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ORIGINAL ARTICLE

Associative growth behavior of dahi and yoghurt starter cultures with *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in buffalo skim milk

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Abstract We report here for the first time variations in the viability and biochemical activity of dahi and yoghurt cultures, when grown together with therapeutic cultures, such as *Lactobacillus acidophilus* I and *Bifidobacterium bifidum* R, in buffalo skim milk. Nearly one log reduction in mesophilic lactic count was observed in dahi supplemented with probiotic cultures after 18 h of incubation at 30 °C. Associative growth increased the titratable acidity (TA) of dahi marginally (from 0.93 to 1.18 % lactic acid) but reduced the TA in yoghurt (from 1.68 to 1.44 % lactic acid). Probiotic culture supplementation reduced volatile acidity (VA) (from 36.0 to 15.8 ml) and diacetyl (from 4.05 to 2.80 ppm) and tyrosine (from 0.46 to 0.36 μg tyrosine/g curd) content in dahi, whereas it increased VA (from 8.2 to 8.6 ml of 0.01 % NaoH/50 g) and acetaldehyde (from 28.4 to 34.6 ppm) production in yoghurt. Based on these results, the associative growth had no effect on proteolytic activity of probiotic yoghurt.

Keywords Associative growth · Acidophilus · Bifidobacteria · Dahi · Yoghurt · Buffalo skim milk

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Introduction

Selected intestinal therapeutic cultures, such as bifidobacteria and *Lactobacillus acidophilus*, which are capable of growth and acid production in milk, are being used in the manufacture of various fermented milks (Leroy and De Vuyst 2004), with stress being placed on the potential beneficial role of these fermented milks in the human gut (Goldin and Gorbach 1984). The use of such cultures, which can synthesize bacteriocins, vitamins and essential amino acids and establish a stable normal flora in the intestines has been advocated to enhance the therapeutic value and dietary qualities of the fermented milk products and infant formulae (Gilliland 1989; Santosa et al. 2006; Shah 2007; Shamekhi et al. 2011; Tham et al. 2011; Vijayendra and Gupta 2011). Many reviews on functional dairy products describing the beneficial role of these organisms are available (Marshall and Tamime 1997; Ooi and Liong 2010; Özer and Kirmaci 2010; Shah 2007; Sarkar 2008).

A wide variety of fermented milk products containing bifidobacteria and/or acidophilus bacteria along with either mesophilic or thermophilic starters have been developed in the past few years. Different strains of bifidobacteria and *L. acidophilus* have been used together with the mesophilic lactic starters to prepare the progurt and acidophilus buttermilk products (Kurmann and Rasic 1988). Klupsch (1984) reported a fermented milk drink that was fermented by lactic mesophilic bacteria (*Lactococcus lactis*, *L. cremoris*, *L. lactis* ssp. *lactis* biovar. *diacetylactis*) along with a mixed culture of *L. acidophilus* and bifidobacteria (in a ratio of 1:3)). Yadav et al. (2007) reported the preparation of probiotic dahi using *L. acidophilus* and *L. casei* as adjunct cultures along with mesophilic lactic cultures. In order to improve the nutritive and therapeutic value of yoghurt,

attempts have been made to supplement *Bifidobacterium bifidum* and *Lactobacillus acidophilus* to yoghurt cultures (Tamime and Robinson 1988; Vijayendra and Gupta 2011).

Numerous instances of associative inter-relationships among microorganisms are found in nature. Lactic acid bacteria (LAB) in particular have the ability to enter into associative relationships with other microbes (Ramachandran and Shah 2010). Yet the associative and especially symbiotic relationships of LAB with probiotic populations remain very poorly explored (Oliveira et al. 2011, 2012a, b), although the strong influence of co-cultures on fermented milk quality has been reported (Oliveira et al. 2012a). The chemical nature of the associative action in terms of its effect on acidity development, flavor production and proteolytic activity, which in turn determines the technological quality of the fermented milk products, is not known. Likewise, reports dealing with the effect of associative growth between dahi cultures with bifidobacteria and *L. acidophilus* are not available. However, the fermentation characteristics of individual probiotic cultures have been studied in soymilk (Li et al. 2012). Although the biological, biochemical, technological and therapeutical properties of these cultures in terms of their relevance to dairy products have been reviewed (Gomes and Xavier 1999; Roy 2005; Dewan and Tamang 2007), information is still lacking on the associative growth pattern between therapeutic cultures and dahi and yoghurt. Such information is necessary in order to increase our knowledge of the quality of dahi and yoghurt when therapeutic cultures are used along with the conventional cultures of the respective products. With this background, the aim of our study was to investigate the associative growth pattern of dahi and yoghurt cultures in the presence of bifidobacteria and *L. acidophilus*. This is the first report on the associative behavior of dahi and yoghurt cultures with these cultures in buffalo skim milk (BSM).

Materials and methods

Materials

The starter cultures used for the preparation of dahi (mesophiles, designated as D, containing *Lactococcus lactis* ssp. *lactis* C10, *L. lactis* ssp. *cremoris* C1, *L. lactis* ssp. *lactis* biovar. *diacetylactis* DRC10 in a 1:1:1 ratio), yoghurt (thermophiles, designated as Y, containing *Streptococcus salivarius* ssp. *thermophilus* HST and *Lactobacillus delbrueckii* ssp. *bulgaricus* RTS in a 1:1 ratio), *Bifidobacterium bifidum* I and *Lactobacillus acidophilus* R were collected from the culture collection center of the National Dairy Research Institute, Karnal, India (Vijayendra and Gupta 2011). All mesophilic and thermophilic lactic cultures including *L. acidophilus* were grown in chalk litmus milk, incubated at 30 °C and 37 °C, respectively, and maintained at 4 °C till use. *B. bifidum* I was grown at 37 °C in deMan Rogosa and Sharpe (MRS) broth supplemented with filter-sterilized 0.1 % N-acetyl-D-glucosamine and 0.05 % cysteine hydrochloride solutions and stored at 5 °C until use. For routine use, this culture was grown anaerobically in yeast dextrose milk containing 0.1 % yeast extract and 1.0 % dextrose in BSM. *B. bifidum* I and *L. acidophilus* R were added in the ratio of 3:1 to culture combinations of dahi (D) and yoghurt (Y), and designated as DAB and YAB, respectively.

Preparation of the samples

Samples of freshly prepared BSM (200 ml) were poured into 500-ml Erlenmeyer flasks and sterilized at 121 °C for 15 min. After cooling to room temperature, the milk samples were inoculated with 1 % of either the D, DAB, Y and YAB culture combinations. One set of milk samples mixed with culture combinations D and DAB were incubated at 30 °C; another set of milk samples having culture combinations DAB, Y and YAB were incubated at 37 °C. To evaluate the performance of the cultures prepared in various combinations, samples were withdrawn aseptically at regular intervals (6, 12 and 18 h post-inoculation), and the viability and biochemical activity of the cultures were determined (Fig. 1).

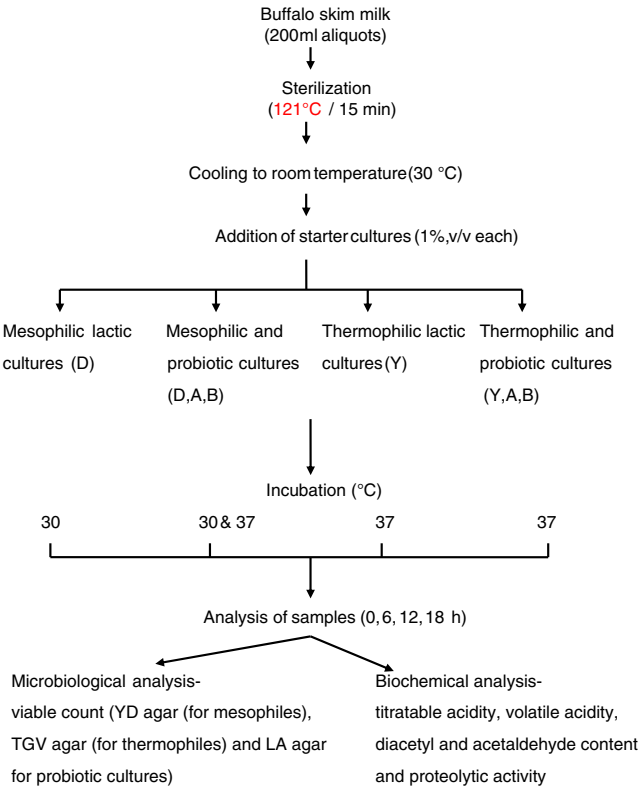

Fig.1 Schematic flow chart of the experiment conducted to study the associative growth behaviour of dahi and yoghurt starter cultures with probiotic cultures in buffalo milk (BSM). See Table 1 for the culture codes. *YD* Yeast dextrose, *TGV* tryptone glucose vegetable

Determination of viable count

To determine the viable count, the samples were serially diluted in sterile phosphate buffer. Appropriate dilutions of the dahi culture combinations was spread on yeast dextrose (YD) agar and LA agar (Klupsch 1984) plates, whereas the formulated yoghurt culture combinations were spread onto tryptone glucose vegetable (TGV) agar and LA agar plates. The YD agar and TGV agar plates were incubated aerobically at 30 °C and 37 °C, respectively, and the LA agar plates were incubated at 37 °C under anaerobic conditions. The viable count was made after 48 h of incubation, and the results were expressed in terms of log colony forming units (CFU) per gram. On LA agar plates, the elevated chocolate brown-coloured colonies were considered to be *B. bifidum* and the larger, flat grayish-coloured colonies were counted as *L. acidophilus*.

Biochemical performance of mixed cultures

The titratable acidity (TA) of the samples (10 ml each) was determined by titration against 0.1 N sodium hydroxide (NaoH); acidity was expressed in terms of lactic acid as %TA. The activity of the cultures was determined according to the procedure of Horrall and Elliker (1950). Volatile acidity (VA) of the samples was determined using the method of Hempenius and Liska (1968) by adding 50 g of the sample to a tube containing 3 ml of 1 N sulphuric acid and antifoam A–silicone oil [poly(dimethylsiloxane-co-methylphenylsiloxane)] and subjecting the combination to steam distilling. The first 100 ml of the fraction collected was titrated against 0.01 N NaoH using 0.1 % phenolphthalein indicator. The VA was expressed as millilitres of 0.01 N NaoH per 50 g of sample. The diacetyl and acetaldehyde contents of the samples were determined by the method of Pack et al. (1964) and Lindsay and Day (1965), respectively, and expressed in parts per million (ppm). The proteolytic activity of the samples was determined by the method of Hull (1947), and the tyrosine content was expressed as milligrams per gram sample.

Statistical analysis

The results are presented as the means of replicates ± the standard deviation (SD). The data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) method (Senedcor and Cochran 1967).

Results and discussion

Effect on viable cell count

Viable cell counts (VCC) increased up to 18 h of incubation but decreased considerably thereafter with incrasing incubation time (Table 1). The mesophilic lactic count of dahi in the combination DAB after 18 h of incubation was comparatively less at 37 °C than when grown at 30 °C, which indicates the necessity of using the optimum temperature for obtaining the maximum VCC. Higher counts of bifidobacteria and *L. acidophilus* of dahi in the combination DAB were obtained at an incubation temperature of 37 °C than at 30 °C, possibly due to the higher optimum temperature requirement for these cultures. There was nearly a one log difference in mesophilic count in dahi in the combinations D and DAB after 18 h of incubation at 30 °C, which can be attributed to the presence of the comparatively high acid-producing *L. acidophilus* in the latter combination, which might have affected the growth of the mesophiles. This effect was more prominent at 37 °C, the temperature at which the maximum growth rate of *L. acidophilus* was achieved. Higher VCC were achieved for both *L. acidophilus* R and *B. bifidum* I at 37 °C than at 30 °C, indicating the need for incubation at 37 °C to achieve higher counts of these cultures and ensure adequate therapeutic properties. Czulak and Hammond (1954) found increased starter activity of the same mesophilic cultures when grown together. Kim (1990) found higher counts of *Streptococcus lactis* and *S. lactis* ssp. *cremoris* or of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus* when cultured in mixed cultures than when grown individually.

The observations pertaining to yoghurt culture combinations indicated a very slight decrease in the VCC of yoghurt made with the combination YAB than with the combination Y after 18 h of incubation at 37 °C. At this temperature the VCC of therapeutic cultures, especially of bifidobacteria, was comparatively less in yoghurt made with YAB than in dahi in the combination DAB, which may be due to the presence of high acid-producing cultures of yoghurt, the acidity of which might have hampered growth. Similar to our findings, a viability of bifidobacteria exceeding 7 log CFU/ml after 24 h of culture in fermented soy milk was reported recently by Yeo and Liong (2010). In a study involving probiotic bacteria and other starter cultures, Vinderola et al. (2002) observed that starter cultures were more highly inhibited by probiotic bacteria, such as *L. acidophilus* and *Bifidobacterium* spp. In the presence of co-cultures or cocktail cultures, *S. thermophilus* exhibited the shortest generation time (0.22–0.28 h) in reconstituted skim milk, which is due to the synergistic effect associated with the release of organic acids, changes in physiology, among others (Oliveira et al. 2011). *Lactobacillus acidophilus* La-5 had a stimulating effect on the growth of *Bifidobacterium lactis* Bb-12 and the viable count increased significantly by 6 h of fermentation when grown in ultrahigh temperature-treated (UHT) milk (Moayednia and Mazaheri 2011), which may be due to an increased availability of nonprotein nitrogen due to the higher proteolytic activity of the former culture (Shihata and Shah 2000).

Table 1 Effect of associative growth behaviour between starter cultures of dahi/yoghurt and probiotic cultures (*Bifidobacterium bifidum* I and *Lactobacillus acidophilus* R) on viability in fermented buffalo skim milk

Culture combination code	Incubation temperature (°C)	Culture(s)	Viable cell count (log CFU/g)			
			Incubation period (h)			
			0	6	12	18
D	30	Dahi cultures[a]	6.55±0.01 a	7.51±0.03 bA	7.91±0.02 cA	8.56±0.04 dA
DAB	30	Dahi cultures	6.45±0.03 a	7.08±0.02 bB	7.68±0.04 cB	7.93±0.03 dB
		Lactobacillus acidophilus R	5.78±0.02 a	6.34±0.05 bC	6.75±0.03 cD	6.83±0.03 dD
		Bifidobacterium bifidum I	5.90±0.03 a	6.26±0.03 bE	6.48±0.02 cF	6.59±0.05 dF
DAB	37	Dahi cultures	6.45±0.02 a	7.03±0.05 bB	7.46±0.05 cC	7.66±0.06 dC
		Lactobacillus acidophilus R	5.78±0.01 a	7.11±0.02 bD	7.65±0.04 cE	7.94±0.03 dE
		Bifidobacterium bifidum I	5.90±0.03 a	6.60±0.05 bF	7.43±0.04 cG	7.63±0.02 dG
Y	37	Yoghurt cultures[b]	6.69±0.04 a	7.69±0.05 b	7.97±0.03 c	8.64±0.03 d
YAB	37	Yoghurt cultures	6.66±0.03 a	7.45±0.03 bG	7.72±0.04 cK	8.62±0.04 dM
		Lactobacillus acidophilus R	5.81±0.02 a	7.15±0.04 bG	7.51±0.03 cK	7.80±0.05 dN
		Bifidobacterium bifidum I	5.92±0.03 a	6.08±0.02 bJ	6.78±0.04 cL	7.26±0.02 dO

Data are presented as the average ± standard deviation (SD) of five observtions. Values followed by a different lowercase letter within a row are significantly different at $p<0.05$. Values followed by a different uppercase letter within a column and within a culture combination are significantly different at $p<0.05$

[a] D*: Lactococcus lactis* ssp. *lactis* C 10, *L. lactis* ssp. *cremoris* C 1, *L. lactis* ssp. *lactis* biovar. *diacetylactis* DRC 10 (in 1:1:1 ratio). DAB: cultures of D along with *L. acidophilus* R and *B. bifidum* I

[b] Y: *Streptococcus salivarius* ssp. *thermophilus* HST and *Lactobacillus delbrueckii* ssp. *bulgaricus* RTS (in 1:1 ratio). YAB: cultures of Y along with *L. acidophilus* R, *B. bifidum* I

Effect on TA

The TA of dahi and yoghurt prepared with BSM using different cultures is presented in Table 2. The TA produced after 18 h by the combination DAB at 30 °C was slightly higher than that produced by combination D and significantly less ($p<0.05$) than the TA produced by combination DAB incubated at 37 °C, which may be due to this being the optimum temperature for the *L. acidophilus* present in this combination. Dahiya and Speck (1966) reported that *S. lactis* and *Lactobacillus* isolates produced acid at a faster rate in a mixed culture than when either strain was grown individually. Mixed cultures of *S. lactis* ssp. *lactis* and *S. lactis* ssp. *cremoris* perform better in terms of acid production than single cultures (Kim 1990). The stimulation of acid production by paired cultures of *S. lactis* and *Lactobacillus acidophilus* has also been documented (Kothari 1970).

Table 2 Effect of associative growth behaviour between starter cultures of dahi/yoghurt and probiotic cultures (*B. bifidum* I and *L. acidophilus* R) on titratable acidity of fermented buffalo skim milk

Culture Combination	Incubation temperature (°C)	Titratable acidity (%) of fermented skim milk (expressed as lactic acid)		
		Incubation period (h)		
		6	12	18
Dahi culture D	30	0.56±0.1 aA	0.72±0.2 bA	0.93±0.2 cA
Dahi culture DAB	30	0.48±0.1 aB	0.64±0.3 bB	0.99±0.4 cA
Dahi culture DAB	37	0.66±0.2 aC	0.82±0.4 bC	1.18±0.3 cB
Yoghurt culture Y	37	0.91±0.1 aA	1.42±0.3 bA	1.68±0.3 cA
Yoghurt culture YAB	37	0.85±0.2 aB	1.12±0.5 bB	1.44±0.2 cB

Culture details as given in Table 1.

Results are the average ± SD of five observations. Values followed by a different lowercase letter within a row are significantly different at $p<0.05$. Values followed by a different uppercase letter within a column in same product are significantly different at $p<0.05$

The rate of acid development by yoghurt culture Y decreased significantly with the addition of *L. acidophilus* and *B. bifidum*, which is in agreement with the observations of Yu and Nakanishi (1975) who also recorded a decrease in TA from 2.75 to 1.62 % with the addition of *L. acidophilus* to yoghurt culture. However, no significant difference in the TA of yoghurt cultures alone and in association with *L. acidophilus* after 12 h of incubation at 37 °C in skim milk was observed by Sharma and Singh (1982). The incorporation of *Bifidobacterium* into yoghurt starter was found to result in a slight reduction of acidity (Kisza et al. 1978). Similarly, while preparing yoghurt from ewe's milk, Bonczar et al. (2002) noticed the production of significantly less acid by supplementing *L. acidophilus* and *B.* for *S. thermophilus*. Souza and Saad (2009) obtained higher TA values in minas cheese prepared with a combination of *S. thermophilus* and *L. acidophilus* than in that prepared with the former culture alone. *S. thermophilus* stimulated the growth of other LAB either in co-culture (*B. lactis*, *Lactobacillus acidophilus*, *L. rhamnosus* or *L. bulgaricus*) or in cocktail cultures, and *S. thermophilus* in the presence of *L. acidophilus* or *Bifidobacterium* had lower V_{max} (maximum rate of acidification) values than with other cultures and took longer (10 h) to complete the fermentation (to reach pH 4.5) than with other cultures (4–5 h) (Oliveira et al. 2009a). According to the authors of this latter study, either in the presence or absence of inulin, a co-culture of *L. acidophilus* and *S. thermophilus* had higher acidity than with other combination of co-cultures. This may be due to the homolactic metabolism of the former culture (Zhao et al. 2007), and the low acidity development in a co-culture of *S. thermophilus* and *Bifidobacterium* may be due to the heterolactic nature of the latter culture (Bongaerts et al. 2005). Moayednia and Mazaheri (2011) noticed higher TA (77.25 °D) of the fermented UHT milk when *L. acidophilus* La-5 and *B. lactis* Bb-12 were grown together than when they were grown individually (41.83 and 25.60 °D, respectively).

Effect on VA

The pleasing flavour of dahi and yoghurt can be attributed to the optimum level of volatile acid production by the LAB. With regard to VA production, variations were observed between dahi culture combinations D and DAB (Table 3). The addition of therapeutic cultures to dahi culture D drastically decreased VA production from 36.0 to 15.8 ml after 18 h of incubation at 30 °C. No significant difference in VA production by dahi culture DAB was observed, irrespective of incubation temperature, but an increase in VA in yoghurt prepared with YAB cultures was noticed. Similarly, an increased VA development (3.6 ml/50 g) by mixed cultures of yoghurt compared to that of the single cultures (0.6 ml by *S. thermophilus* and 2.0 ml by *Lactobacillus bulgaricus*) was found by Singh et al. (1982). Conversely, no significant difference in the VA in mixed cultures of either yoghurt or yoghurt cultures following the addition of *L. acidophilus* was reported by Yu and Nakanishi (1975) and Sharma and Singh (1982). Bonczar et al. (2002) did not find a significant difference in the production of free fatty acid content with the supplementation of *L. acidophilus* and *B. bifidum* to *S. thermophilus*. However, Güler-Akin (2005) noticed higher VA production (9.1 ml/100 g) in bioyoghurt prepared in combination with *L. acidophilus* and *B. bifidum* along with regular yoghurt cultures than in plain yoghurt (7.2 ml) after 14 days of storage at 4 °C.

Effect on flavour production

The most important flavour components of fermented dairy products are diacetyl and acetaldehyde. The diacetyl content of BSM curds prepared by dahi cultures D and DAB is shown in Fig. 2. From the results, it is evident that the amount of diacetyl liberated by dahi culture D fell

Table 3 Effect of associative growth behaviour between starter cultures of dahi/yoghurt and probiotic cultures (*B. bifidum* I and *L. acidophilus* R) on volatile acidity of fermented buffalo skim milk

Culture combination	Incubation temperature (°C)	Volatile acidity of fermented skim milk (ml of 0.01 % NaoH/50 g)		
		Incubation period (h)		
		6	12	18
Dahi culture D	30	8.4±0.6 aA	19.0±0.8 bA	36.0±1.1 cA
Dahi culture DAB	30	10.0±0.7 aB	12.5±0.8 bB	15.8±0.5 bB
Dahi culture DAB	37	13.1±0.6 aC	14.9±0.7 bC	16.5±0.9 cC
Yoghurt culture Y	37	7.0±0.4 aA	7.5±0.3 bA	8.2±0.2 cA
Yoghurt culture YAB	37	7.8±0.5 aB	8.4±0.4 bB	8.9±0.6 cB

Culture details as given in Table 1

Results are the average ± SD of five observations. Values followed by a different lowercase letter within a row are significantly different at $p<0.05$. Values followed by a different uppercase letter within a column in same product are significantly different at $p<0.05$

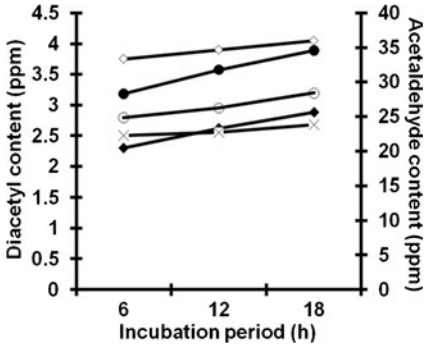

Fig. 2 Effect of associated growth among dahi and yoghurt cultures with *B. bifidum* I and *L. acidophilus* R on the production of diacetyl and acetaldehyde in fermented BSM. *Open diamond* Dahi fermented at 30 °C with culture combination D, *filled diamond* dahi fermented at 30 °C with culture combination DAB, *X* dahi fermented at 37 °C with culture combination DAB, *open circle* yoghurt fermented at 37 °C with culture combination Y, *filled circle* yoghurt fermented at 37 °C with culture combination YAB. See Table 1 for the culture codes

significantly ($p<0.05$) from 4.05 to 2.80 ppm following the addition of the therapeutic cultures. This fall in diacetyl production might be due to the decrease in the lactococci count in the presence of probiotic cultures. However, with the change in incubation temperature from 30 to 37°C, dahi culture DAB did not show any significant change in diacetyl content. In contrast, Pack et al. (1968) reported higher levels of diacetyl at 22 °C than at 30 °C. In a comparison of single verus mixed cultures, Lindsay and Day (1965) found that diacetyl was produced in the range of 0.1–3.24 ppm by strains of *S. lactis* ssp. *lactis*, *S. lactis* ssp. *cremoris*, *S. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc citrovorum* grown individually, whereas the mixed cultures of these same species produced as much as 5.45 ppm diacetyl. Contrary to this, Kneifel et al. (1992) found that slightly less diacetyl was produced (3.1–3.4 ppm) by mixed mesophilic cultures (*S. lactis* ssp. *lactis*, *S. lactis* ssp. *cremoris* and *S. lactis* ssp. *lactis* biovar. *diacetylactis*) than by *S. lactis* ssp. *lactis* biovar. *diacetylactis* alone (3.7 ppm). Nevertheless, this value is relatively very high compared to that produced by the combined culture of *S. lactis* ssp. *lactis* and *S. lactis* ssp. *cremoris* (0.3 ppm) in cultured milk.

The levels of acetaldehyde, which is the main flavouring compound in yoghurt, in BSM yoghurt prepared by cultures Y and YAB are shown in Fig. 2. Contrary its effect of decreasing diacetyl production by dahi cultures DAB, *L. acidophilus* and *B. bifidum* supplementation to yoghurt cultures significantly increased the acetaldehyde content ($p<0.05$) from 28.4 to 34.6 ppm after 18 h of incubation at 37 °C. Yuguchi et al. (1989) also found a stimulation of acetaldehyde production in yoghurt cultures supplemented with *B. bifidum* 15696. These results are contradictory to the observation of Kisza et al. (1978) who reported the production of less acetaldehyde by a mixed yoghurt culture in the presence of *Bifidobacterium*. However, Sharma and Singh (1982) found no appreciable difference in the acetaldehyde content with yoghurt starters supplemented with *L. acidophilus*. While preparing yoghurt from ewe's milk, Bonczar et al. (2002) obtained the production of significantly higher amounts of acetaldehyde by supplementing *L. acidophilus* and bifidobacteria to *S. thermophilus*. Similarly, Güler-Akin (2005) noticed the production of a higher acetaldehyde content (> 5 ppm) in bioyoghurt compared to plain yoghurt. There was no significant difference in the taste or sensory quality of dahi and yoghurt with the addition of *L. acidophilus* and *Bifidobacterium* to regular starter cultures of yoghurt and dahi. However, a slightly increased acidic taste was sensed in dahi supplemented with probiotic cultures, possibly because of the higher acid production by *L. acidophilus* (Vijayendra and Gupta 2011). An increased production of conjugated linoleic acid (38 % higher than the control) was observed by a co-culture of the probiotic *L. acidophilus* in the presence of maltodextrin as a prebiotic (Oliveira et al. 2009b). The composition of the co-culture influences the level of diacetyl and acetoin production (Oliveira et al. 2012a, b). The addition of a prebiotic, inulin, to a co-culture containing *S. thermophilus* and *L. acidophilus* suppressed acetoin production and hindered that of diacetyl due to the catbolic repression of α-acetolactate synthase expression in the former culture (Oliveira et al. 2012a). In a co-culture of *S. thermophilus* and *B. lactis*, inulin also increased the amount of volatile compounds and other organic acids, such as lactic and acetic acids, which contribute to the pleasant flavour of the fermented milks, indicating the symbiotic effect between pre- and probiotics (Oliveira et al. 2012b). In the same study, associative growth increased the viable count of *S. thermophilus* and *B. lactis* by 15 and 38 % compared to their respective pure cultures, possibly due to mutual interactions.

Effect on proteolytic activity

Lactic acid bacteria are nutritionally fastidious organisms which require an exogenous supply of pre-formed amino acids to initiate growth. They show varying degrees of proteolytic activity, which is generally strain- rather than species-specific. We observed a significant reduction ($p<0.05$) in the tyrosine content liberated by culture combination DAB, especially when incubated at 37 °C rather than 30 °C (Table 4), which may be due to the high VCC of lactococci in this combination, particularly at 37 °C. Rajagopal and Sandine (1990) observed that lactobacilli possessed greater proteolytic activity than streptococci. The range of tyrosine produced per millilitre of curd was 25–144.6 μg by lactobacilli and 2.4–34 μg by

Table 4 Effect of associative growth behaviour between starter cultures of dahi/yoghurt and probiotic cultures (*B. bifidum* I and *L. acidophilus* R) on proteolytic activity of fermented buffalo skim milk

Culture combination	Incubation temperature (°C)	Proteolytic activity of fermented skim milk (μg tyrosine/g curd)		
		Incubation period (h)		
		6	12	18
Dahi culture D	30	0.36±0.06 aA	0.41±0.03 bA	0.46±0.03 cA
Dahi culture DAB	30	0.28±0.03 aB	0.33±0.02 bB	0.41±0.04 cA
Dahi culture DAB	37	0.25±0.04 aB	0.29±0.03 bC	0.36±0.03 cC
Yoghurt culture Y	37	0.27±0.03 aA	0.33±0.05 bA	0.38±0.05 cA
Yoghurt culture YAB	37	0.22±0.02 aA	0.28±0.01 bA	0.33±0.04 cA

Culture details as given in Table 1

Results are the average ± SD of five observations. Values followed by a different lowercase letter within a row are significantly different at $p<0.05$. Values followed by a different uppercase letter within a column in same product are significantly different at $p<0.05$

streptococci. Decrease in the proteolysis by mixed mesophilic culture compared to that exhibited by one of the component culture *L. lactis* ssp. *lactis* biovar. *diacetylactis* was observed by Kneifel et al. (1992).

We observed no significant decrease in proteolytic activity with the addition of therapeutic cultures to the yoghurt culture combination, and this observation is in agreement with that of Sharma and Singh (1982) with mixed yoghurt culture combination containing *L. acidophilus*. However, the highest reported proteolytic index was obtained with a combination of *L. acidophilus* and *S. thermophilus* in yoghurt (Souza and Saad 2009). Similarly, in another study, the presence of probiotic organisms (*L. acidophilus* and *B. lactis*) enhanced proteolysis significantly in comparison with the control batch containing *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342 only (Donkor et al 2006). According to these latter authors, the proteolytic activity varied due to the termination pH, but it also appeared to be strain related, and the increased proteolysis improved the survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* during storage, resulting in a lowering of the pH and the production of higher levels of organic acids. Bergamini et al. (2009) noticed significant influence of *L. acidophilus* on secondary proteolysis from the beginning of ripening of a semi-hard cheese; this caused an increase in the levels of small nitrogen-containing compounds and free amino acids and changed the peptide profiles. The effect was more noticeable when it was added to cheese–milk after pre-incubation in an enriched milk fat substrate. Enhanced proteolysis with the three-strain mixed culture (*L. acidophilus*, *L. paracasei* and *B. lactis*) was also observed, suggesting that *L. acidophilus* played a major role in the secondary proteolysis of probiotic cheeses. However, the addition of *L. acidophilus* R to yoghurt culture was found to reduce the proteolytic activity (from 0.32 to 0.26 mg tyrosine/g curd) when grown in skim milk for 18 h at 37 °C (Sharma and Singh 1982). These authors found that the rate of proteolysis was comparatively less in acidophilus yoghurt than in plain yoghurt. Ramachandran and Shah (2008) observed a higher proteolytic activity in *Bifidobacterium longum* 5022 than in *L. acidophilus* 4461. However, it was less than that of *S. thermophilus* 1275 and higher than that of *S. thermophilus* 285 and *L. bulgaricus* 1092, indicating that the proteolytic activity is strain- rather than species-dependent.

Conclusions

Associative growth behaviour among probiotic cultures and starter cultures of dahi and yoghurt was observed in this study, but there was no significant difference in the taste or sensory quality of dahi and yoghurt with the addition of *L. acidophilus* and *Bifidobacterium* to regular starter cultures of yoghurt and dahi. As the probiotic cultures have not hampered the technological quality of dahi and yoghurt, it is advantageous to use these cultures along with starter cultures to improve the therapeutic quality of the fermented dairy products, thereby providing health benefits to consumers.

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