivarius* subsp. *thermophilus* (Clemson University, Food Microbiology Culture Collection) were grown in M-17 medium (Difco Laboratories). *S. salivarius* was grown at 40˚C and propagated twice for 24 hours. *L. lactis* was grown at 32˚C and propagated twice for 24 hours. Both of these were maintained as freezer stocks in M-17 medium with 20% glycerol.

2.2. Culture Filtrate Preparation

B. infantis cultures were inoculated (10 mL/L) into sterile MRSc broth or TPY broth and allowed to grow with CO_2 (0.4 LPM) at 37˚C for 48 to 72 hours. Culture supernatants were prepared by centrifuging at 10,000 × g, 4˚C, for 30 minutes.

The supernatant was then passed through a Supor-450, 47 mm, 0.45 μm membrane filter (Gelman Sciences, Ann Arbor, MI). The activity of the culture filtrate was measured by the agar well assay technique [14]. The culture filtrates were analyzed for total acidity by titration. This filtrate was titrated using 10 N NaOH and 1% phenolphthalein and titrating to the first recognizable pink color.

2.3. Agar Well Assay Technique

Pour plates were made using 1 mL of a 1:100 dilution (10^6 to 10^7 cells/mL) of a 24-hour culture of *E. coli*, *S.* Choleraesuis, *S. thermophilus*, and *L. lactis*, with Tripticase Soy Agar used to grow *S.* Choleraesuis and *E. coli*, and M17 agar used for growth of other organisms. A 6.0 mm diameter well was bored into each plate using a sterile hollow borer, and 40 μl of the *B. infantis* filtrate was transferred to these wells using sterile pipettes. Plates were immediately incubated aerobically for 24 hours at 37˚C, 32˚C, and 40˚C for *E. coli* and *S.* Choleraesuis, *L. lactis* and *S. thermophilus*, respectively. Zones of inhibition were measured using calipers.

2.4. Effect of pH on Filtrate Inhibitory Activity

The pH of the *B. infantis* filtrate obtained from growth in MRSc broth and TPY broth was adjusted using 10 N NaOH or 1 N HCl. The unadjusted filtrate had a pH of 4.2 and served as the control. The filtrate was divided into twenty-two 5 mL samples and placed into sterile beakers, where the pH was then adjusted, ranging from 1 to 8 at every 0.5 pH interval and monitored using a pH meter (Orion, Model 420A). All samples, including the unaltered control, were tested for inhibitory activity against *Salmonella* Choleraesuis and *Escherichia coli* using the agar well assay technique.

2.5. Acetic and Lactic Acid Inhibition

Acetic acid (0.05 M) and lactic acid (0.05 M) were combined in a 3:2 ratio (pH 4.2) and tested for inhibitory activity against *S.* Choleraesuis, *L. lactis*, *S. thermophilus*, and *E. coli* using the agar well assay technique. The diameters of inhibition were measured and compared to the zones of inhibition produced when using *B. infantis* filtrate.

2.6. Stability of Filtrate Inhibitory Activity

The pH of *B. infantis* MRSc filtrate was adjusted to 7.0 using 10 N NaOH, then, by using 1 N HCl, the pH was readjusted to the initial pH of 4.2. The pH-adjusted filtrate (at pH 4.2) was then tested for inhibitory activity against *S.* Choleraesuis and *E. coli* by using the agar well assay technique.

2.7. Heat Stability

Sterile 10 × 130 mm test tubes with 5 mL of MRSc culture filtrate were held in a water bath held at 50˚C, 70˚C, or 100˚C for 0 (control), 1, 2, 4, 8, 12, 24, 48, and 96 minutes. The filtrate from TPY broth was held in a water bath held at 100˚C

for 96 minutes. Time of exposure began as soon as the tubes were placed into the water bath. Test tubes were cooled immediately after heat exposure in ice water and assayed for activity against *E. coli*. The control, which had no heat exposure, was also tested for antimicrobial activity and compared to these heat-treated filtrates. Plates were incubated at 37˚C for 24 hours after which zones of inhibition were measured.

2.8. Treatment with Pepsin and Papain

Proteolytic enzymes, pepsin and papain, were obtained from Sigma Chemical Company (St. Louis, MO). Positive controls were performed by procedures outlined in The Worthington Manual (Worthington Enzyme Manual 1903. This was done using hydrolysis of benzoyl-L-arginine ethyl ester (BAEE) for papain and hydrolysis of bovine hemoglobin for pepsin. Culture filtrates (100 μL) were added to citrate-phosphate buffer (pH 5.2) with papain in the following ratios: 0 mg/mL (control), 1 mg/mL, 2 mg/mL and 5 mg/mL. Final concentrations were: 0 mg/mL, 0.91 mg/mL, 1.8 mg/mL, and 5.4 mg/mL. Culture filtrates (100 μL) were also added to glycine-HCl buffer (pH 2.3) with pepsin in the same ratios as those used for papain. The pepsin and papain cultures were allowed to incubate overnight at 37˚C. Activity was then tested using the agar well assay technique.

2.9. Dialysis of Culture Filtrate

Culture filtrates of *B. infantis* propagated from MRSc medium and TPY medium were dialyzed using 500 MWCO (molecular weight cut-off) tubing. Five mL of filtrate were placed into the tubing and were dialyzed against 500 mL citrate-phosphate buffer (pH 4.3 - 4.5) with 3 changes of buffer. The filtrate was dialyzed at 4˚C for 48 hours.

2.10. Ammonium Sulfate Precipitation

Solid ammonium sulfate was added to the filtered supernatant from MRSc and TPY to a final concentration of 85% saturation. This solution was held at 4˚C overnight and then centrifuged at 10,000 × g for 30 minutes to collect the precipitated proteins. Pellet and supernatant were analyzed for inhibitory activity. The precipitate was redissolved using distilled water or piperazine (pH 5.8, 0.025M).

2.11. Beta-Glycerol Phosphate Treatment

Beta-glycerol phosphate is a buffer that binds to acids. One mL of beta-glycerol phosphate (1 gm/5mL distilled water, pH 9.1) was added to 10 mL of Tripticase Soy Agar. Agar well assay techniques were then utilized to determine inhibition zones against *S.* Choleraesuis and *E. coli*.

2.12. *B. infantis* Filtrate Tested for Bacterial Inhibition in a Food Product

Bifidobacterium infantis MRSc filtrate was examined for potential to inhibit micro-

bial growth in turkey thigh meat. Turkey thigh meat was purchased from a local retail store and separated into thirds using a sterile knife. Two separate pretreatments were applied using each half of the turkey meat. For one half, no pre-sterilization procedures were utilized and were referred to as the "no pretreatment" group. The second half was washed with a 50% ethanol solution. No pretreatment group and ethanol rinsed separated into nine, 100 g samples, which were diced into small pieces and the following treatments were performed for each experimental trial. One third of the meat was not inoculated, one third was inoculated with 10^8 cfu/g *S.* Choleraesuis and one third was inoculated with 10^8 cfu/g *E. coli*, 10^8 cfu/g. Each of these groups was exposed to three treatments: control (no treatment), 1 mL addition of filtrate, or 1 mL addition of acetic acid and lactic acid (3:2 ratio, 0.05 M). The 10 g samples were subjected to treatments while in a sterile weighing pan. Treatments were performed by completely covering the meat surfaces with treatment solutions, which were applied with sterile pipettes. The diced, treated samples were placed into Ziploc bags and incubated at 4˚C.

After 1, 3, 5 and 7 days of storage, 1 g from each sample was removed and added to 9 mL of sterile peptone water (0.1%). These samples were stomached (Seward 400) at 250 RPM for 2 min. Serial dilutions were made for each sample and added to Tripticase Soy Agar (TSA). These plates were incubated for 24 hours at 37˚C and colonies were enumerated.

2.13. Statistical Analyses

T-tests were used to determine differences in acetic acid and lactic acid in comparison to filtrate inhibition, comparison of MRSc to TPY medium for production of inhibitory substance(s), comparison of enzyme-treated filtrate to non-treated filtrate, and comparison of ammonium sulfate precipitate to non-precipitated filtrate. A General Linear Model (GLM) using an ANOVA was used to determine treatment effects ($p < 0.05$) in the pH stability, heat stability (50˚C and 70˚C), and the three turkey meat application experiments. When treatment effects were significant, means for the GLM experiments were separated using the p diff command of SAS at the $p < 0.05$ level [15]. No statistical analyses were conducted on beta-glycerol phosphate treatment since no zones of inhibition appeared for any treatment comparison.

3. Results and Discussion

3.1. Production of Antimicrobial Activity by *B. infantis* Culture Filtrates

Both *E. coli* and *S.* Choleraesuis were inhibited by *B. infantis* culture filtrates produced from TPY and MRSc medium, but *S. salivarius* subsp. *thermophilus* and *L. lactis* subsp. *lactis* were not inhibited by the MRSc or TPY-produced filtrates (**Table 1**). When *B. infantis* culture filtrates collected from MRSc and TPY media were compared, the filtrate produced on MRSc had larger inhibition zones against *S.* Choleraesuis and *E. coli* that were approximately twice the diameter produced by

the filtrate from the TPY medium. Scardovi found that TPY was the medium type that promoted favorable growth conditions for all bifidobacteria [16]. Additionally, MRSc media have been used to cultivate bifidobacteria species in several other studies [15] [17]. *B. infantis* likely produced higher levels of inhibitory substances on MRSc medium because this medium contains more sugar (20%) than TPY (5%), and that could have resulted in a greater production of acid during bacterial growth. The titration of these two media types also supported this theory. The total acidity of the MRSc filtrate was 1.27%, while that of the TPY-filtrate was 0.68%. The total acidity produced by *B. infantis* when propagated in MRSc medium was nearly twice as great as that produced from the TPY-filtrate. T-test verified that this difference was significant (t_{crit} = 4.303, t = 7.39).

Table 1. Inhibition of selected bacteria by *Bifidobacterium infantis* culture filtrate when propagated in De Man, Rogosa, and Sharpe cysteine (MRSc).

Indicator Strains[1]	Assay Medium	Zones of Inhibition (mm)	
		TPY Medium	MRSc Medium
E. coli	TSB	9.9[b]	19.5[a]
S. Choleraesuis serotype *typhi*	TSB	9.6[b]	23.5[a]
L. lactis subsp. *lactis*	M-17	0	0
S. salivarius subsp. *thermophiles*	M-17	0	0

[1]*Escherichia coli* (*E. coli*), *Salmonella* Choleraesuis (*S.* Choleraesuis serotype typhi), Lactobacillus lactis (*L. lactis* subsp. lactis), and *Streptococcus salivarius* (*S. salivarius* subsp. thermophiles) were evaluated. [a,b]means within rows with different superscripts are significantly different at the 0.05 alpha level (t_{crit} =2.571; t = 28.37, *S.* Choleraesuis; t = 19.56, *E. coli*; df = 4).

3.2. pH Effects on Filtrate Inhibitory Activity

Filtrate from growth in MRSc showed inhibitory zones against *E. coli* and *S.* Choleraesuis from pH 1 to 5.5 (**Figure 1**). Activity gradually decreased as the pH increased. The zones of inhibition at pH 5.5 were approximately half that of pH 1.0 filtrate, and there was no activity present at a pH of 6.0. Filtrates from TPY cultures also showed a gradual decline with increasing pH values and no activity was detected at a pH of 4.8 (**Figure 1**). A significant negative correlation (r = −0.8670) was found between the size of the inhibition zone and pH. *Bifidobacterium* spp. produced 3 moles of acetic acid and 2 moles of lactic acid per 2 moles of glucose, so a 3:2 ratio of acetic and lactic acids was chosen to evaluate the acidic-based inhibition hypothesis [18]. Growth of *Salmonella* Choleraesuis serotype *typhi* and *E. coli* was inhibited by acetic and lactic acid at the 3:2 ratio, but this inhibition was about 60% as effective as the inhibition with the filtrate, that is, acid mean zone area was 60% as large as filtrate area (**Table 2**). Neither the filtrate nor the acetic/lactic acid combination was able to inhibit *S. thermophilus* and *L. lactis*, as these bacteria were able to grow very efficiently at low pH levels. *Salmonella* has a pH growth optimum between 6.2 and 8.2, and with acetic acid present, does not

grow below a pH of 5.4 [19]. This supports the results found in the present study, with *Salmonella* inhibition only taking place when the pH of the filtrate was 5.5 or less. *E. coli* does not survive at a pH of less than 5.5 [8]. This also supports the results found in the present study, showing the filtrate does not inhibit *E. coli* at a pH above 5.5. When the pH of the filtrate was altered to 7.0 and then lowered back to 4.2, the filtrate did not lose its inhibitory activity, implying that the inhibitory substance was not proteinaceous, but related to the acids present.

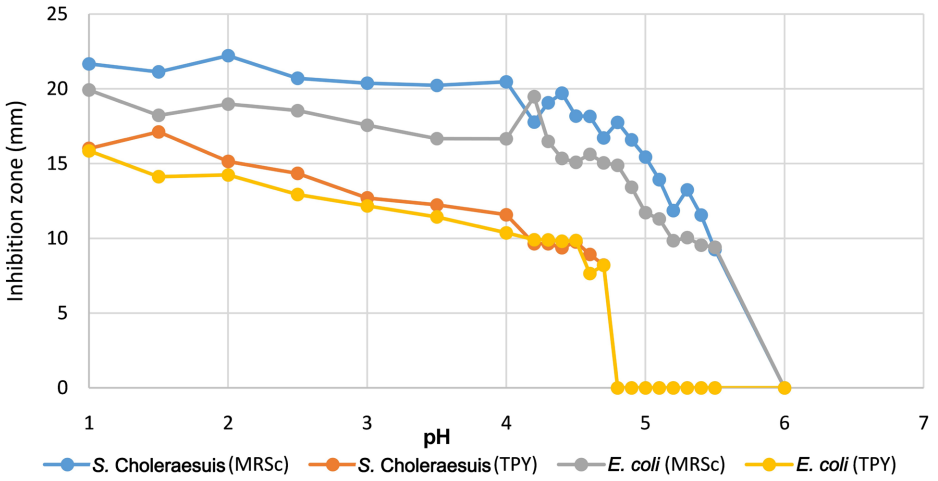

Figure 1. Effect of pH on the inhibitory activity of *Bifidobacteria infantis* extracts.

Table 2. Inhibitory capability of a 3:2 ratio of acetic and lactic acids against selected micro-organisms[1].

Inhibitory Substance	Zones of Inhibition (mm)			
	S. Choleraesuis	*E. coli*	*S. thermophilus*	*L. lactis*
Acetic acid and lactic acid	11.96[b]	10.64[b]	0	0
B. infantis filtrate	17.17[a]	15.10[a]	0	0

[1]*Escherichia coli* (*E. coli*), *Salmonella* Choleraesuis (*S.* Choleraesuis serotype typhi), Lactobacillus lactis (*L. lactis* subsp. lactis), and *Streptococcus salivarius* (*S. salivarius* subsp. thermophiles) were evaluated. [a,b]indicates within columns with different superscripts are significantly different at the 0.05 alpha level (t_{crit} = 2.447; *S.* Choleraesuis, t = 10.35; *E. coli*, t = 9.60; df = 2).

3.3. Beta-Glycerol Phosphate and Enzyme Treatment

Using the agar well assay for inhibitory activity, no zones of inhibition were present against *S.* Choleraesuis or *E. coli* after beta-glycerol-phosphate buffer (pH 9.1) treatment. This indicates that the acids found within the filtrate were the only inhibitory components present and were neutralized by the added buffer.

B. infantis bacterial inhibitor produced in MRSc was treated with pepsin and papain to determine if activity could be destroyed by proteolytic enzymes. Culture filtrate treated with these enzymes showed no significant decrease in activity after the treatment (Table 3). If inhibition was due to a proteinaceous bacteriocin with

the appropriate amino acid sequence, then the inhibition would no longer be apparent [11]. Had the enzyme decreased activity, a proteinaceous inhibitory presence would be implied. However, it would still have the potential of having a bacteriocin present with amino acid sequences that are unable to be broken down by these two particular enzymes.

Table 3. *Bifidobacteria infantis* culture filtrate tested for inhibitory activity after treatment with proteolytic enzymes.

Enzyme	Zones of Inhibition (mm)[1]	
	S. Choleraesuis	*E. coli*
Pepsin (4.5 mg/mL)	8.84	9.82
Pepsin Control	9.63	9.35
Papain (4.5 mg/mL)	15.25	14.82
Papain (Control)	15.01	14.21

[1]*Salmonella* Choleraesuis (*S.* Choleraesuis serotype typhi), and *Escherichia coli* were evaluated.

3.4. Heat Stability of Antimicrobial Component of *B. infantis*

When exposed to 50˚C, 70˚C, and 100˚C for up to 96 minutes, the *B. infantis* filtrate grown in MRSc remained stable with no significant decrease in activity against *E. coli* (Figure 2). The filtrate, which had been propagated in TPY, then held at 100˚C for 90 minutes, showed no significant decline in activity. This indicates that the inhibitory substance found within the filtrate is heat-stable.

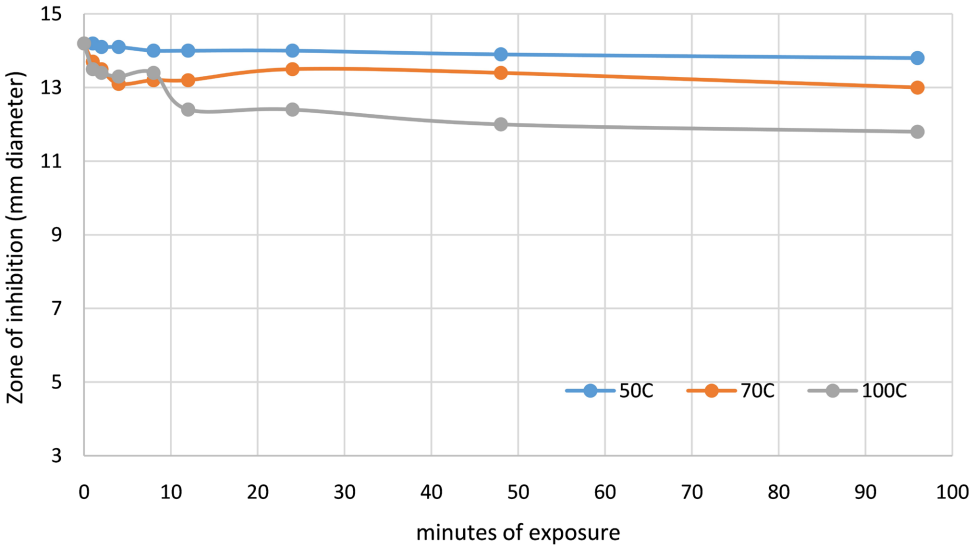

Figure 2. Effect of temperature and time (minutes of exposure) on the inhibitory activity of *Bifidobacteria infantis* extracts.

3.5. Dialysis and Ammonium Sulfate Precipitation

Proteins were precipitated from *B. infantis* filtrate by adding ammonium sulfate

to 85% saturation. Redissolved precipitate and supernatant originating from the MRSc-filtrate had limited activity (Table 4). This activity was significantly less than the activity present in the culture filtrate control with that of the supernatant containing approximately 50% as much activity as the control, and the pellet containing approximately 55%. Redissolved pellet originating from TPY-filtrate contained no apparent activity, however, the supernatant had the same approximate activity as that found in the MRSc produced filtrate (Table 4). These results are conflicting, since the TPY-produced filtrate supports the theory that the inhibitory component is acidic, while the MRSc-produced filtrate indicates that the inhibitory substance could be a combination of acids and proteins or other non-proteinaceous compounds. This supports the results showing the filtrates contained non-proteinaceous compounds since they also retained inhibitory activity after exposure to heat and protease treatments. Corr *et al.* found that the supernatant from selected species of Bifidobacterium lost their ability to prevent *Listeria monocytogenes* from invading C2Bbe1 epithelial cells after the supernatant was treated with trypsin, but Lactobacillus species retained the ability to inhibit invasion by secreting unidentified proteinaceous molecule(s) active at low pHs [20]. These researchers suggest that the mode of inhibition of listeria infection prevented by Bifidobacterium was due to a secreted protein and not low pH, supporting the observation that different modes of action work under different environmental conditions.

Table 4. Inhibitory activity of 85% saturated ammonium sulfate pellet and supernatant of *Bifidobacterium infantis* culture filtrates against *Salmonella* Choleraesuis and *Escherichia coli.*

Growth medium	Indicator Organism	Zone of Inhibition (mm)			
		Control	Supernatant	Pellet Redissolved in Distilled H_2O	Pellet Redissolved in Piperazine Buffer
TPY	*S.* Choleraesuis	12.15^a	10.12^b	0^c	0^c
TPY	*E. coli*	13.21^a	10.20^b	0^c	0^c
MRSc	*S.* Choleraesuis	17.17^a	10.44^b	9.54^b	11.27^b
MRSc	*E. coli*	15.10^a	10.56^b	10.61^b	10.18^b

^{a,b,c}indicates within columns with different superscripts are significantly different at the 0.05 alpha level ($t_{crit} = 4.303$).

3.6. Inhibition of Bacterial Growth on Turkey Thigh Meat Using *B. infantis* Culture Filtrate and Acids

B. infantis culture filtrate or a 3:2 ratio of acetic acid and lactic acid was added to turkey thigh meat and tested for its ability to inhibit bacterial growth. The bacterial counts on ground, turkey thigh meat stored for 7 days at 4˚C and inoculated with *E. coli* had significantly higher bacterial counts for the non-treated groups as compared to meat treated with filtrate or the acid (Table 5). Table 6 shows the results of bacterial growth after 7 days of storage at 4˚C when the turkey thigh meat

was inoculated with *Salmonella* Choleraesuis. These results are similar to that found within the *E. coli* treated group with bacterial counts were much lower in the groups with acid or filtrate added to this inoculum. However, these numbers did not differ significantly from the control, except within the filtrate rinsed group. For *E. coli*, the filtrate was superior to the acid blend in the agar well assay but not on the food matrix. This difference may be due to potential interactions between the active component and food components (e.g., fats, proteins).

Table 5. Population of *Escherichia coli* after exposure to *Bifidobacteria infantis* filtrate or 3:2 acetic acid:lactic on ground turkey meat during 7 days of storage at 4˚C.

Treatment	No Pretreatment	50% Ethanol Rinse Pretreatment
	Log_{10} cfu/g	
Control	4.1[a]	3.9[a]
3:2 acetic:lactic acids	3.8[b]	3.7[b]
B. infantis filtrate	3.6[c]	3.5[c]

[a,b,c]means within a column with different superscripts are significantly different at the 0.05 alpha level.

Table 6. Population of *Salmonella* Choleraesuis after exposure to *Bifidobacteria infantis* filtrate or 3:2 acetic acid:lactic acid ratio on ground turkey meat during 7 days of storage at 4˚C.

Treatment	No Pretreatment	50% Ethanol Rinse Pretreatment
	Log_{10} cfu/g	
Control	4.3[a]	3.9[a]
3:2 acetic:lactic acids	4.0[b]	3.7[b]
B. infantis filtrate	3.9[c]	3.5[c]

[a,b,c]means within a column with different superscripts are significantly different at the 0.05 alpha level.

Table 7. Population of total aerobic bacteria after exposure to no treatment, *Bifidobacteria infantis* filtrate or 3:2 acetic acid:lactic on ground turkey meat during 7 days of storage at 4˚C.

Treatment	No Pretreatment	50% Ethanol Rinse Pretreatment
	Log_{10} cfu/g	
Control	4.3[a]	3.7[a]
3:2 acetic:lactic acids	3.9[b]	3.7[a]
B. infantis filtrate	3.8[c]	3.4[b]

[a,b,c]means within a column with different superscripts are significantly different at the 0.05 alpha level.

Table 7 shows the comparison of the control to the groups in which there was no inoculum, only the addition of filtrate and acid. In this experiment, it was found that the control had significantly lower numbers than the filtrate group when the

turkey sample was ethanol rinsed. It was also shown that the control had lower bacterial numbers than the acid group when the turkey was not pretreated or when acid rinsed.

This experiment indicates that if there are high bacterial numbers already present within a product, the filtrate and acids can slow down this bacterial growth. However, for shelf-life extension in turkey thigh meat, there does not seem to be an added advantage.

4. Conclusion

This study examined the inhibitory effects of *Bifidobacterium infantis* filtrates on the growth of various microorganisms and found that *B. infantis* filtrates inhibited *Salmonella* Choleraesuis and *E. coli* but had no effect on growth of *Streptococcus salivarius* and *Lactobacillus lactis*. A acid blend (3:2 ratio acetic and lactic) was 60% as effective at inhibiting *Salmonella* Choleraesuis and *E. coli* as *B. infantis*. Furthermore, treating the *B. infantis* filtrate with proteolytic enzymes (pepsin and papain) or heat (up to 100˚C for 96 min) had no impact on the inhibitory capacity, suggesting that the filtrate was non-proteinaceous in nature. Findings suggest that there may be an additional minor component that enhances the *B. infantis* filtrate's antimicrobial capacity beyond that demonstrated with acid alone. Non-proteinaceous exopolysaccharides produced by *Leuconostoc* and *Weissella* bacteria species inhibited the growth of kimchi spoilage bacteria despite being exposed to proteolytic enzymes and heat treatment (121˚C for 15 min) [21].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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