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Effects of blackberry juice on growth inhibition of foodborne pathogens and growth promotion of *Lactobacillus*



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ABSTRACT

Berries such as blueberry, blackberry and raspberry possess several biological activities including antimicrobial and nutritional effects. In this study, the antimicrobial activities of blackberry (*Rubus fruticosus*) juice against foodborne pathogens including *Listeria monocytogenes*, *Salmonella* Typhimurium and *Escherichia coli* O157:H7 were investigated. Inhibition of growth of these foodborne pathogens was measured in broth (Luria—Bertani broth for *E. coli* O157:H7 and *S. Typhimurium*, and brain heart infusion broth for *L. monocytogenes*), skim milk and whole milk supplemented with 10% blackberry juice at different time points (0, 24, 48 and 72 h). The effects of blackberry juice on the growth of *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were also investigated in Man—Rogosa—Sharpe (MRS) broth and skim and whole milk supplemented with blackberry juice. The growth of *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 were significantly inhibited by blackberry juice by 1—3 logs in both milk and broth. We also observed that the growths of *Lactobacillus* strains were significantly stimulated (1—4 logs CFU/mL) by blackberry juice in both milk and MRS broth. These data clearly demonstrate that diluted blackberry juice can be used as a preservative in food processing and a preventive in foodborne infections as a natural antimicrobial.

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1. Introduction

Foodborne pathogens are one of the major causes of morbidity and mortality all over the world. In the United States, 31 foodborne pathogens caused about 9.4 million illness, 56,000 hospitalizations, and 1300 deaths each year (Scallan et al., 2011). Salmonella enterica, Listeria monocytogenes and enterohemorrhagic Escherichia coli O157:H7 are on the top three of the lists of bacterial pathogens responsible for foodborne illnesses (Scallan et al., 2011; Yang, Feirtag, & Diez-Gonzalez, 2013).

Probiotics, specifically lactic acid bacteria (*Lactobacillus casei*, *Lactobacillus plantarum*, *and Lactobacillus rhamnosus*), are widely used in the food industry for fermentation but have gained attention from health professionals because of their potential beneficial effects. Now probiotic therapy is thought to be an effective way to improve the gut health and an alternative to antibiotic treatments.

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Recently, Dhanani, Gaudana, and Bagchi (2011) reported that probiotic strains could cure acute diarrhea and prevent inflammatory bowel diseases. In addition, prebiotic and natural antimicrobials are also attractive alternatives to synthetic chemical antibiotics in treating many diseases. They contain many safe bioactive compounds to combat with bacterial pathogens and stimulate growth of probiotic species (Nohynek et al., 2006; Saarela, Virkajarvi, Nohynek, Vaari, & Matto, 2006). Several studies have demonstrated that blueberry juice can inhibit the growth of foodborne bacterial pathogens but does not substantially inhibit the growth of probiotic bacteria (Biswas et al., 2012; Puupponen-Pimia et al., 2001).

Rubus fruticosus, commonly referred to as the blackberry, has a high abundance of healthy antioxidants and nutrients such as anthocyanins, proanthocyanidins and other flavonoids, salicylic acid, ellagic acid, and fiber (Hager, Howard, Liyanage, Lay, & Prior, 2008; Hager, Howard, & Prior, 2008; Rommel, Wrolstad, & Heatherbell, 1992; Thielman & Guerrant, 2004; Wang & Lin, 2000). Many of these compounds have been recognized as anti-cancer effects (Vattem, Ghaedian, & Shetty, 2005). Blackberries are popular for being low in calories as about 11 kcal/100 g and much unique in that they contain high levels of ellagitannins compared to other

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berries (Asaduzzaman, Islam, & Islam, 2010; Hager, Howard, Liyanage, et al., 2008; Hager, Howard, & Prior, 2008).

Increased consumer demand for flavored milks has led to the use of natural plant extracts as aroma and flavor enhancers (Biswas et al., 2012; Goff & Griffiths, 2006). Blackberry juice is promising to be applied as one of these flavor enhancers. It is also a natural antimicrobial product (González et al., 2013).

The aims of this study were to investigate the effects of blackberry juice on bacteria including pathogens and probiotic bacteria in additional to nutritional value. The effectiveness of blackberry juice, produced by thermal processing of blackberries, in inhibiting growth of foodborne bacterial pathogens including *L. monocytogenes*, *Salmonella* Typhimurium, and *E. coli* O157:H7 was examined. Its role on the growth of the beneficial bacteria *L. casei*, *L. plantarum*, *and L. rhamnosus* were also tested. The results would help to utilize blackberry juices as natural antimicrobials for food safety.

2. Materials and methods

2.1. Bacterial strains and growth conditions

S. enterica serovar Typhimurium LT2 (ATCC19585, Manassas, VA, USA), *L. monocytogenes* LM2 (gift from the University of Arkansas Center for Food Safety), and *E. coli* O157:H7 EDL933 (gift from Dr. Michael Doyle, University of Georgia) were used in this study. *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* were grown on Luria—Bertani (LB) agar, MacConkey agar, and brain—heart infusion (BHI) agar (Gibco, Grand Island, NY, USA), respectively, at 37 °C overnight under aerobic conditions. Three lactic acid bacterial strains, *L. casei* (ATCC 7517), *L. plantarum*, and *L. rhamnosus* (gift from Dr. John A. Lindquist at the University of Wisconsin Madison), were also used in this study as probiotics. *Lactobacillus* strains were grown on de Man—Rogosa—Sharpe (MRS) agar (EMD, Rockland, MA, USA) overnight at 37 °C after three consecutive transfer from MRS broth at 37 °C for 48 h.

2.2. Blackberry juice extraction

Organic highbush blackberry (*R. fruticosus*) was purchased from local retail shops (College Park, MD, USA) and juice was collected following the method described by Biswas et al. (2012). Briefly, fruit was washed with sterile deionized water and added to a steam jacketed kettle and stirred continuously. Clean blackberry puree was heated to 95 °C, kept for 3 min and then cooled to 40 °C. Pectinex 3XL enzyme (Novozyme Corp., Bagsværd, Denmark) was added at a usage level of 0.0827 mL/kg. The must was held for 1 h at room temperature and pressed in a 70-L Enrossi bladder press (Enoagricol Rossi, Calzolaro, Italy) using screens and press cloths. Juice was filtered with a Flowjet Model T2913 (Buon Vino Inc., Cambridge, Ontario, Canada) with 9–10 mm filter pads. Juice was subsequently bottled in sterile glass jars and stored at 4 °C.

2.3. Inhibition assay of foodborne pathogens by blackberry juice

Bacterial strains were grown on agar plates at 37 °C overnight under aerobic conditions, and cells were collected in 10 mL of phosphate buffered saline (PBS) using a sterile 10 μ L loop under a biosafety cabinet. The optical density (OD) of the bacterial suspension was adjusted to an absorbance value of 0.10 at 600 nm on a LAMBDA BIO/BIO+ spectrophotometer (PerkinElmer, Beaconsfield, UK). An aliquot of 100 μ L bacterial suspension was added to a sterile 24-well cell culture plate containing either 900 μ L of broth (LB broth for *E. coli* O157:H7 and *S.* Typhimurium, and BHI broth for *L. monocytogenes*), skim milk or whole milk. Both skim milk and whole milk were purchased from local supermarket, College Park, MD and sterilized by UV light in a biosafety cabinet. Blackberry juice (10%, 100 μ L) or same

volume of sterile deionized water (control) was added to each of the wells and incubated for different time points (0, 24, 48 and 72 h) at 37 °C (Biswas et al., 2012). After incubation, serial dilutions were preformed in PBS. Serial dilutions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were plated on MacConkey, LB, and BHI agar, respectively to count bacterial colony forming units (CFU). Triplicate plates were performed for each dilution in each trial. Three trials were performed for each bacterium.

2.4. Growth and survival of Lactobacillus strains in blackberry juice

L. casei, L. plantarum, and *L. rhamnosus* were grown on MRS agar plates overnight (EMD, Rockland, MA) at 37 °C, and then cells were collected in 10 mL of PBS using a sterile 10 μ L loop under a biosafety cabinet. The OD of the bacterial suspension was adjusted to an absorbance approximately 0.10 at 600 nm on a LAMBDA BIO/BIO+ spectrophotometer (PerkinElmer). An aliquot of 100 μ L bacterial suspension was added to each of the wells of a sterile 24-well cell culture plate containing either 900 μ L of MRS broth or blackberry juice and incubated for different time points (0, 24, 48 and 72 h) at 37 °C (Biswas et al., 2012). After incubation, serial dilutions were made in PBS and plated on MRS agar to count bacterial colony forming units (CFU). Triplicate plates were performed for each dilution in each trial, Three trials were performed for each bacterium.

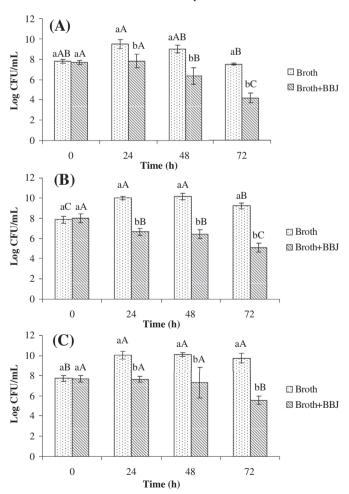
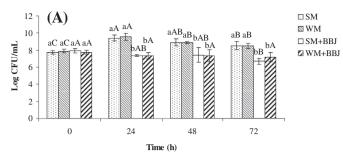
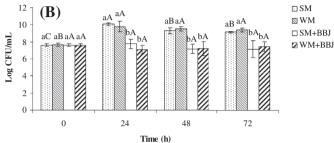


Fig. 1. Growth inhibition of *Escherichia coli* O157:H7 (A), *Salmonella* Typhimurium (B), and *Listeria monocytogenes* (C) in selective broth and selective broth with 10% blackberry juice at 0, 24, 48, and 72 h. $^{\rm a}$ Within each time period, means with different lowercase letters are significantly different (P < 0.05) among different groups. Within each group, means with different capital letters are significantly different among different time points (P < 0.05). BBJ, blackberry juice.





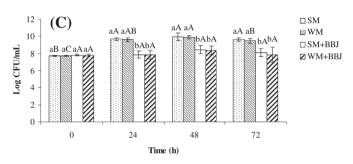


Fig. 2. Comparative growth and survival of *Escherichia coli* O157:H7 (A), *Salmonella* Typhimurium (B), and *Listeria monocytogenes* (C) in skim milk, whole milk, skim milk with 10% blackberry juice, and whole milk with 10% blackberry juice at 0, 24, 48, and 72 h. ^aWithin each time period, means with different lowercase letters are significantly different (P < 0.05) among different groups. Within each group, means with different capital letters are significantly different among different time points (P < 0.05). BBJ, blackberry juice. SM, Skim milk; WM, whole milk.

2.5. Statistical analysis

Bacterial growth was analyzed by comparing the viable cell counts between the treatment and control at 0, 24, 48 and 72 h. Growth in broth or milk without blackberry juice was used as the control. Data were presented as average and standard deviation. Analysis of variance (ANOVA) was performed to determine the effects of addition of blackberry juice and storage time on survival and growth of *L. monocytogenes*, *S.* Typhimurium, *E. coli* O157:H7, and *L. casei*, *L. plantarum* and *L. rhamnosus*, Pearson's correlation analysis between the results of these bacteria survival and growth was performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, US). Mean comparisons were carried out using Tukey's test to examine the differences between treatments and storage time. Comparisons that yielded P < 0.05 were considered significant.

3. Results

3.1. Effect of blackberry juice on the growth of E. coli O157:H7, S. Typhimurium and L. monocytogenes

Antimicrobial activity of blackberry juice against above mentioned enteric bacterial strains was measured in liquid cultures by plate count method. Fig. 1 shows the antimicrobial effect of

blackberry juice on the growth of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7. Growth of these foodborne bacterial pathogens was inhibited significantly after 24 h incubation in liquid bacterial culture media supplemented with blackberry juice. We observed that in the presence of 10% blackberry juice, growths of all three bacterial pathogens were inhibited significantly (P < 0.05). We found that growth of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were reduced by 1.65, 3.34 and 2.40 logs CFU/mL, respectively after 24 h as compared with the growth in broth alone.

The inhibitory effects of 10% blackberry juice in broth remained throughout the 72 h period. For *E. coli* O157:H7, we observed that 10% blackberry juice in broth inhibited the growth of this bacterial pathogen 1.65, 2.61 and 3.35 logs CFU/mL at 24 h, 48 h and 72 h, respectively (Fig. 1). Results of *Salmonella* and *L. monocytogenes* also had similar trends. This finding indicated that the role of blackberry juice in the reduction of growth of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in broth were significant and time dependent. The inhibitory effects were more effective at 72 h than at 24 h (Fig. 1).

Fig. 2 shows the growth of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in skim milk, and whole milk alone or skim and whole milk supplemented with 10% blackberry juice. We observed

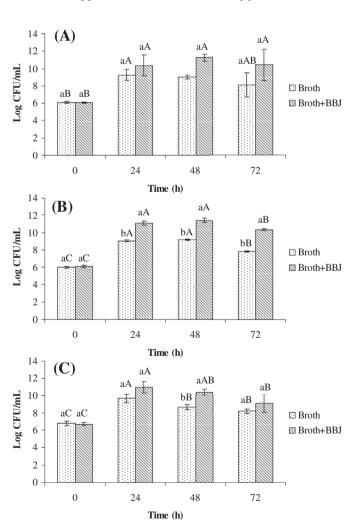
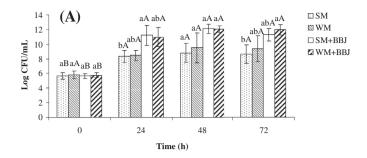
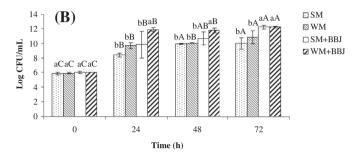


Fig. 3. Effect of blackberry juice on the growth of *Lactobacillus* strains in MRS broth and MRS broth with 10% blackberry juice at 0, 24, 48, and 72 h. *Lactobacillus casei* (A), *Lactobacillus rhamnosus* (B), and *Lactobacillus plantarum* (C). ^aWithin each time period, means with different lowercase letters are significantly different (P < 0.05) among different groups. Within each group, means with different capital letters are significantly different among different time points (P < 0.05). BBJ, blackberry juice.





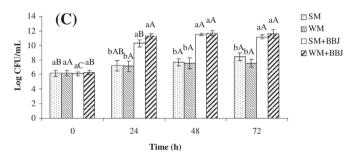


Fig. 4. Comparative growth and survival of *Lactobacillus casei* (A), *Lactobacillus rhamnosus* (B), and *Lactobacillus plantarum* (C) in skim milk, whole milk, skim milk with 10% blackberry juice, and whole milk with 10% blackberry juice at 0, 24, 48, and 72 h. ^aWithin each time period, means with different lowercase letters are significantly different (P < 0.05) among different groups. Within each group, means with different capital letters are significantly different among different time points (P < 0.05). BBJ, blackberry juice. SM, Skim milk; WM, whole milk.

that blackberry juice also inhibits the growth of these foodborne bacterial pathogens in both skim and whole milk.

We found that the role of blackberry juice on the growths of these three bacterial pathogens in both skim and whole milk had similar trend. Both skim and whole milk supplemented with 10% blackberry juice decreased the growth of L. monocytogenes with time dependent manner during the incubation period (Fig. 2). The inhibitory effects of blackberry juice on L. monocytogenes were pretty stable. In this study, we observed from 1.5 to 1.8 logs CFU/mL reduction (P < 0.05) in both skin and whole milk supplemented with 10% blackberry juice. We found that milk supplemented with

10% blackberry juice also inhibited growth of S. Typhimurium (>2 logs CFU/mL) and E. coli O157:H7 (1.3—2.0 logs CFU/mL). Effects of blackberry juice in skim and whole milk were similar (Fig. 2).

3.2. Effect of blackberry juice on the growth of Lactobacillus

The effects of blackberry juice on the growth of *Lactobacillus* strains (*L. casei, L. plantarum*, and *L. rhamnosus*) are shown in Fig. 3. We observed that effect of blackberry juice on *Lactobacillus* is species specific. All three *Lactobacillus* species showed different growth pattern in the MRS broth supplemented with blackberry juice. The growth of *L. rhamnosus* was significantly (P < 0.05) stimulated (>2 logs CFU/mL) by blackberry juice at all three time points (24 h, 48 h and 72 h) as compared with the growth in MRS broth alone. For *L. plantarum* and *L. casei*, we observed that 10% supplemented blackberry juice in MRS broth promoted the growth significantly only at 48 h time point (Fig. 3). These results revealed that blackberry juice significantly stimulated (P < 0.05) the growth of *Lactobacillus* in a time dependent manner for some species.

Fig. 4 shows the growth of *L. casei*, *L. plantarum*, and *L. rhamnosus* in both skim and whole milk with or without 10% blackberry juice. We found that the growth of *L. plantarum* was significantly stimulated (2.74–4.20 logs CFU/mL) by blackberry juice in both skim and whole milk during all time (24 h, 48 h and 72 h) points. Compared to skim milk, whole milk supplemented with 10% blackberry juice had more stimulating effect on the growth of *L. plantarum* (Fig. 4). Both skim and whole milk supplemented with blackberry juice stimulated the growth of *L. casei* and *L. rhamnosus* less efficiently (2.5–3.0 and 0.77–2.24 logs CFU/mL for *L. casei* and *L. rhamnosus*, respectively) compared to *L. plantarum*.

3.3. Effect of blackberry juice on the correlations of growth of pathogenic and Lactobacillus bacteria

Pearson correlation analysis among these bacteria including both pathogens and lactobacillus was performed to better understand their relationships. Tables 1 and 2 show the correlation matrixes of survival and growth of bacteria (in broth and milk) without and with 10% blackberry juice, respectively. After addition of blackberry juice, the correlation coefficients were significant among the three pathogens or three *Lactobacillus* species, but there was no statistically significant correlation between species of pathogens and species of *Lactobacillus* (P > 0.05). In control groups (without addition of blackberry juice), however, all correlation groups except between *L. plantarum* and *E. coli* O157:H7, and between *L. plantarum* and *L. rhamnosus* had significant correlation coefficients.

4. Discussion

Disease caused by consuming microbial contaminated food has increased significantly in recent years due to changes in the livelihoods and eating habits of the human populations (Carbas,

Table 1Correlation matrix of survival and growth of bacteria without addition of blackberry juice.

	Escherichia coli O157:H7	Salmonella Typhimurium	Listeria monocytogenes	Lactobacillus casei	Lactobacillus rhamnosus	Lactobacillus plantarum
Escherichia coli O157:H7	1.00					
Salmonella Typhimurium	0.81**	1.00				
Listeria monocytogenes	0.68*	0.95***	1.00			
Lactobacillus casei	0.69*	0.91***	0.95***	1.00		
Lactobacillus rhamnosus	0.65*	0.79**	0.85***	0.94***	1.00	
Lactobacillus plantarum	0.45	0.72**	0.78**	0.74**	0.57	1.00

Table 2Correlation matrix of survival and growth of bacteria with addition of 10% blackberry juice.

	Escherichia coli O157:H7	Salmonella Typhimurium	Listeria monocytogenes	Lactobacillus casei	Lactobacillus rhamnosus	Lactobacillus plantarum
Escherichia coli O157:H7	1.00					
Salmonella. Typhimurium	0.86***	1.00				
Listeria monocytogenes	0.84***	0.79**	1.00			
Lactobacillus casei	-0.31	-0.36	0.10	1.00		
Lactobacillus rhamnosus	-0.32	-0.41	0.06	0.95***	1.00	
Lactobacillus plantarum	-0.15	-0.26	0.24	0.97***	0.96***	1.00

Note: *, ** and *** indicate significance at P < 0.05, P < 0.01 and P < 0.001, respectively.

Cardoso, & Coelho, 2013; Gerner-Smidt & Whichard, 2010; Jones & Angulo, 2006). Salmonella, L. monocytogenes and E. coli O157:H7 are major causative agents and account for over half of the foodborne illnesses. Control of these foodborne enteric pathogens is a real challenge for public health agency and food industry. Moreover, the resurgence of multidrug resistant strains of foodborne pathogens has made it more difficult to ensure the safety of the food supply chain (Donado-Godoy et al., 2012; Mirzaagha et al., 2011).

Natural products as antimicrobials is a promising alternative way to control foodborne bacterial infections (Caillet, Cote, Sylvain, & Lacroix, 2012; Cote et al., 2011; Lacombe, Wu, Tyler, & Edwards, 2010; Lacombe, Wu, White, Tadepalli, & Andre, 2012;). However, limited information is available about the role of natural products such as berries and other fruits and vegetables as antimicrobials. Role of antimicrobial plant products in inhibition of growth and control of cross contamination of foodborne bacterial pathogens is an emerging area of research. The present study demonstrated with strong evidence that a small amount of blackberry juice (10%) has strong inhibitory effects against several foodborne pathogens and stimulatory effects on the growth of beneficial bacterial species. The effects of blackberry juice are in contrast to blueberry juice which displayed no stimulating effects on the growth of probiotic bacteria (Biswas et al., 2012).

In the present study, we have shown that pathogenic bacterial E. coli O157:H7, S. Typhimurium and L. monocytogenes were significantly inhibited by blackberry juice (2-4 logs CFU/mL in broth and 1-2 logs CFU/mL in milk). This inhibitory effect might be due to phenolic components. This is supported by previous reports that found that phenolic compounds, especially ellagitannins of berries, are strong inhibitory compounds against S. enterica and L. monocytogenes (Puupponen-Pimia, Nohynek, Alakomi, & Oksman-Caldentey, Puupponen-Pimia. 2005; Nohynek, Hartmann-Schmidlin, et al., 2005). In their reports, they also found that phenolic compounds, especially ellagitannins of berries, were inhibitory compounds against S. enterica L. monocytogenes. Our previous studies have also demonstrated that blueberry juice inhibits the growth of L. monocytogenes, S. Typhimurium, Campylobacter jejuni, and E. coli O157:H7 (Biswas et al., 2012). The possible reason for this inhibition could be the partial hydrophobicity of phenolics, anthocyanins, and proanthocyanidins, which allows them to bind to the outer membrane of the bacteria causing changes in fluidity (Kwon, Apostolidis, Kim, & Shetty, 2007). This membrane interface interaction may be important in the mechanism of inhibition (Lacombe et al., 2010, 2012).

Many recent studies have shown that the growth of probiotic bacteria was not inhibited by the presence of phenolic compounds found in berries (Biswas et al., 2012; Lacombe et al., 2010, 2012; Puupponen-Pimia et al., 2001). In this study, we also observed that instead of inhibition, the blackberry juice showed positive effects on the growth of *Lactobacillus* species. The growth of *Lactobacillus* species, used in this study, were significantly stimulated in the presence of 10% blackberry juice (approximately 1.0—

2.5 logs CFU/mL in MRS and 1–4 logs CFU/mL in milk). The possible reason for this stimulation could be the diverse nutritional options of probiotics. In this case, blackberry juice could be utilized as sugars by *Lactobacillus* strains in slightly acidic pH (Demir, Bahceci, & Acar, 2006; Pereira, Maciel, & Rodrigues, 2011) or rhamnosidase-producing activity of *Lactobacillus* (Beekwilder et al., 2009; Puupponen-Pimia, Nohynek, Alakomi, et al., 2005; Puupponen-Pimia, Nohynek, Hartmann-Schmidlin, et al., 2005; Puupponen-Pimia et al., 2001). Different effects between broth and milk could be partially due to their different components of media.

The change of correlation coefficients from Pearson analysis demonstrated that addition of 10% blackberry juice changed the trends of survival and growth of pathogens and lactobacillus. Without addition of blackberry juice, all bacterial strains grew without inhibiting effects. Therefore, most of the bacteria had significant correlation coefficients (Table 1). For groups supplemented with blackberry juice, within three pathogens or three lactobacillus species, the trend of inhibitions or stimulations were much similar, therefore their correlation coefficients were statistically significant (P < 0.05) (Table 2). However, due to the inhibiting effects on pathogens and promoting effects on *Lactobacillus*, the correlation between the pathogens and the *Lactobacillus* bacteria were not statistically significant (Table 2).

Our previous study showed that skim milk supplemented with blueberry juice reduced the growth of foodborne bacterial pathogens, and it did not have any effect on the growth of *Lactobacillus bulgaricus* (Biswas et al., 2012). In this study, we showed that the growth of all pathogenic bacterial strains used in this experiment was significantly inhibited in both skim milk and whole milk in the presence of 10% blackberry juice. Interestingly, we also observed that the growth of all *Lactobacillus* strains significantly increased in both broth and milk media supplemented with blackberry juice. Therefore, blackberry juice has potential to be used as a promising natural antimicrobial against foodborne bacterial pathogens as well as a growth promoter for beneficial bacteria, specifically *Lactobacillus*. This information is especially important for organic foods, considering many chemical antimicrobial agents are not allowed to be used due to the strict regulation for organic foods.

During antimicrobial treatments for diseases, it is important to consider possible impacts upon the beneficial microorganisms. Antibiotic treatments usually result in detrimental reduction to the population of natural and normal flora in human gut, causing the growth of non-beneficial bacteria to high numbers (Lacombe et al., 2010, 2012). Our study clearly demonstrated blackberry juice can significantly promote the growth of probiotic species. These data have been clarified by the several previous reports of Pereira et al. (2011) and Demir et al. (2006). Pereira et al. (2011) reported that growth of *L. casei* increased 0.52 log CFU/mL after 16 h incubation at 30 °C with cashew apple juice and further increased 0.3 log CFU/mL at 4 °C for 28 days. Demir et al. (2006) also found the similar effect of carrot juice on the growth of *L. plantarum*.

Future research needs to be conducted to elucidate the inhibitory mechanism of blackberry juice against these foodborne pathogens and mechanism of promoting the growth of probiotic *Lactobacillus*.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Acknowledgement

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