حيوية بكتيريا بفيديوبكتيريوم بفيدم (ب. ب) في الزيادي المحضر من حليب البقر والابل والمتبل بالقرفة والثوم

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الملخص:

تم دراسة تأثير المستخلص المائي للثوم والقرفة في الزيادي على بكتيريا بفيديوبكتيريوم بفيدم (ب. ب) طيلة 21 يوم من التخزين المبرد ومن خلال محاكاة الهمض المعدي- الممثلي. وتเสนอ النتائج نوعين من الزيادي المحضر من حليب البقر أو الألبال في وجود المستخلص المائي للثوم أو القرفة. وقد جد أن عدد الخلايا الحيوية من ب. ب في اليوم الأول للزيادي المحضر من حليب البقر وماضي المستخلص الثوم أو القرفة كانت أعلى معنويًا (8.1 × 10^9 وحدة- مكونة مستعمرة/مل) و (6.6 × 10^9 وحدة/مل على التوالي) من الزيادي العادي (1.9 × 10^9 وحدة/مل). بالمقابل فإن عدد الخلايا الحيوية من بكتيريا ب. ب في الزيادي الطازج المحضر من حليب البقر قبل التجفيف كانت 1.99 × 10^9 وحدة/مل بينما أدى وجود مستخلص الثوم أو القرفة في الزيادي إلى زيادة معنوية في عدد البكتيريا إلى 1.961 × 10^9 وحدة/مل والثوم والصامد في الزيادي العادي و (5.25 × 10^9 وحدة/مل على التوالي). وكذلك فإن عدد الخلايا الحيوية من ب. ب في كل أنواع الزيادي الحاوي على مستخلص الإعشاب والمحضر من حليب البقر أو الابل انخفضت معنويًا خلال التخزين المبرد في الطلاء. كان عدد الخلايا الحيوية من ب. ب تقريبًا 1.3 × 10^9 وحدة/مل في نوعي زيادي الإعشاب الطازج المحضر من حليب البقر بعد فترة من الهمض في المعدة. أما الهضم المعدي لمدة ساعة أي 7 أيام (الزيادي العادي والزيادي الحاوي على خلاصة الثوم). ولكن فإن الطاقة الهضم المعدي لمدة أخرى أدى إلى خفض معنوي في عدد الخلايا الحيوية من ب. ب في كل أنواع الزيادي الطازج ولكن ليس في أنواع الزيادي التي عمرها 7 أيام (الزيادي العادي والزيادي الحاوي على خلاصة الثوم). ولكن فإن إطالة الهضم المعدي لمدة أخرى أدى إلى خفض معنوي في عدد الخلايا الحيوية من ب. ب. في كل أنواع الزيادي الطازج والصامد. بالمقابل فإن الزيادي الطازج المحضر من حليب البقر يظهر اعدادًا من الخلايا الحيوية من ب. ب أقل أو يساوي 10^9 وحدة/مل بعد الهمض في جهاز محاكاة الهمض المعدي- المعدي. هذا وقد أظهرت الزيادي الثوم المحضر من حليب البقر والمخلز في الطلاء لمدة 7 أيام خلايا حيوية أقل لعدد البكتيريا بعد الهمض في المعدة مقارنة بالزيادي العادي والزيادي القرفة. كذلك فإن إعداد الخلايا الحيوية من ب. ب انخفضت معنويًا في كل أنواع الزيادي المحضر من حليب البقر بعد ساعتين من الهمض في الامعا. في الختام فإن المستخلص المائي للثوم القرفة قد حسن نمو ب. ب. في كلا النوعين من الزيادي أثناء التخزين في الطلاء ولكن ليس أثناء الهمض.
Survival of Bifidobacterium bifidum in cow- and camel-milk yogurts enriched with Cinnamomum verum and Allium sativum

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Abstract The effects of Allium sativum and Cinnamomum verum water extracts on the survival of Bifidobacterium bifidum during 21 days of refrigerated storage and after simulated gastrointestinal digestion (SGD) were investigated. Two types of yogurt (cow- and camel-milk yogurts) were prepared in the presence of A. sativum or C. verum. The viable cell counts (VCC) of B. bifidum in fresh A. sativum- or C. verum-cow milk yogurt (1 day) were higher than plain-yogurt (1.9 x 10^9 cfu/ml). In contrast, B. bifidum VCC in fresh plain-camel milk yogurt was 1.99 x 10^9 cfu/ml whereas the presence of A. sativum or C. verum in yogurt increased (p < 0.05) VCC to 19.61 x 10^9 cfu/ml and 25.55 x 10^9 cfu/ml, respectively. The VCC of B. bifidum in both herbal-yogurts decreased (p < 0.05) during refrigerated storage for both types of yogurt. The VCC of B. bifidum was 1.3 x 10^9 cfu/ml in all fresh cow milk yogurts after 1 h gastric digestion. Intestinal digestion (1 h) increased VCC of B. bifidum in all fresh yogurts but not in 7 day old yogurts (plain- and A. sativum-yogurts). However, prolonged digestion to another 1 h in intestine reduced (p < 0.05) VCC of B. bifidum in all fresh and storage yogurts. In contrast, all fresh camel milk yogurts showed VCC of B. bifidum ≤1 x 10^9 cfu/ml after SGD. Seven day old A. sativum – camel milk yogurt showed the lowest survival of B. bifidum after gastric digestion compared to plain- and C. verum-yogurt. The VCC reduced (p < 0.05) in all camel milk-yogurts after 2 h intestinal digestion.

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1. Introduction

Nowadays, there has been a worldwide increasing interest about the survival of probiotic bacteria in yogurt. Probiotics are live microorganisms that provide health benefits on the host when administered in sufficient amounts (Wang et al., 2012). Yogurts containing probiotics are claimed to provide
several health benefits such as improve lactose utilisation (De Vrese et al., 2001), prevent cancer (Rafter, 2003), maintain intestinal microflora balance (Mainville et al., 2005) and reduce serum cholesterol level (Baroutkoub et al., 2010). Moreover, yogurt containing *Bifidobacterium bifidum* Bb-12 improved immunoglobulin A (IgA) production in the intestine that enhances local immunity against gastrointestinal infection (Kabeerdoss et al., 2011). It also has inhibitory effects on commonly known food borne pathogens (Goderska and Czarnecki, 2007) and ability to control intestinal infections by producing inhibitory/antimicrobial substances such as organic acids, hydrogen peroxide, deconjugated bile acids, antibiotics and bacteriocins (Schiffrin and Blum, 2001).

Viable numbers of probiotics in the final product suggested being at least 10^{9–10} CFU/g to be accepted as the therapeutic and bacteriocins (Schiffrin and Blum, 2001). The ability of probiotic bacteria to survive through the gastrointestinal tract varies according to species and even strain-dependent (Wattiaux and Howard, 2000). In addition, functional properties of this probiotic can be affected by the food matrix used in delivery (Lahtinen et al., 2007; Ranadheera et al., 2012) because the buffering capacity of food would help to enhance the viability of probiotics during gastric transit (Kailasapathy and Chin, 2000; Mainville et al., 2005). Ranadheera et al. (2012) reported that the addition of certain ingredients such as cocoa powder and stabilizers guar gum and dextrose in the ice cream enhanced the viability of probiotics by providing some protection. Other study showed that the presence of *Allium sativum* or *Cinnamomum verum* in yogurt enhanced the growth of lactic acids’ bacteria (Shori and Baba, 2012). The objective of this work is to evaluate the viability of *B. bifidum* in *C. verum* - or *A. sativum*-yogurt during 21 days of refrigerated storage and the survival of these bacteria after simulated gastrointestinal digestion.

2. Materials and methods

2.1. Plant water extraction

Commercially available dried *A. sativum* or *C. verum* powder was mixed with sterile dH_{2}O in the ratio of 1:10 in a 250 ml bottle. The final concentration of both herbal extracts was 0.1 g/ml. The mixture was left for 12 h (Shori and Baba, 2011a) in a water bath at 70 °C (Julabo, Model Sw-21c) followed by centrifugation (1000 rpm, 15 min at 4 °C). The supernatant was removed and used as herbal water extract in the making of herbal-yogurt.

2.2. Preparation of starter culture and bio-yogurt

Commercially available direct vat set (DVS) starter culture powder used in yogurt preparation consisting of a mixture of *Lactobacillus acidophilus* LA-5, *Bifidobacterium Bb-12*, *Lactobacillus casei* LC-01, *Streptococcus thermophilus* Th-4 and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Chris-Hansen, Denmark) was in the ratio of 4:4:1:1:1. The preparation of starter culture from cow or camel milk was carried out using the method described by Shori and Baba (2011b). Two groups of bio-yogurt made from cow and camel milk and three types of yogurt (plain-, *A. sativum*- and *C. verum*-yogurts) were prepared in each group as described by Shori and Baba (2011b).

2.3. In vitro gastrointestinal model

2.3.1. Preparation of gastric and duodenum juices

The gastric and duodenum solutions were freshly prepared according to the protocols described by Huang and Adams (2004). To simulate the *in vivo* saliva, 100 ml of a sterile electrolyte solution (6.2 g/l NaCl, 2.2 g/l KCl, 0.22 g/l CaCl_{2}, 1.2 g/l NaHCO_{3}) was added to lysozyme (10 mg) to obtain a final concentration of 100 ppm. To simulate the stomach environment (gastric juice), the electrolyte solution was added to 0.3% pepsin and the pH was adjusted to 3 by adding 5 M HCl. To simulate the intestinal digestion (duodenum juice), the electrolyte solution (6.4 g/l NaHCO_{3}, 0.239 g/l KCl, 1.28 g/l NaCl) containing 0.3% bile salts and 0.1% pancreatin (v/w concentrations) was adjusted to pH 7.2 by using 5 M NaOH.

2.3.2. Simulation of gastrointestinal digestion (SGD)

Yogurt samples were mixed with the artificial saliva solution in the ratio of 1:1 followed by incubation at 37 °C for 5 min. Samples were then mixed with artificial gastric fluid solution in the ratio of 3:5 prior to a second incubation at 37 °C for 1 h. After 1 h, 30 ml of samples from the “stomach digestion” was taken out for analysis. The remaining solutions from “stomach digestion” were then mixed with artificial duodenal secretion in the ratio of 1:4 followed by a third incubation at 37 °C for 2 h. Samples (30 ml) were taken out for analysis after every hour interval of “intestinal digestion”. All samples were manually agitated and stirred intermittently during the incubation time in order to ensure adequate enzymatic digestion to mimic gastrointestinal movement.

2.4. Viable cell counts (VCC) of *B. bifidum*

Cultures of *B. bifidum* were enumerated using MRS-LP agar. The formulation of MRS-LP was prepared according to Vinderola et al. (2000) where 0.2% (w/v) of lithium chloride (solid–powder) and 0.3% (w/v) of sodium propionate (solid–powder) were added to the MRS media (62 g/930 L dH_{2}O, 45 °C). Yogurt samples (1 ml) were mixed with 9 ml of 0.15% sterile buffered peptone water (20 g/L dH_{2}O). The mixture was thoroughly stirred and serial decimal dilutions were prepared by using buffered peptone water. One millilitre of diluted yogurt with buffered peptone water was mixed with 15 ml of autoclaved melted MRS–LP media using the pour plate method. The probiotic cultures were anaerobically incubated (GasPak System-OXOID) at 37 °C for 72 h. The viable *B. bifidum* counts were calculated (Sivakumar and Kalaiaarasu, 2010) as follow:

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\text{CFU} = \text{Number of colonies formed} \times \text{dilution factor of sample} / 1 \text{ ml of sample}
\]

*CFU: colony forming unit.*
2.5. Statistical analysis

The experiment was carried out using three different batches of yogurt (n = 3). Data were expressed as mean ± SE. The statistical analysis was performed using one way analysis of variance (ANOVA, SPSS 14.0), followed by Duncan’s post hoc test for mean comparison. The criterion for statistical significance was p < 0.05.

3. Results

3.1. VCC of B. bifidum in yogurt during storage (4°C)

Both fresh A. sativum- and C. verum-yogurts made from cow milk showed higher VCC of B. bifidum (8.10 × 10⁹ cfu/ml and 6.59 × 10⁹ cfu/ml, respectively) than plain-yogurt (1.89 × 10⁹ cfu/ml; Fig. 1). Refrigerated storage of plain-yogurt increased B. bifidum VCC to the highest counts (2.68 × 10⁹ cfu/ml) on day 7 followed by significant decrease to 0.26 × 10⁹ cfu/ml by day 21 of storage. The VCC of B. bifidum in A. sativum- and C. verum-yogurts decreased during refrigerated storage but they were still higher than plain-yogurt even on day 21 of storage (0.48 × 10⁹ cfu/ml and 1.01 × 10⁹ cfu/ml, respectively; Fig. 1).

The VCC of B. bifidum in fresh plain-yogurt made from camel milk was 1.99 × 10⁹ cfu/ml (Fig. 2). The presence of A. sativum or C. verum in yogurt increased the VCC to 19.61 × 10⁹ cfu/ml and 25.55 × 10⁹ cfu/ml, respectively. Refrigerated storage of yogurt up to 7 days increased the VCC of B. bifidum in plain-yogurt (6.05 × 10⁹ cfu/ml) followed by reduction to 0.75 × 10⁹ cfu/ml on day 21 of storage (Fig. 2). The VCC of B. bifidum in both A. sativum- and C. verum-yogurts decreased to 1.41 × 10⁹ cfu/ml and 1.11 × 10⁹ cfu/ml for A. sativum- and C. verum-yogurts, respectively on day 21 of storage.

3.2. VCC of B. bifidum after SGD

The VCC of B. bifidum in cow milk both in the presence and absence of A. sativum or C. verum water extract were less than 1 × 10⁹ cfu/ml during 3 h SGD (Fig. 3). Similar VCC was shown in all fresh yogurts (~1.3 × 10⁹ cfu/ml, 1 day) after 1 h gastric digestion. Intestinal digestion (1 h) increased the VCC of B. bifidum to the highest value in plain-yogurt (33.4 × 10⁹ cfu/ml) followed by C. verum- (30.4 × 10⁹ cfu/ml) and A. sativum- (6.6 × 10⁹ cfu/ml) yogurts. Prolonged digestion for another 1 h reduced the VCC of B. bifidum to 2.4 × 10⁹ cfu/ml for both plain- and C. verum-yogurts and to 0.8 × 10⁹ cfu/ml for A. sativum-yogurts (Fig. 3). The VCC of B. bifidum in refrigerated storage (7 days) plain-yogurt was 49.9 × 10⁹ cfu/ml after 1 h gastric digestion. The VCC was not affected by either the presence of A. sativum or C. verum in yogurt (0.6 × 10⁹ cfu/ml and 12.4 × 10⁹ cfu/ml, respectively). Intestinal digestion (1 h) decreased VCC of B. bifidum in plain-
and A. sativum-yogurts (32.9 × 10⁹ cfu/ml and 0.2 × 10⁹ cfu/ml, respectively) but not in C. verum-yogurt (44.5 × 10⁹ cfu/ml; p < 0.05). A further one hour digestion in intestinal section decreased VCC to the lowest value in A. sativum-yogurt (0.04 × 10⁹ cfu/ml) followed by plain- (13.69 × 10⁹ cfu/ml) and C. verum- (19.04 × 10⁹ cfu/ml) yogurts (Fig. 3).

The VCC of B. bifidum in all camel milk treatments at initial time of fermentation (0 h) and after fermentation (1 day) were ≤1 × 10⁹ cfu/ml (Fig. 4). Refrigerated storage (7 days) of plain- and C. verum-yogurts showed similar VCC of B. bifidum after 1 h gastric digestion (66.0 × 10⁹ cfu/ml) whereas A. sativum-yogurt had only 9.7 × 10⁹ cfu/ml VCC of B. bifidum. Intestinal digestion for 2 h reduced VCC to 4.85 × 10⁹ cfu/ml, 0.50 × 10⁹ cfu/ml and 5.55 × 10⁹ cfu/ml for plain-, A. sativum- and C. verum-yogurts, respectively.

4. Discussion

The survival of probiotic microflora in yogurt is governed by physicochemical factors such as yogurt acidity, dissolved oxygen, species interaction and storage conditions (Rybka and Kailasapathy, 1995). The present study showed that the addition of A. sativum or C. verum water extract in both cow- and camel-milk yogurts increases (p < 0.05) the VCC of B. bifidum compared to the respective plain-yogurts during 21 days of storage. This could be related to the essential growth factors present in A. sativum or C. verum such as vitamins, minerals, amino acids and polyphenolics (Abdullah et al., 1988; Al-Numair et al., 2007). The presence of higher free amino groups in herbal-yogurt made from camel- than cow-milk (Shori and Baba, 2011a,b) may explain the higher VCC of B. bifidum in the former than in the latter. Furthermore, the higher buffering capacity in camel milk than cow milk (Ramet, 2001) may help to stabilise the pH in yogurt (Shori and Baba, 2011a,b) thus allowing more B. bifidum growth prior to the development of inhibitory acidic environment. The present study showed significant reduction in B. bifidum VCC of A. sativum- and C. verum-yogurts made from either cow or camel milk during refrigerated storage. This observation was in agreement with Vinderola et al. (2000) whereby the reduction of VCC of B. bifidum was shown dependent on the milk type. Thus, the faster reduction (p < 0.05) of these bacteria in herbal- camel-milk- than cow milk-yogurts after 7 days of storage would suggest that specific milk composition in the former may be responsible for the reduction. In particular, the anti-microbial compounds are present in higher concentrations in camel milk than other mammalian milk (El-Agamy et al., 1992). In vitro limited tolerance of probiotic strains to gastric acid has been demonstrated elsewhere (Mishra and Prasad, 2005; Madureira et al., 2011). In the present study, the effect of A. sativum or C. verum on the survival of B. bifidum was dependent on milk type and type of herbal extract used. During intestinal digestion, B. bifidum in C. verum-cow milk yogurt showed the ability to grow in such condition and/or recover from sub lethally-injured cells. This may suggest possible interaction between phenolic compounds and cow milk proteins that could provide considerable protection for B. bifidum against exposure to intestinal juice. This possibility shown also from Ranadheera et al. (2012) suggested that the addition of ingredients i.e., cocoa powder and stabilizers (guar gum and dextrose) in goat milk ice cream has provided protection towards probiotic survival during simulated gastrointestinal digestion. Camel milk was reported to have a higher antimicrobial lactoperoxidase system (Anonymous, 2003) which may be caused by further inhibitory effects on B. bifidum growth. Prolonged exposure to intestinal digestion (2 h) showed substantial reduction of B. bifidum VCC in both types of yogurt. This is in agreement with other studies of (Saxelin et al., 2010; Vinderola et al., 2011; Ranadheera et al., 2012) who related that to the antimicrobial nature of bile salt that arises mainly from its detergent property.

5. Conclusion

The growth of B. bifidum improved in the presence of A. sativum or C. verum water extract in both cow- and camel-milk yogurts during fermentation and they continued to survive even during refrigerated storage. A. sativum- and C. verum-yogurts made from either cow or camel milk provided higher viable B. bifidum over 2 weeks of storage. Therefore, these yogurts may be considered as probiotic yoghurt with promising therapeutic properties upon daily consumption.

References


