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Original article

Cranberry seed fibre: a promising prebiotic fibre and its fermentation by the probiotic *Bacillus coagulans* MTCC 5856

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Summary Cranberry, a versatile fruit, is known for nutritional as well as medicinal properties due to the presence of bioactive compounds. However, cranberry fruit has not been fully explored for its prebiotic potential. Therefore, this study was conducted to evaluate prebiotic potential of cranberry seed fibre (CSF) and also evaluate its fermentability by the probiotic strain *Bacillus coagulans* MTCC 5856. The resistance to gastric acid and porcine pancreatic enzymatic (PPE) hydrolysis of CSF was investigated using an *in vitro* model. It was found that CSF was resistant to gastric acid and also nondigestible to PPE hydrolysis. CSF as sole nutrition source was evaluated for the fermentability by *B. coagulans* MTCC 5856. A significant amount of short-chain fatty acids was produced by the *B. coagulans* MTCC 5856 while fermenting cranberry fibres anaerobically. CSF supported the growth of *B. coagulans* MTCC 5856 and also inhibited the growth of *E. coli* ATCC 25922 when cocultured in an anaerobic environment. CSF from the cranberry fruit exhibited prebiotic potential and also found to be fermentable by *B. coagulans* MTCC 5856. This study provided the scientific evidence of CSF as a prebiotic fibre and also its suitability with the probiotic *B. coagulans* MTCC 5856 for an ideal synbiotic preparation.

Keywords *Bacillus coagulans*, cranberry seed fibre, prebiotic, probiotic, short-chain fatty acids, synbiotic.

Introduction

Cranberry (*Vaccinium macrocarpon* Ait. Ericaceae), an evergreen dwarf shrub, is a native fruit of North America. However, in Britain, cranberry is referred to the native species *Vaccinium oxycoccos*. Cranberry is cultivated in central and northern Europe and also throughout the northern United States, Canada and Chile (Vattem *et al*., 2005). More than 90% of the total cranberry fruit is produced by mainly the north-eastern part of North America and Canada (Vattem *et al.,* 2005). Cranberries product range includes fresh fruits, dried fruits and products such as juices or food ingredients in cereals, meat, milk products and sauces (Sona *et al*., 2015). Traditionally, cranberry fruit has attracted much attention due to its biological properties reported to prevent and treat urinary tract infections, stomach ulcers, reduction in the cardiovascular disease risks, protection against lipoprotein oxidation, anticancer activity and antitumorigenic activity (Burger *et al*., 2000; Seeram, 2008; Novotny *et al*., 2015). In a recent systematic review with meta-analysis, it was concluded that product derived from cranberry fruit may decrease the incidence of urinary tract infections, particularly in individuals with recurrent urinary tract infections (Luís *et al*., 2017). Further, cranberry may also reduce the administration of antibiotics, which could be beneficial, as excessive use of antibiotics can lead to the worldwide emergence of antibiotic resistance in microorganisms (Luís *et al*., 2017). The biological properties of cranberry fruit have been linked with the presence of organic and phenolic acids, flavonols, polyphenols, pentacyclic triterpenoids, quercetin, anthocyanins and proanthocyanidins (PACs) (Neto *et al*., 2000). Cranberry is also a good source of vitamins, dietary fibre and the essential dietary mineral manganese as well as other essential micronutrients (Dorofejeva *et al*., 2011). Cranberry fruit is a transformational functional food and a nutraceutical due to the presence of various biologically active substances (Howell *et al*., 1998). The use of cranberries in

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foods is very common in Canada and in the United States as a common ingredient in baking goods (muffins, scones, cakes and breads), soups and stews (Mildner-Szkudlarz *et al*., 2016).

Probiotics are live microorganisms which are defined as 'live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO, 2002). Microorganisms including from the genus Lactobacillus, Bifidobacterium, *Enterococcus, Bacillus, Streptococcus, Escherichia* and also *Saccharomyces boulardii* have been used as probiotics for humans (Makarova *et al*., 2006; McFarland, 2007). *Bacillus coagulans*, a spore-forming, Gram-positive, rod-shaped bacteria, has long history of safe and effective use as probiotics (Makarova *et al*., 2006; McFarland, 2007). LactoSpore®, a commercial probiotic preparation of Sabinsa Corporation, USA, which contains the spores of *B. coagulans* MTCC 5856, was marketed for over two decades worldwide (Majeed & Prakash, 1998). *B. coagulans* MTCC 5856 was found to be phenotypically and genotypically consistent over multiple years of commercial production and also stable in various functional foods during processing and storage (Majeed *et al*., 2016b,d) *B. coagulans* MTCC 5856 exerted significant antidiarrhoeal activity and also inhibited gastrointestinal motility in rodent model (Majeed *et al*., 2016c). In double-blind human clinical trial, *B. coagulans* MTCC 5856 at a dose of 2×10^9 spore day^{-1} was found to be safe and effective in patients with diarrhoea-predominant IBS for 90 days of supplementation (Majeed *et al*., 2016e). It is a well-known fact that diet and/or dietary fibres play a pivotal role in the clinical efficacy of probiotics and help them to colonise in the gut. As per American Association of Cereal Chemists, a dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine (AACC, 2001) Typically, dietary fibres are the polysaccharides, oligosaccharides, lignin and associated plant substances (Devinder *et al*., 2012). Gibson *et al*., 2004 defined prebiotics as 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits'. Further, prebiotics confer these benefits by supporting the growth of probiotics (beneficial microbes) in the colon by serving as a substrate. Moreover, the association of dietary fibres and prebiotics with the beneficial health effect has been well documented specifically to promote physiological effects including laxation, and/or blood glucose and cholesterol attenuation (James & Mark, 2010). Thus, it is essential to study the role of dietary fibres/prebiotics on the growth/colonisation of probiotics. Hence, this study was aimed to investigate the CSF for its prebiotic potential and also its utilisation by the probiotic *B. coagulans* MTCC 5856.

Material and methods

Lysozyme, pepsin, fructooligosaccharide (FOS), pancreatin from porcine, bile salt, copper sulphate, SCFA standards (acetic, propionic and butyric acids) were procured from Sigma-Aldrich (St. Louis, MO, USA). Potato soluble starch, de Man, Rogosa and Sharpe (MRS) broth, glucose yeast extract agar, trypticase soy agar, eosin methylene blue agar (EMB agar) and glucose yeast extract agar (GYEA) were purchased from HiMedia, Mumbai, India. Oxyrase was procured from Oxyrase, Inc, Mansfield, OH, USA. Probiotic bacterium *B. coagulans* MTCC 5856 is a patented strain of Sami Labs Limited and deposited to Microbial Type Culture Collection and GenBank (MTCC), Chandigarh, India. *E. coli* ATCC 25922 was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Trypticase soy agar (TSA; Becton–Dickinson, Cockeysville, MD, USA) was used for culturing of bacteria, and culture stocks were maintained in aqueous glycerol (15% v/v) at −80 °C.

Methods

Gastric acid hydrolysis

Cranberry seed fibre (CSF) used in this study was obtained from Fruit d'Or Nutraceuticals, Quebec, Canada, which contained 50% fibre, 25% protein, 4.8% moisture, 16 essential amino acids and fatty acids such as omega-3, 6 and 9 along with 3% proanthocyanidins (Majeed *et al*., 2016a). CSF (2 gm) was dissolved in 100 mL of a sterile electrolyte solution (6.2 g L^{-1} NaCl, 2.2 g L^{-1} KCl, 0.22 g L^{-1} $CaCl_2$, 1.2 g L^{-1} $NaHCO_3$) containing 0.01% lysozyme (Sigma-Aldrich) and 0.3% pepsin (Sigma-Aldrich). Samples were taken at 0, 30, 60, 90, 120 and 180 min. Fructooligosaccharide (FOS; Sigma-Aldrich) was taken in the study as reference to compare with CSF. Potato soluble starch (HiMedia) was also taken as positive control in the study. The release of reducing sugar was measured according to the previously described method (Miller, 1959; Nilsson & Bjorck, 1998).

Enzymatic hydrolysis

Pancreatin from porcine (Sigma-Aldrich) 100 mg was dissolved in 100 mL of phosphate buffer (50 mM; pH 7.0) containing bile salt (0.3%, w/v, Sigma-Aldrich). Further, CSF (2 gm) was dissolved in above pancreatin solution and incubated at 37 °C with 100 rpm for 180 min. Samples were taken at 0-, 30-, 60-, 90-, 120- and 180-min interval. FOS was taken in the study as reference to compare with CSF, and potato starch was

also taken as positive control. The release of reducing sugar was measured according to the previously described method (Miller, 1959; Oku *et al*., 1988).

Fermentation

The fermentation of prebiotic fibre by the probiotic is an important aspect for the determination of prebiotic activity of a prebiotic fibre. The fermentation of cranberry seed fibre was evaluated by growing probiotic *B. coagulans* MTCC 5856 in an anaerobic environment as per the method described earlier (Crittenden *et al*., 2002) with little modification. A single isolated colony of *B. coagulans* MTCC 5856 was inoculated into MRS broth (pH 7.0; HiMedia) and incubated in an orbital shaker at 37 °C with 120 rpm for overnight. Different concentrations (0.5, 1.0 and 2.0%, w/v) of cranberry seed fibre were prepared in demineralised water, and pH was adjusted to 7.0 followed by the sterilisation at 121 °C for 15 min. Similarly, MRS media were prepared and pH was adjusted to 7.0 and sterilised. After sterilisation, oxygen-reducing enzyme Oxyrase (Oxyrase® for Broth) was also added to each flask. Five per cent of overnight grown *B. coagulans* MTCC 5856 culture was inoculated to all flasks and incubated in an orbital shaker at 37 °C with 120 r.p.m. for 24 h. pH values at 0 h and after fermentation (24 h) were recorded. Viable count was determined by serial dilution using glucose yeast extract agar (HiMedia) at 0, 6, 12, 18 and 24 h as per the previously described method (Majeed *et al*., 2016b). Average mean of viable counts is presented in Log_{10} cfu mL^{-1}.

Production of short-chain fatty acids

The production of short-chain fatty acids by the *B. coagulans* MTCC 5856 while fermenting cranberry seed fibre was carried out as per the method described earlier (McBurney & Thompson, 1987) with some modifications. Briefly, 2.0 g of FOS or CSF was added to 100 mL of demineralised water. The pH was adjusted to 7.0 ± 0.2 and autoclaved at 121 °C for 20 min. After sterilisation, oxygen-reducing enzyme Oxyrase (Oxyrase® for Broth) was added to each flask to induce anaerobic conditions. Similarly, MRS media were prepared and used as control in the study to compare with CSF. Five per cent of overnight grown *B. coagulans* MTCC 5856 culture was inoculated to all the flasks and incubated at 37 °C with 40 r.p.m. for 24 h. The bottles were tightly closed and sealed with parafilm to maintain anaerobic condition generated by the enzyme supplement. The pH values at 0 h of incubation and after fermentation (24 h) were recorded. One millilitre of copper sulphate (10 g L^{-1}) was added to each sample to inhibit further microbial growth (Sigma-Aldrich). Further, 5.0 mL of sample was added to 5 mL of distilled water and pH was adjusted to 1.5 using 3 M H_2SO4. Chilled (−20 °C) diethyl ether 10 mL was added to samples and then mixed in a vortex mixer for 1 min. Sodium chloride was added and then centrifuged at 3000× ***g*** for 10 min. After centrifugation, organic layer was separated and transferred to the fresh vial. This was used to quantify SCFAs. The SCFA standards were purchased from Sigma-Aldrich and similarly processed. SCFA production (acetic, propionic and butyric acids) was measured by gas chromatography (GC) with the use of a Agilent Technologies 6890N gas chromatograph (Stevens Creek Blvd Santa Clara, CA, USA) containing a DB-FFAP (Terephthalic acid modified polyethylene glycol) column. The column temperature was 200 °C. The injector and detector port temperatures were 250 °C. The carrier gas was N_2 at a flow rate of 1.0 mL min^{-1}. SCFA (acetic, propionic and butyric acids) concentrations were expressed in mg per g of CSF.

Inhibition of E. coli *ATCC 25922 growth*

The *in vitro* study was designed to evaluate inhibitory effect of *B. coagulans* MTCC 5856 on the growth of Gram-negative pathogenic bacteria *E. coli* ATCC 25922 while growing in cranberry seed fibre. CSF (2.0 g) was dissolved in 100 mL of demineralised water, and pH was adjusted to 7.0 ± 0.2 and then sterilised at 121 °C for 15 min. After sterilisation, oxygen-reducing enzyme Oxyrase (Oxyrase® for Broth) was added to each flask. Glucose yeast extract agar (HiMedia) and trypticase soy agar (HiMedia) media were used to grow *B. coagulans* MTCC 5856 and *E. coli* ATCC 25922, respectively. Suspension was made from single isolated colony of both the cultures, and turbidity was adjusted to 0.5 McFarland standards (equivalent to 1.5×10^8 cfu mL^{-1}). In group 1, 1 mL of *E. coli* ATCC 25922 was added to the flask containing cranberry seed fibre. In another group, 1 mL of *E. coli* ATCC 25922 and 1 mL of *B. coagulans* MTCC 5856 were added to flask containing cranberry seed fibre and then incubated at 37 °C with 100 rpm for 24 h. Samples were serially diluted in sterile saline, and the viable count of *E. coli* ATCC 25922 was enumerated by plating on eosin methylene blue agar (EMB agar; HiMedia) at 0, 6, 12, 18 and 24 h. The plates were incubated at 37 °C for 48 h. Each analysis was performed in triplicate at two different occasions. Average mean of viable counts is expressed in log_{10} cfu per mL.

Statistical analysis

The values were calculated as the mean of individual experiments in triplicate, and viable count of *B. coagulans* MTCC 5856 and *E. coli* ATCC 25922 was expressed in log_{10} CFU. The data presented are the average of the three determinations. Differences between two mean values were calculated by Student's

t-test. The chosen level of significance for all statistical tests was 5% ($P < 0.05$).

Results

Gastric acid and pancreatic enzyme digestibility of cranberry seed fibre

The effect of gastric acid hydrolysis on CSF was determined in an *in vitro* model mimicking *in vivo* conditions. There were 26.6% increase and 11% increase in the reducing sugars after 180 min of acid hydrolysis in potato starch and FOS, respectively. However, in the case of cranberry seed fibre, there was only 1.4% increase in the reducing sugars after 180 min of gastric acid treatment (Fig. 1). This suggested the nondigestibility of CSF during gastric acid hydrolysis. Further, CSF was evaluated for porcine pancreatic enzyme digestibility along with potato starch and FOS. The increase in reducing sugar was 4.4% and 2.2% in cranberry seed fibre and FOS, respectively, after 180 min of pancreatic enzyme hydrolysis (Fig. 2). However, potato starch which was taken as the positive control for the above study showed 47% increase in reducing sugars after 180 min of pancreatic enzyme hydrolysis. The data of the study indicated that the CSF was comparable with FOS for pancreatic enzyme nondigestibility.

Fermentation

An *in vitro* experiment was designed to evaluate cranberry seed fibre as sole source of nutrition for the growth of *B. coagulans* MTCC 5856. The effect of CSF on the viability of *B. coagulans* MTCC 5856 was concentration- and time-dependent (Fig. 3). There was 2.3 Log_{10} cfu mL^{-1} increase in the viable count of *B. coagulans* MTCC 5856 at a concentration of 2.0% of CSF after 24 h of incubation (Fig. 3). The increase in the viable count of *B. coagulans* MTCC 5856 was comparable in CSF group and in nutrient media (MRS) (Fig. 3).

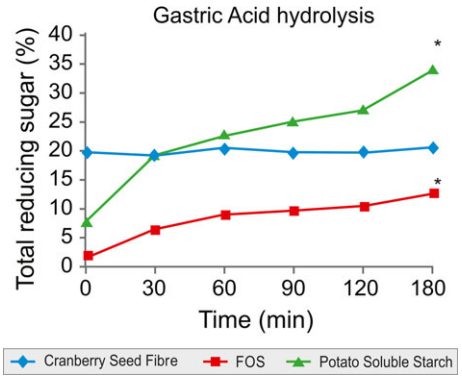

Figure 1 Effect of gastric acid hydrolysis on the cranberry fibres. Potato starch and FOS showed significant increase in the reducing sugars after 180 min of gastric acid treatment compared to initial content ($P < 0.05$). Each value is the mean ± SD ($n = 3$). *$P < 0.05$ (Student's t-test). [Colour figure can be viewed at wileyonlinelibrary.com]

Growth inhibition of *E. coli* ATCC 25922

The effect of *B. coagulans* MTCC 5856 on the growth of *E. coli* ATCC 25922 was investigated in a coculture model when growing in cranberry seed fibre. The

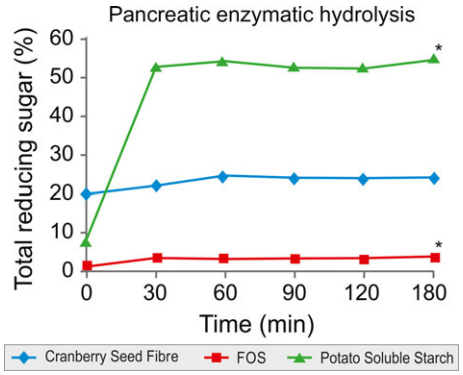

Figure 2 Effect of pancreatic enzyme digestibility on the cranberry fibres. Potato starch used as control in the experiment showed significant increase in the reducing sugars after 180 min of gastric acid treatment compared to initial content ($P < 0.05$). Each value is the mean ± SD ($n = 3$). *$P < 0.05$ (Student's *t*-test). [Colour figure can be viewed at wileyonlinelibrary.com]

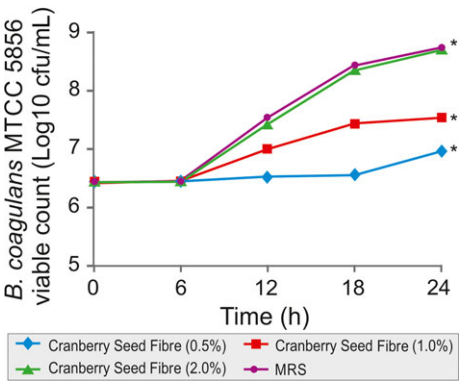

Figure 3 Effect of the cranberry fibre as a sole nutritional source on the viable count of *B. coagulans* MTCC 5856. The effect of CSF on the viable count of *B. coagulans* MTCC 5856 was in concentration-dependent manner. *B. coagulans* MTCC 5856 showed significantly higher viable count at 2.0% compared to 0.5% of CSF ($P < 0.05$). Values are average mean of triplicate performed at two different occasions and represented in log_{10} CFU per mL. *$P < 0.05$ (Student's *t*-test). [Colour figure can be viewed at wileyonlinelibrary.com]

viable count of *E. coli* ATCC 25922 was significantly higher in the MRS broth (9.321 Log_{10} cfu mL^{-1}) compared to CSF group (7.71 Log_{10} cfu mL^{-1}) after 24 h of incubation (Fig. 4). However, a significant difference in *E. coli* ATCC 25922 count was noticed when cocultured with *B. coagulans* MTCC 5856 in cranberry seed fibre group compared to MRS and CSF ($P < 0.05$). This suggested that the CSF supported the growth of the probiotic strain *B. coagulans* MTCC 5856 when cocultured with *E. coli* ATCC 25922 using cranberry seed fibre as growth medium.

Production of SCFAs

The production of SCFAs (acetic, propionic and butyric acids) while fermenting CSF by *B. coagulans* MTCC 5856 was evaluated at different time intervals and compared with FOS and dextrose (MRS media). There was a time-dependent production of SCFAs by the *B. coagulans* MTCC 5856 while fermenting CSF, FOS and dextrose (MRS media). At 24 h, maximum production of propionic acid was recorded in CSF group (69.07 ± 0.3 mg g^{-1}) followed by FOS (21.61 ± 0.4 mg g^{-1}) and MRS (1.04 ± 0.01 mg g^{-1}) (Table 1). However, the production of acetic acid was highest in MRS group (113.07 ± 1.0 mg g^{-1}) followed by CSF (8.64 ± 0.01 mg g^{-1}) and FOS group (1.07 ± 0.01 mg g^{-1}). The lowest amount of SCFAs produced in all groups was butyric acid. However, CSF had the highest amount (0.22 ± 0.01 mg g^{-1}) of butyric acid produced after 24 h of incubation compared to FOS (0.07 ± 0.001 mg g^{-1}) and MRS (0.05 ± 0.001 mg g^{-1}).

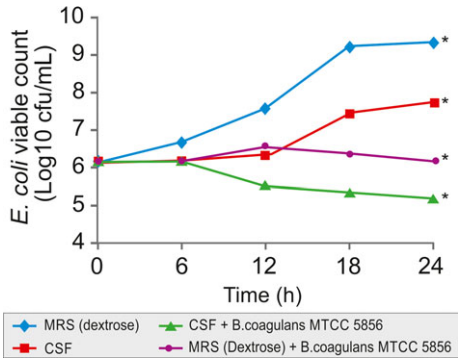

Figure 4 Inhibitory effect of *B. coagulans* MTCC 5856 on the viability of *E. coli* ATCC 25922 when cocultured in cranberry seed fibres. *B. coagulans* MTCC 5856 significantly inhibited *E. coli* ATCC 25922 growth when cocultured in MRS media and CSF compared to respective controls ($P < 0.05$) after 24 h of incubation. Values are average mean of triplicate performed at two different occasions and represented in log_{10} CFU per mL. *$P < 0.05$ (Student's *t*-test). [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

The term prebiotic was defined as 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health' (Gibson *et al.*, 2004). Prebiotics have been reported for various health benefits such as relieving constipation, reducing the risk of cardiovascular diseases, boosting immunity, helping to reduce cholesterol, production of bacteriocin and to enhance gut health (Gibson *et al.*, 2004). In the current study, we report the prebiotic potential of cranberry seed fibre from cranberry fruit. One of the important criteria to qualify a fibre as prebiotic fibre is that it must be resistant to digestion in upper gastrointestinal tract and made available to the colonic bacteria as a substrate for the fermentation process (Patel & Goyal, 2012). Further, fermentation of dietary fibres and prebiotics in colon by the probiotics is an important mechanism of action to confer the health benefits (Gibson *et al.*, 2010). This unique feature of a prebiotic fibre helps to modulate the gut flora by increasing the probiotic count and reducing the pathogenic organisms count in the gut (Joanne, 2013). Growth of the *B. coagulans* MTCC 5856 was concentration-dependent when subjected to different concentrations (0.5–2.0%, w/v) of cranberry seed fibre. The difference in the growth of *B. coagulans* MTCC 5856 might be due to the difference in the available quantity of prebiotic fibre. The presence of fermentable prebiotic fibre in cranberry seed fibre may be responsible for the increase in the viable count of *B. coagulans* MTCC 5856 in an *in vitro* fermentation study. The rate of fermentation is influenced by the degree of polymerisation, glycosidic linkage, nature of fermentation and relationship among the bacteria with fibre (Holscher, 2017). The fermentation in colon by the microflora is important on the host health. The growth and metabolism of microorganisms, specifically probiotic bacterial species inhabiting the large intestine, depend on the substrates available to them (George & John, 1999). Intake of fermentable dietary fibre plays an important role to modify the structure and metabolic activities of such microbial community in gut. Consequently, the fermentation of such fibre by the probiotic bacteria leads to the production of several micronutrients which are responsible for host health such as boosting the host natural resistance, promoting healthy digestion and improving the balance of gut microflora (Holscher, 2017). This is first time that the utilisation and fermentation of CSF by the probiotic bacteria *B. coagulans* MTCC 5856 have been reported, and the report suggested that *B. coagulans* grew very well by utilising CSF as sole nutritional source. Furthermore, the

Table 1 Production of short-chain fatty acids (acetic acid, propionic acid and butyric acid) from the cranberry fibre, FOS, dextrose (MRS) after 6, 12 and 24 h *in vitro* batch culture fermentation with *B. coagulans* MTCC 5856

Short-chain fatty acids	Substrates	Short-chain fatty acids (mg g^{-1} of substrate)		
		6 h	12 h	24 h
Acetic acid	Cranberry fibre	2.01 ± 0.01^{a}	3.21 ± 0.02^{a}	8.64 ± 0.01^{b}
	FOS	0.41 ± 0.001^{c}	0.51 ± 0.01^{c}	1.07 ± 0.01^{d}
	MRS (dextrose)	10.21 ± 0.02^{e}	42.11 ± 0.2^{f}	113.07 ± 1.0^{g}
Propionic acid	Cranberry fibre	11.11 ± 0.03^{h}	31.21 ± 0.1^{i}	69.07 ± 0.3^{j}
	FOS	3.21 ± 0.01^{k}	9.54 ± 0.01^{l}	21.61 ± 0.4^{m}
	MRS (dextrose)	0.32 ± 0.005^{n}	0.41 ± 0.01^{n}	1.04 ± 0.01^{o}
Butyric acid	Cranberry fibre	0.05 ± 0.001^{p}	0.12 ± 0.01^{p}	0.22 ± 0.01^{q}
	FOS	0.01 ± 0.001^{r}	0.02 ± 0.001^{r}	0.07 ± 0.001^{o}
	MRS (dextrose)	0.005 ± 0.0001^{s}	0.01 ± 0.001^{s}	0.05 ± 0.001^{t}

Values are average mean of triplicate performed at two different occasions and represented in mg per g of substrate. Different letters in the same row indicate significant differences ($P < 0.05$). Values in a given row which are followed by the same letter are not statistically different ($P > 0.05$). The production of acetic acid and propionic acid by the *B. coagulans* MTCC 5856 was significantly higher in the CSF group compared to FOS and MRS (dextrose) after 24 h of incubation ($P < 0.05$).

production of short-chain fatty acids (acetic, propionic and butyric acids) by the *B. coagulans* MTCC 5856 while fermenting CSF has been reported in this experiment. Formation of high amount of SCFAs stimulates mineral uptake and water absorption which result in faster recovery from diarrhoea and prevention of mineral deficiency (Topping & Clifton, 2001). Propionic acid was reported to be the highest short-chain fatty acids produced by *B. coagulans* MTCC 5856 while fermenting the cranberry seed fibre. Propionic acid has a metabolic effect that may inhibit the synthesis of cholesterol in liver (Stark & Madar, 1993). Propionic acid and acetic acid play an important role in rumen and also induce apoptosis in human colorectal carcinoma cell lines through the loss of mitochondrial transmembrane potential (Hosseini *et al*., 2011). Further, *B. coagulans* MTCC 5856 produced notable amount of acetic acid, propionic acid and also a minimal amount of butyric acid while fermenting with CSF as the sole source of nutrition. Propionic acid and butyrate play an important role in cell cycle processes such as proliferation, differentiation and apoptosis (Comalada *et al*., 2006).

Regular consumption of cranberry fruit-derived products has been reported to suppress and prevent the infections by inhibiting uropathogenic P-fimbriated *Escherichia coli* to uroepithelial cells in the urinary tract (Howell, 2002; Shmuely *et al*., 2004). There are number of recent clinical studies conducted on cranberry for the effectiveness in reducing reoccurrence of urinary tract infections, pyuria and bacteriuria (Bianco *et al*., 2012; Takahashi *et al*., 2013). However, primarily, consumption of cranberry has been used as a strategy to reduce the clinical UTI recurrence in women with a recent history of a UTI (Stapleton *et al*., 2012). Our data suggested that the fermentation of CSF by the *B. coagulans* MTCC 5856 leads to the growth inhibition of *E. coli* ATCC 25922. The inhibition of *E. coli* ATCC 25922 could be due to the production of antimicrobial compounds produced by the *B. coagulans* MTCC 5856 or the competitive exclusion while fermenting the cranberry fibres. The combination of the CSF and *B. coagulans* MTCC 5856 could be a strategy to reduce the infection involving pathogen *E. coli*. The *E. coli* inhibition and increase in the number of *B. coagulans* MTCC 5856 while fermenting CSF in our study indicated that the application of this combination before (as prophylactic agent) or during (therapeutic) the onset of *E. coli* infection is critical and may provide better health benefits in clinical conditions.

This study has investigated functional aspects of CSF along with probiotic *B. coagulans* MTCC 5856. The application of spores of *B. coagulans* MTCC 5856 provides practical advantages as their incorporation does not require encapsulation as the spores are resistant to food processing temperatures, storage and the hostile GIT environment (Majeed *et al*., 2016b,d). Further, the use of *B. coagulans* MTCC 5856 in baking confectionary such as cake, muffins and chocolates is well established (Majeed *et al*., 2016b). Moreover, cranberry has also been one of the common ingredients of baking goods in Canada and in the United States (Mildner-Szkudlarz *et al*., 2016). Therefore, the combination of probiotic *B. coagulans* MTCC 5856 spores and CSF would be really ideal in various functional foods specifically baking foods. Thus, *B. coagulans* MTCC 5856 spores and CSF can serve in formulating shelf-stable foods as well as products that require high temperature processing. As CSF contains dietary fibre, protein, essential amino acids, fatty acids and proanthocyanidins, the combination product is expected to deliver nutritional as well as functional health benefits.

Conclusion

Cranberry seed fibre (CSF) showed resistant to gastric acid and pancreatic enzymatic hydrolysis and also increased the number of probiotic bacteria *B. coagulans* MTCC 5856, thus exhibiting prebiotic activity. The current study provides the first scientific evidence for CSF as prebiotic fibre, which is suitable in synbiotic preparation along with commercial probiotic strain *Bacillus coagulans* MTCC 5856. This study warrants further investigation of CSF to confirm the prebiotic properties with other probiotics (*Lactobacillus* spp. and *Bifidobacterium* spp.) and any cooperative activity for modulating the gut microbiota.

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The authors also recognise the following intellectual property rights for ingredients used in this study: (i) LACTOSPORE® is a registered brand name (U.S Trademark Registration No. 1701366) of Sabinsa Corporation, 20 Lake Drive, East Windsor, NJ, USA 08520. (ii) LACTOCRAN™ is a registered brand name (U.S Trademark Serial No 87681973) of Sabinsa Corporation, 20 Lake Drive, East Windsor, NJ, USA 08520. The PCT application (PCT/US2015/047608) of this study has been filed on 29 August 2015, and the USPTO has assigned (US 9 717 766) the same invention to Sabinsa Corporation/Sami Labs Limited.

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Competing interests

The authors are employees of Sabinsa Corporation/ Sami Labs Limited, manufacturer and marketer of LactoSpore®.

Authors' contributions

The manuscript was written through contributions of all authors. All authors approved the final version of the manuscript.

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