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The antimicrobial properties of the lowbush blueberry (*Vaccinium angustifolium*) fractional components against foodborne pathogens and the conservation of probiotic *Lactobacillus rhamnosus*

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ABSTRACT

The antimicrobial properties of lowbush blueberry (*Vaccinium angustifolium*) were studied against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Lactobacillus rhamnosus* to determine which fractional components have antimicrobial effects and which microorganisms are most susceptible to these antimicrobial properties. Lowbush blueberry extract (F1) was separated using a C-18 Sep-Pak cartridge into monomeric phenolics (F2) and anthocyanins plus proanthocyanidins (F3). Fraction 3 was further separated into anthocyanins (F4) and proanthocyanidins (F5) using a LH-20 Sephadex column. Each fraction was initially screened for antimicrobial properties using agar diffusion assay. Treatments that demonstrated inhibition were further analyzed for inhibition in liquid culture. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a two-fold dilution series and viable cell counts taken at 0 and 24 h to examine growth reduction. Fraction 3 demonstrated the lowest MICs/MBCs followed by F1, F2, F4, and F5. *L. monocytogenes* was the most susceptible to blueberry fraction treatment, followed by *E. coli* O157:H7, and *S.* Typhimurium. *L. rhamnosus* was the least susceptible to each fraction treatment. The results can be applied to the field of preventive medicine, food safety, and enrich the understanding of the health benefits of lowbush blueberries.

1. Introduction

Vaccinium angustifolium, commonly referred to as the lowbush blueberry, has distinguished itself from other berries in its family based upon its high concentration of phenolics and antioxidant capabilities (Wolfe and Liu, 2008). The health benefits of lowbush blueberries have been accredited to their high phytochemical composition. Research demonstrates the potential of blueberries to alleviate of a wide range of maladies such as irritable bowel disease (IBS), urinary tract infections (UTI), cardiovascular disease, Alzheimer's, and cancer (Schmidt et al., 2004; Neto, 2007; Badjakov et al., 2008; Osman et al., 2008; Del Bo et al., 2010). Lowbush blueberries have been recognized for anti-adhesion properties in previous studies (Schmidt et al., 2004), with particular emphasis the prevention microbial adhesion to gut epithelial tissue. These effects were mainly attributed to phenolic acids and flavonoids, especially anthocyanins and proanthocyanidins (Schmidt et al., 2004; Tisane et al., 2009). Although these findings hold promise for the field of preventative medicine, researchers have yet to learn which pathogens are susceptible, which classes of antimicrobial compounds are responsible for bacterial inhibition, and these antimicrobial compounds' effects on potentially beneficial probiotic species.

The resurgence of multidrug resistance (MDR) strains of foodborne pathogens has made it more difficult to ensure the safety of the food supply (Mirzaagha et al., 2011). Cook et al. (2011) sampled 538 veal chops, and found that resistance to one or more antibiotic was found in 29% *Salmonella* isolates and 54% *Escherichia coli* isolates. Recent studies have uncovered the antimicrobial effects of many essential oils and plant polyphenols, and have demonstrated their effect on virulence genes and antibiotic resistance (Johny et al., 2010; Amalaradjou et al., 2011). These studies demonstrated that plant derived plant compounds can target the molecular machinery of multiple pathogens.

Lactic acid bacteria (LAB), such as *Lactobacillus rhamnosus* are used in the food industry for fermentation, but have gained notice

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by the medical profession because of their probiotic effects. Probiotic therapy is thought to be a potentially attractive alternative to antibiotic treatments, and *L. rhamnosus* is one the best studied bacterial strains (Dhanani et al., 2011). This strain originated from the intestinal tract of a healthy human and is being studied for the treatment of acute diarrhea and the prevention of inflammatory bowel diseases (Dhanani et al., 2011). When developing therapeutic treatment for infectious maladies, it is important to both conserve the beneficial microbial population and not to incur resistance in the target pathogen. Many of the chemotoxic pharmaceutical antibiotic treatments have harsh side effects such as diarrhea due to the overpopulation of *Clostridium difficile* (Bergogne-Berezin, 2000). Natural antimicrobials are attractive alternatives in many disease models, such as UTIs and IBS because they contain dynamic combinations of bioactive compounds to combat resistance, and have demonstrated the conservation of probiotic species (Nohynek et al., 2006). To date no one has studied the antimicrobial properties of lowbush blueberries' fractional components against foodborne pathogens and observed conservation of probiotic bacteria.

Other berries in the *Vaccinium* family have demonstrated antimicrobial action against foodborne pathogens. Recent studies have demonstrated that cranberry fractional components can permeate the extracellular membrane of *E. coli* O157:H7 at doses of 5% v/v (Lacombe et al., 2010). Total cranberry extract demonstrated membrane damage against *E. coli* O157:H7, *Listeria monocytogenes*, *S.* Typhimurium, and *Staphylococcus aureus* at concentrations of 5 μl/ml (Wu et al., 2008). Bilberry, the European counterpart of lowbush blueberry, is a common folk remedy for gastro-intestinal disorders (Nohynek et al., 2006). Bilberry extract was shown to strongly inhibit *Bacillus cereus*, *Clostridium perfringens*, *Helicobacter pylori*, and *Staph. aureus*, but produced no inhibition against *L. rhamnosus* (Nohynek et al., 2006).

The objectives of this study were to i) separate *V. angustifolium* into its phytochemical constituents and screen for inhibition using agar disc diffusion ii) determine the minimum inhibitory bactericidal concentration and growth reduction of each separated component against *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium, and iii) observe the conservation of the probiotic *L. rhamnosus*.

2. Materials and methods

2.1. Preparation, fractionation, and characterization of lowbush blueberry fractions

2.1.1. Blueberry preparation

Unprocessed freeze-dried lowbush blueberry powder (Durham Research Londonderry, NH, USA), 40 g, was stirred overnight at 25 °C in 100 ml 80% v/v methanol. The mixture was then centrifuged at 5800 × g (Eppendorf 5810 R-series, Hamburg, Germany) for 25 min at 4 °C. The solution was vacuum filtered with No. 3 Whatman paper (Schleicher & Schuell, Maidstone, England), with a recovery of 75 ml. The liquid filtrate was transferred to a sterile round-bottom flask and the methanol was entirely removed on a rotor evaporator (Buchi, Flawii, Switzerland) at 45 °C for 1 h. To ensure the removal of all solvent, the solution was placed in a desiccator until completely dried and then resuspended in 40 ml of distilled water. From this solution, 4 ml was removed and designated as fraction 1, total blueberry extract. Four C-18 Sep-Pak Vac 35cc (Waters, Milford, MA, USA) columns were sequentially primed with ethyl acetate, acidified methanol (0.01% v/v HCl), and acidified water (0.01% v/v HCl), and loaded with 9 ml of total blueberry extract. The columns were then washed with 60 ml of acidified distilled water to remove any organic sugars and acids remaining in solution. Monomeric phenolic acids were eluted with 60 ml of ethyl acetate and were designated as fraction 2. Anthocyanins plus proanthocyanidins (fraction 3) were eluted with 60 ml of acidified methanol and divided into two round-bottom flasks. Fraction 2 was rotor-evaporated for 20 min and fraction 3 was rotor-evaporated for 30 min. After overnight storage in a desiccator, fraction 2 was resuspended in 4 ml of distilled water and stored at 4 °C. One of the fraction 3 samples was resuspended in 6 ml of distilled water. The other fraction 3 was resuspended in 4 ml of 40% v/v ethanol for further separation into anthocyanins and proanthocyanidins. A LH-20 Sephadex column (GE Healthcare, Schenectady, NY, USA) was used, according to the procedure by Kalt et al. (2008). The column was swelled for 3 h using 1 g of Sephadex in 5 ml of 40% v/v ethanol. For each column prepared, 1 ml of the fraction 3 sample was loaded and separated with a peristaltic pump (Ecoline, Glattbrugg, Switzerland) set at 1 ml/min. Anthocyanins (fraction 4) were eluted with 5 ml of 40% v/v ethanol and rotor-evaporated for 30 min at 60 °C to remove all the solvent. Proanthocyanidins (fraction 5) were eluted with 4 ml of 80% v/v acetone, and rotor-evaporated for 30 min at 40 °C to remove all the solvent. After rotor-evaporation and overnight drying in a desiccator, fractions 4 and 5 were resuspended in 4 ml of distilled water and stored for use in experiments.

2.1.2. Quantification of fractions

To determine the relative concentration and purity of each fraction, several assays were performed. Each fraction was analyzed for total phenolics using the Folin–Ciocalteu (F–C) method (Slinkard and Singleton, 1977), anthocyanins using the pH differential method (Lee et al., 2008), and proanthocyanidins using the DMAC (4-dimethlylaminocinnamaldehyde) method (Payne et al., 2010). Folin–Ciocalteu measurement of the phenolic extract demonstrated a correlation of $R^2 = 0.79$ with their 280 nm HPLC peaks, suggesting F–C is a reasonable method for characterizing phenolics (Kalt et al., 2008). Both the pH differential and DMAC methods are approved by the AOAC for the determination of anthocyanins and proanthocyanidins (Lee et al., 2008; Payne et al., 2010).

The F–C assay is a non-specific method for the quantification of total reducing phenolics (Slinkard and Singleton, 1977). Total phenolics were determined by comparing samples against a standard curve of gallic acid (Slinkard and Singleton, 1977). Samples were read at 725 nm in a 96-well microplate (Biotech, Winoskii, VT, USA) and results were reported in equivalents of gallic acid (GAE). The pH differential method determined the total monomeric anthocyanins content, based on the structural change of the anthocyanins chromophore between pH 1.0 and 4.5 (Lee et al., 2008). A buffer solution of KCl (Fisher Scientific, Pittsburgh, PA, USA) was adjusted to pH 1.0 and a buffer solution of sodium acetate (Fisher Scientific, Pittsburgh, PA, USA) was adjusted to pH 4.5. These solutions were added to the samples and read in a 96-well microplate at 525 and 700 nm (Biotech, Winoskii, VT, USA). The differences in absorbance reading between each wavelength were used as an indication of relative anthocyanins content (Lee et al., 2008). Results were calculated according to Lee et al. (2008), and were reported in equivalents of cyanidin-3-galactoside (C3G) (Lee et al., 2008).

Quantification of proanthocyanidins was determined using the P-dimethylaminocinnamaldehyde (DMAC) protocol from Payne et al. (2010). The absorbance of the reaction product between the proanthocyanidins in the sample and DMAC was measured at 640 nm in a 96-well microplate. Results were compared against a standard of procyanidin-A2/DMAC solution (Indofine, Hillsborough, NJ, USA) and reported in equivalents of procyanidin-A2 (PA2). The pH of each fraction was measured at dilutions of 1:1, 1:2, 1:4, and 1:8 (v/v) in Brain Heart Infusion (BHI) after each

experimental repeat using the Accumet Electrode (Fisher Scientific, Pittsburgh, PA, USA).

2.2. Bacterial culture preparation

Cultures of *E. coli* O157:H7 (ATCC 129000 and 35150, Manassas, VI, USA), *L. monocytogenes* (ATCC 49594 and 7644), *S.* Typhimurium (ATCC 6962 and 14028), *Yersinia enterocolitica* (ATCC 27724), and *Staph. aureus* (ATCC 25923) were grown in BHI to late stationary phase. The *L. rhamnosus* (ATCC 9595 and 7469) was grown anaerobically in a 5% CO_2 incubator (Thermo Scientific, Asheville, NC, USA) in de Man, Rogosa, and Sharpe (MRS, Neogen, Lansing, MI, USA) broth for 48 h at 37 °C and cultured on MRS agar. *E. coli* O17:H7, *L. monocytogenes*, *S.* Typhimurium, *Y. enterocolitica*, and *Staph. aureus* were cultured on MacConkey Sorbitol Agar (MSA, Neogen), Modified Oxford Medium (MOX, Neogen), Xylose Lysine Deoxycholate Agar (XLD, Neogen), *Yersinia* isolation agar, and Baird–Parker Agar (BPA, Neogen), respectively.

2.3. Screening for the antimicrobial properties of wild blueberry fraction using agar disc diffusion assay

Each strain of *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium, *Y. enterocolitica*, and *Staph. aureus* were individually diluted to 6 log CFU/ml to prepare an even lawn on the petri plate. After allowing time for the sample to dry and using flamed forceps, four ½ inch sterile paper disks (Schleicher & Schuell, Maidstone, England) were placed on the agar diagonal from one another. After inoculation, 50 μl of each fraction was placed on each disk on the plate to create duplicate sets. A positive control was assigned using 10% v/v bleach and a negative control using distilled water. The plates were then incubated upside down at 37 °C for 24 h. After incubation, the inhibition zones were recorded in millimeters.

2.4. Antimicrobial evaluation of individual fraction for minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and bacterial growth reductions

To determine the MIC and MBC, strains of *E. coli* O157:H7 (ATCC 129000 and 35150, Manassas VI, USA), *L. monocytogenes* (ATCC 49594 and 7644), *S.* Typhimurium (ATCC 6962 and 14028) and *L. rhamnosus* (ATCC 9595 and 7469) were selected and diluted to 6 log CFU/ml. Strains from the same species were mixed to create bacteria cocktails. Each fraction was diluted to 1:1, 1:2 1:4, and 1:8 with BHI, and then by an additional factor of two with bacterial cocktails, yielding 1:2, 1:4, 1:8, and 1:16. Samples were prepared to a total volume of 200 μl in a microplate (Biotech, Winoskii, VT, USA).

A control was established to be equal parts BHI broth and bacterial cocktail, without addition of blueberry fractions. Viable cell counts were performed at 0 and 24 h on their respective media for *E. coli* 157:H7, *L. monocytogenes*, and *S.* Typhimurium. Plates were spread in duplicate and incubated at 37 °C before enumeration. For *L. rhamnosus*, viable cell counts were taken at 0 h and 48 h, and a control was established to be equal parts MRS broth and bacterial cocktail. Plates were incubated anaerobically in a 5% CO_2 incubator. Bacterial growth log reduction was analyzed by comparing viable cell counts between control and treatments at 24 h/48 h and to the initial inocula at 0 h.

2.5. Statistical analysis

Bacterial growth log reduction was analyzed by comparing the 0 h and 24 h viable cell counts, and well as viable cell counts between the treatment and control at 24 h. Each experiment was individually repeated three times. The number of cells was translated into log CFU/ml and treatments were assigned for comparison. Analysis of variance (ANOVA) was performed on cell counts using the SAS General Linear Models (GLM) procedure with SAS software 8.0 (Statistical Analysis System. Inst. Inc., Cary, NC, USA). Significance of differences was defined as $P < 0.05$. Differences among treatment means were examined for the level of significance using Tukey's Honest Squared Difference (HSD).

3. Results

3.1. Characterization of fractions

In this experiment multiple assays were conducted to determine the relative concentration and composition of each fraction as indicated in Table 1. The phenolic constituents of lowbush blueberries have been previously studied using F–C method, which has demonstrated fidelity when compared to HLPC–DAD analysis (Prior et al., 1998; Kalt et al., 1999, 2008). The C18 SPE extraction was conducted according to Prior and Kalt's methods, which demonstrated no selective loss of phenolic constituents during the extraction process (Kalt et al., 2008; Prior et al., 1998). In order to facilitate the extraction of suitable concentrations of fractions (F2–F5) for use in experiments, the total blueberry extract (F1) had a more concentrated version than commercially available blueberries or blueberry products.

The pH for each fraction dilutions was record prior to inoculation with bacteria (Table 2). The results demonstrated that anthocyanins plus proanthocyanidins have the lowest pH followed by, anthocyanins, phenolic acids, total blueberry extract, and proanthocyanidins. Blueberries naturally have a low pH, which could inhibit the growth of bacteria. Therefore the fraction treatments were diluted with BHI, which has some buffering capacity, helping to neutralize treatments prior to inoculation.

3.2. Agar diffusion assay

Agar disc diffusion was conducted in order to screen each fraction for antimicrobial properties. Each fraction demonstrated significant ($P < 0.05$) inhibition zones compared to sterile water and 10% v/v bleach (Table 3). Results were analyzed by comparing the treatment to bleach and distilled water (0 mm). Total blueberry

Table 1
Analysis of the concentration of lowbush blueberry fractions using Folin–Ciocalteu, pH differential, and DMAC methods. Fractions were analyzed as undiluted blueberry fractions. Results represent the average of three repeats.

Method	Total blueberry extract	Monomeric phenolics	Anthocyanins plus proanthocyanidins	Anthocyanins	Proanthocyanidins
Folin–Ciocalteu					
(Gallic acid equivalents (g/L)	8.9 ± 0.4	8.0 ± 0.2	11.6 ± 0.5	8.1 ± 0.8	4.0 ± 0.2
pH differential					
(Cyanidin-3-glucoside equivalents (mg/L)	51.0 ± 13.5	15.1 ± 6.3	173.08 ± 3.5	139.82 ± 10.7	8.23 ± 1.4
DMAC					
(Equivalents of procyanidin-A2 (mM)	0.60 ± 0.02	0.40 ± 0.007	2.50 ± 0.08	0.30 ± 0.001	1.0 ± 0.045

Table 2
The average ($n = 3$) of treatment pH of isolated blueberry fractions. Readings were taken before inoculation with bacteria cocktail and after being diluted in a two-fold series with BHI.

Fraction[a]	1:1	1:2	1:4	1:8
Total blueberry extract	3.37 ± 0.01	3.69 ± 0.03	4.34 ± 0.1	5.29 ± 0.173
Monomeric phenolics	2.11 ± 0.01	3.73 ± 0.04	4.71 ± 0.01	5.90 ± 0.11
Anthocyanins plus proanthocyanidins	0.88 ± 0.04	2.35 ± 0.01	3.99 ± 0.05	5.31 ± 0.06
Anthocyanins	1.16 ± 0.00	3.30 ± 0.04	4.91 ± 0.01	6.25 ± 0.01
Proanthocyanidins	3.56 ± 0.06	6.89 ± 0.02	7.08 ± 0.03	7.15 ± 0.03

[a] Relative quantitative data for fraction treatments is reported in Table 1. Ratios represent the dilution factors applied the original concentration of 8.9 g/L GAE for total blueberry extract, 8.0 g/L GAE for monomeric phenolics, 173.08 mg/L C3G for anthocyanins and proanthocyanidins, 139.8 mg/L C3G for anthocyanins, and 1.0 mM PA2 for proanthocyanidins.

extract (F1) demonstrated the most inhibition and was the most effective against *E. coli* O157:H7 (ATCC 12900, 35150), *S.* Typhimurium (ATCC 6962), and *L. monocytogenes* (ATCC 49594) strains. Proanthocyanidins were the least effective and only demonstrated inhibition zones for *L. monocytogenes* and *Y. enterocolitica*. For all cultures screened in the agar diffusion assay, *E. coli* O157:H7 and *L. monocytogenes* demonstrated the highest zone of inhibition and *Staph. aureus* (ATCC 25922) demonstrated the least inhibition.

3.3. Assessment of growth reduction and determination of MIC and MBC using liquid culture and dilution method

From the agar diffusion assay, *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were the chosen pathogens for further analysis. Two strains of *L. rhamnosus* were chosen to represent probiotics (Dhanani et al., 2011). The results from the liquid culture methods are presented in Figs. 1–5 for each fraction and were assessed for viable cell counts (log CFU/ml) at 24 h compared to the initial inoculums (0 h) and the control at 24/48 h. The minimum inhibitory concentration (MIC) was established as the lowest concentration of fraction that inhibited the visible growth of the bacteria (Table 4). The minimum bactericidal concentration (MBC) was established as the lowest concentration of fraction that demonstrated no growth of bacteria on fraction-free media after incubation.

Of the cultures analyzed, *L. monocytogenes* was the most susceptible to blueberry fraction treatments, with the highest reduction in growth and lowest MICs/MBCs. When compared to the control, *L. monocytogenes* demonstrated an 8-log CFU/ml reduction in growth for all fractions at their highest and second highest concentrations. The MBCs for *E. coli* O157:H7 were equal or twice the amount of *L. monocytogenes* (Figs. 1–5). When compared to the control, *E. coli* O157:H7 demonstrated an 8-log CFU/ml reduction in growth for all fractions at their highest concentrations and an 8-log reduction in growth for fractions 1 and 3 at their second highest concentrations. *S.* Typhimurium demonstrated similar log reductions in growth to *E. coli* O157:H7, except for being more susceptible to monomeric phenolics and anthocyanins and less susceptible to proanthocyanidins. *L. rhamnosus* was the least susceptible to the treatment with the lowest log reduction in growth and MICs twice that of *L. monocytogenes* for total blueberry extract, monomeric phenolics, and anthocyanins plus proanthocyanidins. *L. rhamnosus* did not demonstrate any MBC, unlike the other cultures tested, and for anthocyanins and proanthocyanidins, *L. rhamnosus* demonstrated no MIC or MBC.

4. Discussion

Lowbush blueberries have been utilized in preventative medicine for their high phenolics and antioxidant concentrations. These phenolics are typically the secondary metabolites developed by plants to protect them from UV light, parasites, and oxygen free radicals (Lila, 2006). Lowbush blueberries are rich in these compounds due to the acidic soil and limited nutrient environment in which they naturally exist (Lila, 2006). The capability lowbush blueberry to prevent the growth of foodborne pathogen and preserve probiotic species has not been previously reported. The present study presents evidence that treatments with lowbush blueberries antioxidant constituents have bactericidal effects against several foodborne pathogens and not *L. rhamnosus*. This study is the first to demonstrate the capability of lowbush blueberry in preventing the growth of foodborne pathogens and preserving probiotic species.

When considering antimicrobial treatments for diseases, it is important to consider possible impacts upon the beneficial microorganism. Many antibiotic treatments result in detrimental reduction to the population of natural flora, causing the overpopulation of non-beneficial bacteria. This research demonstrates that the probiotic species have twice the resistance to blueberry treatments when compared to pathogenic species. The selective inhibition of pathogenic microorganisms by blueberries helps the probiotic flora outcompete them for space along the intestinal wall, decreasing the probability of host illness.

Pathogens and probiotics that are ingested orally both come into direct contact with innate immune defenses such as low pH, bile, and enzymes. Lactic acid bacteria (LAB) have evolved to thrive in this environment, while the innate immune system tries to eliminate pathogens. The bile-salt hydrolase gene (BSH) has recently been proposed to be an intestinal niche-specific molecular marker for lactobacilli and has been accredited for the species' success colonizing the GI tract (Pfeiler and Klaenhammer, 2009). The ability of LAB to survive in these conditions may be applicable to its resistance to blueberry phenolics.

Recent research has demonstrated that numerous berries high in bioactive phenolic chemicals have antimicrobial effects (Lacombe et al., 2010; Wu et al., 2008; Cesoniene et al., 2009; Heinonen,

Table 3
The average ($n = 3$) of inhibition zones (mm) produced by each fraction during agar diffusion screening. Results were analyzed by comparing treatments to bleach and distilled water. Letters (a, b, c) represents treatments belonging to the same population group, * represent treatments statistically the same as zero (distilled water).

Fraction	Inhibition zone (mm)						
	E. coli O157:H7 35150	*E. coli* O157:H7 129000	*S.* Typ 6962	*S.* Typ 14028	*L. mono* 49594	*S. aureus* 25922	*Y. entero* 27724
Total blueberry extract (F1)	20.1 ± 4.2 a	17.9 ± 2.2 ab	19.7 ± 4.2 a	20.7 ± 1.0 a	20.7 ± 1.6 a	16.3 ± 0.8 a	11.3 ± 8.1 b*
Monomeric phenolics (F2)	21.8 ± 6.9 a	17.6 ± 2.4 ab	11.0 ± 8.0 ab	16.2 ± 0.8 a	21.3 ± 1.8 a	11.8 ± 8.8 ab	11.0 ± 7.8 b*
Anthocyanins plus proanthocyanidins (F3)	20.0 ± 3.5 a	16.4 ± 1.7 b	11.3 ± 8.1 a	12.2 ± 9.1 ab	21.8 ± 5.4 a	0 ± 0 b*	10.7 ± 7.6 b*
Anthocyanins (F4)	17.8 ± 4.4 a	14.6 ± 1.5 b	9.8 ± 7.0 ab	10.8 ± 7.8 ab	19.7 ± 3.4 a	0 ± 0 b*	10.0 ± 7.3 b*
Proanthocyanidins (F5)	0 ± 0 b*	0 ± 0 c*	0 ± 0 b*	0 ± 0 b*	7.2 ± 6.5 ab	0 ± 0 b*	10.8 ± 7.7 b*
10% v/v bleach	14.0 ± 2.6 ab	16.8 ± 0.4 a	21.5 ± 4.3 a	22.2 ± 2.1 a	15.3 ± 2.5 a	21.5 ± 3.0 a	34.2 ± 2.6 a

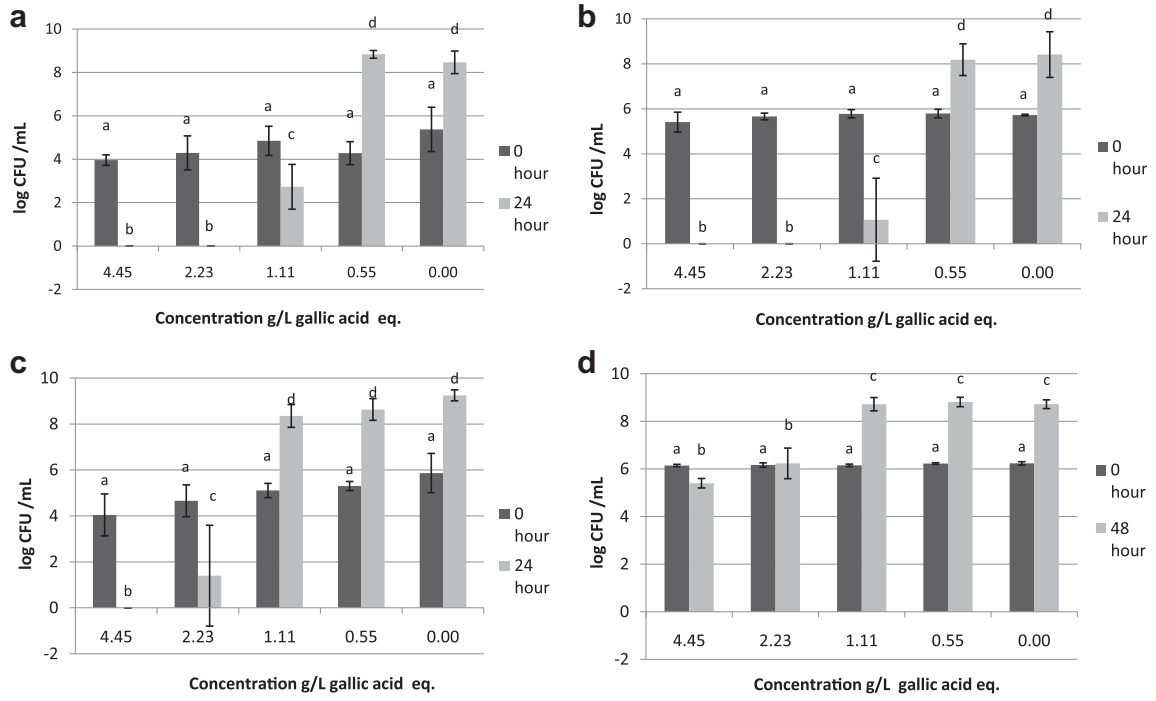

Fig. 1. Evaluation of the antimicrobial effect of total blueberry extract against a) *E. coli* O157:H7, b) *L. monocytogenes*, c) *S.* Typhimurium, and d) *L. rhamnosus*. The experiments were repeated three times, and data are expressed as mean ± standard deviation. Comparisons were made between the 0 h and 24 h, and well as between the treatment and control at 24 h. Means with different letters for the same species are significantly different ($P < 0.05$). Detection limit is <1 log CFU/ml. Concentrations are reported in equivalents of gallic acid (GAE).

2007; Puupponen-Pimiä et al., 2005). Previous work with similar cranberry fractions (Lacombe et al., 2010; Wu et al., 2008) visualized these fractions having a permeabilizing effect against the outer membrane of *E. coli* O157:H7. Transmission electron micrographs demonstrated localized disruption of the outer membrane of cells treated with cranberry phenolics (Lacombe et al., 2010). The partial hydrophobicity of phenolics, anthocyanins, and proanthocyanidins allows them to bind to the outer membrane causing changes in

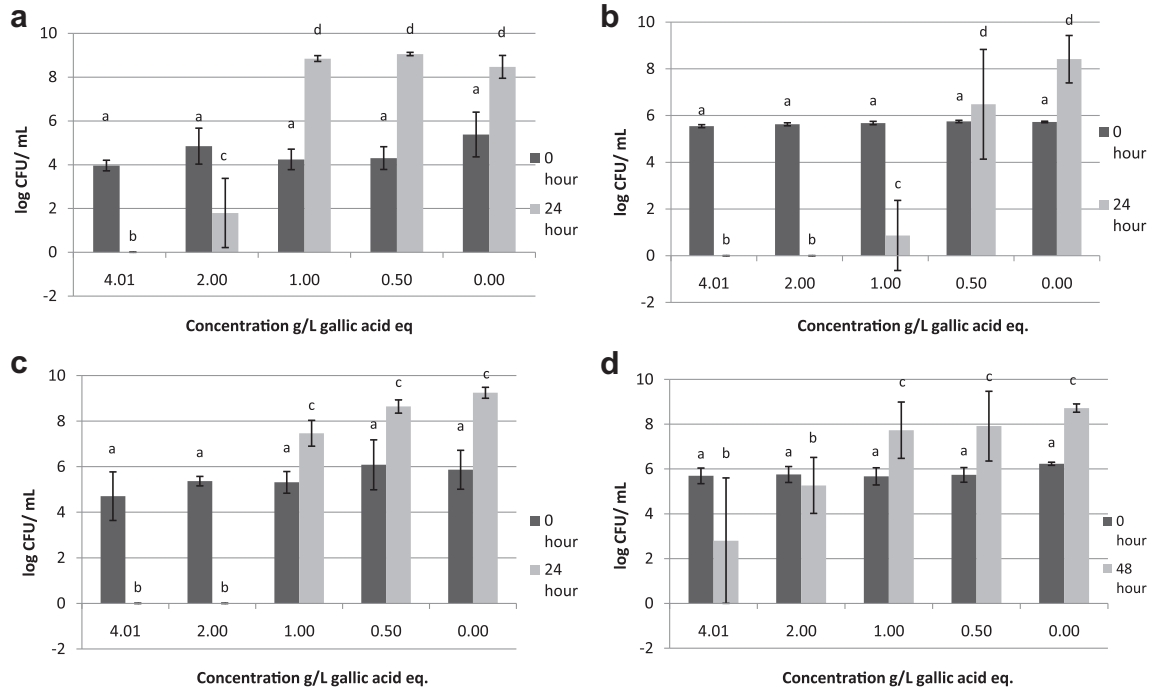

Fig. 2. Evaluation of the antimicrobial effect of blueberry monomeric phenolics against a) *E. coli* O157:H7, b) *L. monocytogenes*, c) *S.* Typhimurium, and d) *L. rhamnosus*. The experiments were repeated three times, and data are expressed as mean ± standard deviation. Comparisons were made between the 0 h and 24 h, and well as between the treatment and control at 24 h. Means with different letters for the same species are significantly different ($P < 0.05$). Detection limit is <1 log CFU/ml. Concentrations are reported in equivalents of gallic acid (GAE).

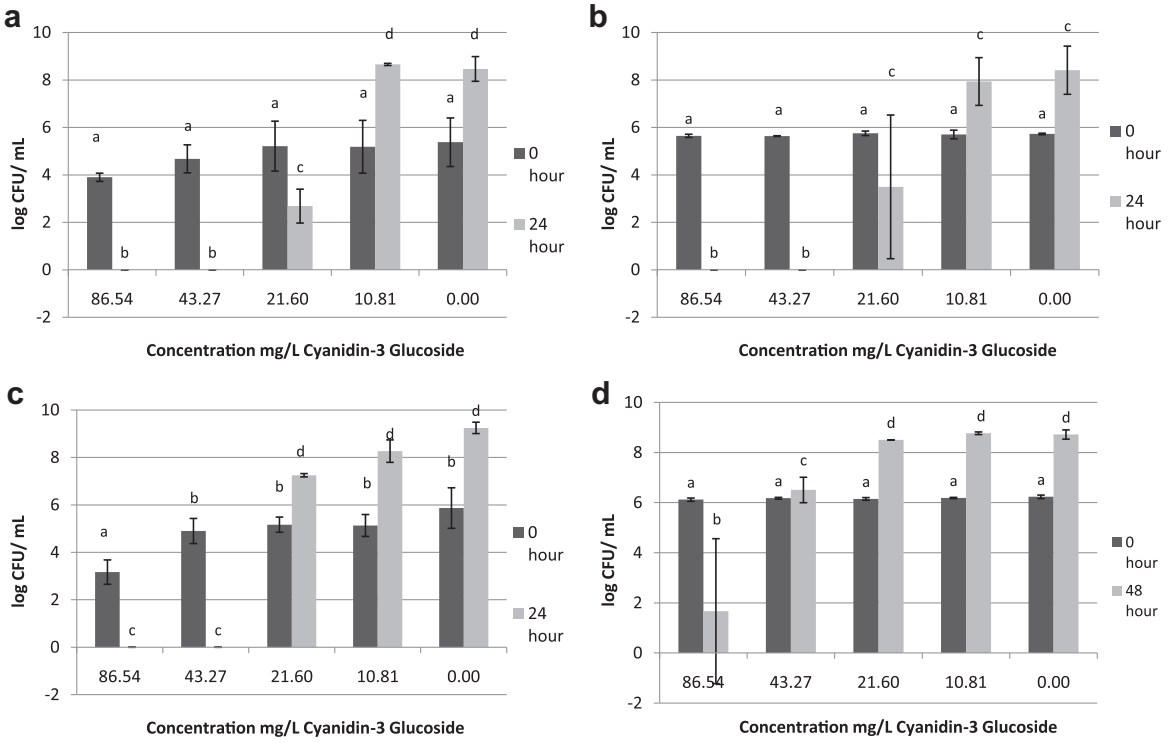

Fig. 3. Evaluation of the antimicrobial effect of blueberry anthocyanins plus proanthocyanidins against a) *E. coli* O157:H7, b) *L. monocytogenes*, c) *S.* Typhimurium, and d) *Lactobacillus rhamnosus*. The experiments were repeated three times, and data are expressed as mean ± standard deviation. Comparisons were made between the 0 h and 24 h, and well as between the treatment and control at 24 h. Means with different letters for the same species are significantly different ($P < 0.05$). Detection limit is <1 log CFU/ml. Concentrations are reported in equivalents of cyaniding-3-glucoside.

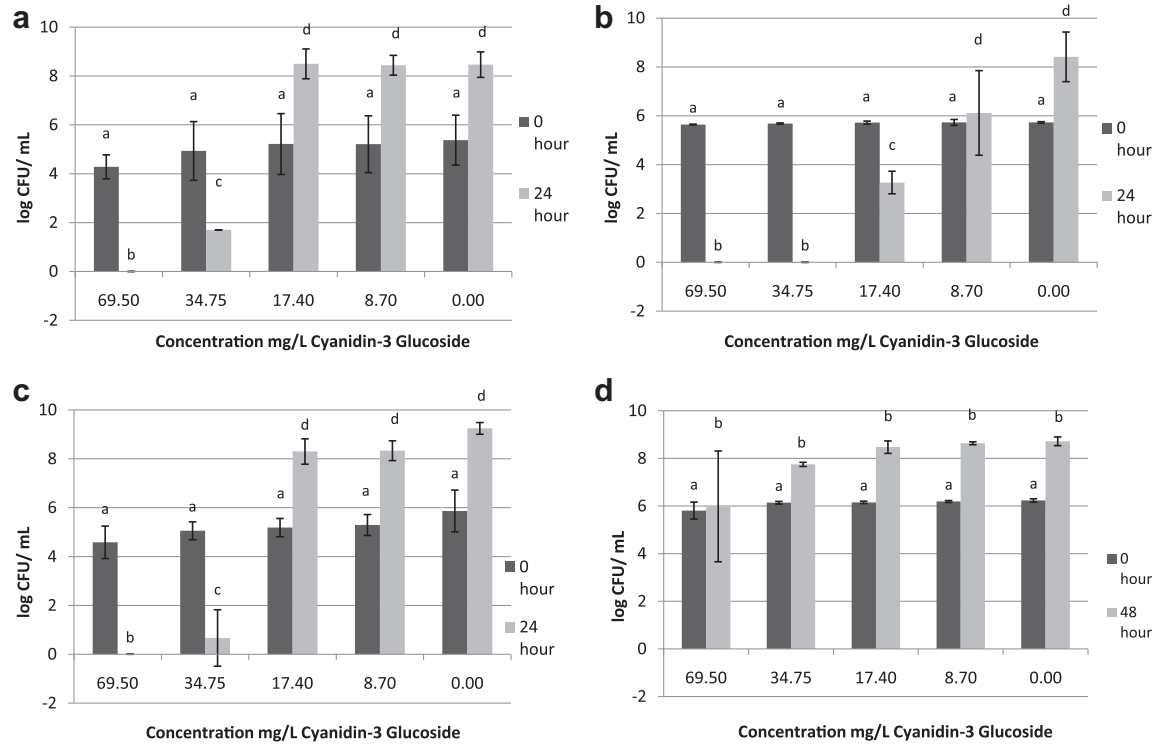

Fig. 4. Evaluation of the antimicrobial effect of blueberry anthocyanins against a) *E. coli* O157:H7, b) *L. monocytogenes*, c) *S.* Typhimurium, and d) *L. rhamnosus*. The experiments were repeated three times, and data are expressed as mean ± standard deviation. Comparisons were made between the 0 h and 24 h, and well as between the treatment and control at 24 h. Means with different letters for the same species are significantly different ($P < 0.05$). Detection limit is <1 log CFU/ml. Concentration are reported in equivalents of cyanidin-3-glucoside.

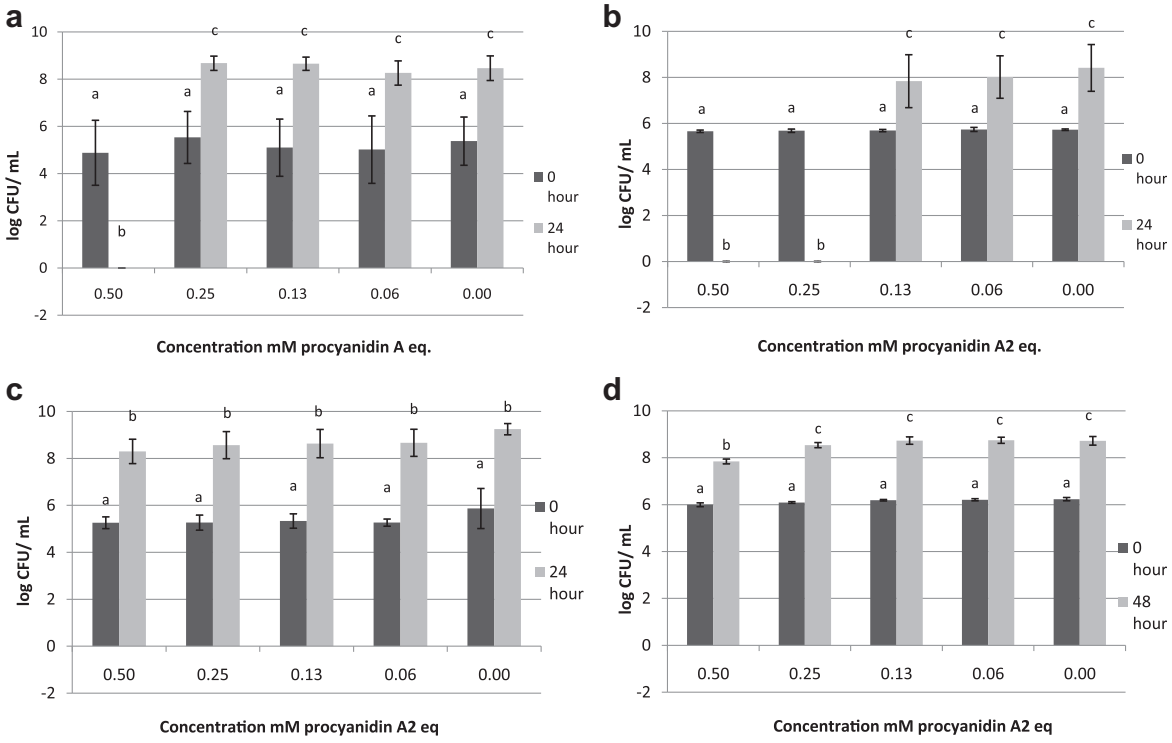

Fig. 5. Evaluation of the antimicrobial effect of blueberry proanthocyanidins against a) *E. coli* O157:H7, b) *L. monocytogenes*, c) *S.* Typhimurium, and d) *L. rhamnosus*. The experiments were repeated three times, and data are expressed as mean ± standard deviation. Comparisons were made between the 0 h and 24 h, and well as between the treatment and control at 24 h. Means with different letters for the same species are significantly different ($P < 0.05$). Detection limit is <1 log CFU/ml. Concentrations are reported in equivalents of procyanidin-A2.

fluidity (Kwon et al., 2007). This observation suggested that the membrane interface interaction is important in the mechanism of inhibition.

Mechanisms of berry phenolics' inhibition against pathogenic bacteria have previously been proposed. The outer bacterial membrane of bacteria provides the intrinsic barrier against chemical assault, and is thought to be the primary target for berry phenolics (Puupponen-Pimiä et al., 2005). Gram-negative bacteria possess lipopolysaccharide (LPS), which provide an inherent resistance to bile salts and hydrophobic antibiotics (Puupponen-Pimiä et al., 2005; Lacombe et al., 2010). Anthocyanins and phenolic compounds have demonstrated permeative action by destabilizing the LPS and increasing the efflux of ATP from the cytoplasm of *S.* Typhimurium (Puupponen-Pimiä et al., 2005). Gram positive bacteria posse thicker peptidoglycan cell walls which serve to protect cells against hostile environments, but *L. monocytogenes* was observed to be the most susceptible to lowbush blueberry fraction treatment in the present study. Blueberry phenolics could have profound effects on membrane fluidity, changes in the fatty acid profile, and disrupt metabolism disruption (Kleerebezem et al., 2010). Although bacteria were treated at relatively low pHs (Table 2), it has been established that just a high concentration of hydronium ions is not the only factor at work in bacterial inhibition (Lacombe et al., 2010; Wu et al., 2008; Kleerebezem et al., 2010; Van Immerseel et al., 2006). Enteropathogeneic bacteria such *E. coli* O157:H7 and *S.* Typhimurium have adapted to survive the harsh environment of the host GI tract, such that these abilities to survive in these conditions are directly associated with their virulence (Audia et al., 2001).

The antimicrobial properties of berries can have a direct effect on the health of the GI tract if ingested in the proper amounts. Blueberries have multi-factorial benefits *in vivo*, including radical oxygen scavenging activity, leading the attenuation of inflammation (Osman et al., 2008). The addition of blueberry phenolics may enhance the body's ability to defend itself against foodborne infection by targeting the surface structures of pathogenic bacteria preventing adhesion to the mucosa. Adhesion of bacteria is a vital prerequisite for successful infection of the intestinal tract and inactivation of outer membrane components, even if transient in nature can, prevent infection (Alakomi et al., 2007).

Table 4
Representation of the MIC and MBC for each culture tested (*E. coli* O157:H7, *Listeria monocytogenes*, *Lactobacillus rhamnosus*, and *S.* Typhimurium) based on the results from liquid culture analysis. Results represent the lowest concentration at which significant ($P < 0.05$) inhibition/bactericidal effect was observed from the average of three repeats.

Fraction	MIC				MBC			
	E. coli O157:H7	*L. mono*	*S.* Typ	*L. rham*	*E. coli* O157:H7	*L. mono*	*S.* Typ	*L. rham*
Total blueberry extract (F1) g/L gallic acid eq	1.11	1.11	2.23	2.23	2.23	2.23	4.45	NA[a]
Phenolics (F2) g/L gallic acid eq	2.00	1.00	2.00	2.00	4.01	2.00	2.00	NA
Anthocyanins plus proanthocyanidins (F3) mg/L C3G eq	21.60	21.60	43.27	43.27	43.27	43.27	43.27	NA
Anthocyanins (F4)	34.75	17.40	34.75	NA	69.50	34.75	69.50	NA
Proanthocyanidins mM (F5)	0.50	0.25	NA	NA	0.50	0.25	NA	NA

[a] NA = not observed.

This experiment observed the ability of fractional components of wild blueberries to inhibit intestinal pathogens and conserve the probiotic species, *L. rhamnosus*. The effects of each fractional could be synergistic or cumulative depending on the mechanism of action. Future work is needed to elucidate how lowbush blueberries can mediate pathogen host responses *in vivo* and how probiotic species can survive these treatments. The outcomes of this and future work will have major implications for preventive medicine and enrich our understanding of the health benefits of lowbush blueberries.

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