Preparation of low galactose yogurt using cultures of Gal⁺ *Streptococcus thermophilus* in combination with *Lactobacillus delbrueckii* ssp. *bulgaricus*

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Abstract *Streptococcus thermophilus* is an important lactic starter used in the production of yogurt. Most strains of *S. thermophilus* are galactose negative (Gal⁻) and are able to metabolize only glucose portion of lactose and expel galactose into the medium. This metabolic defect leads to the accumulation of free galactose in yogurt, resulting in galactosemia among consumers. Hence there is an absolute need to develop low galactose yogurt. Therefore, in this study, three galactose positive (Gal⁺) *S. thermophilus* strains from National Collection of Dairy Cultures (NCDC) viz. NCDC 659 (AJM), NCDC 660 (JM1), NCDC 661 (KM3) and a reference galactose negative (Gal⁻) *S. thermophilus* NCDC 218 were used for preparation of low galactose yogurt. In milk fermented using *S. thermophilus* isolates alone, NCDC 659 released less galactose (0.27 %) followed by NCDC 661 (0.3 %) and NCDC 660 (0.45 %) after 10 h at 42 °C. Milk was fermented in combination with Gal⁻ *L. delbrueckii* subsp. *bulgaricus* NCDC 04, in which NCDC 659 released least galactose upto 0.49 % followed by NCDC 661 (0.51 %) and NCDC 660 (0.60 %) than reference Gal⁻ NCDC 218 (0.79 %). Low galactose yogurt was prepared following standard

procedure using Gal⁺ *S. thermophilus* isolates and Gal⁻ *L. delbrueckii* subsp. *bulgaricus* NCDC 04 in 1:1 ratio. Among which low galactose yogurt by NCDC 659 combination contained less galactose 0.37 % followed by NCDC 661 (0.51 %), NCDC 660 (0.65 %) and reference Gal⁻ NCDC 218 (0.98 %) after 4 h of fermentation. This study clearly reveals that Gal⁺ *S. thermophilus* isolates can be paired with Gal⁻ *L. delbrueckii* subsp. *bulgaricus* for developing low galactose yogurt.

Keywords Galactose · *S. thermophilus* · Low galactose yogurt · *L. delbrueckii* subsp. *bulgaricus*

Introduction

Streptococcus thermophilus, a thermophilic Gram-positive lactic acid bacterium (LAB) is one of the most important starter culture used for yogurt production in combination with *L. delbrueckii* subsp. *bulgaricus*. *S. thermophilus* is highly adapted to lactose but most of the strains are galactose negative (Gal⁻) and are able to efficiently metabolize only glucose portion of lactose and release galactose into the medium. This shortcoming in metabolic pathway is particularly due to the production of insufficient level of galactokinase, a key enzyme of the Leloir pathway and due to the absence of galactose transport system (Thomas and Crow 1984). Presence of excess amount of galactose in dairy products may adversely affect human health, particularly in individuals with galactosemia (Novelli and Reichardt 2000). The other undesirable effects on galactose accumulation in dairy products are browning of mozzarella cheese, production of CO₂ by nonstarter bacteria and

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growth of spoilage microorganisms (Thomas et al. 1980; Tinson et al. 1982; Kindstedt and Fox 1993).

Galactosemia, an inherited disorder of carbohydrate metabolism affects the body's ability to utilize certain sugars like lactose and galactose from food. Individuals with galactosemia are deficient in enzyme that converts galactose to glucose. If galactosemia is left untreated, accumulation of galactose occurs in blood and body tissues, which might lead to jaundice, kidney problems, cataracts, mental retardation and possibly death (Berry et al. 2001; Goodman et al. 2002).

The three different kinds of enzymatic deficiency that leads to galactosemia are i) Classical galactosemia, an autosomal recessive mutant that leads to defect in the utilization of galactose-1-phosphate via galactose-1-phosphate uridylyltransferase (GALT; EC2.7.7.12), ii) galactokinase deficiency (GALK; EC2.7.1.6) and iii) uridine diphosphate (UDP) galactose-4-epimerase (GALE) deficiency (EC5.1.3.2) (Goodman et al. 2002). Galactosemia can be treated by avoiding foods that contain galactose from diet or by consuming lactose or galactose free foods like fruits, vegetables, grains, breads, etc. It is now recommended that persons with galactosemia should avoid eating foods with galactose throughout life (Portnoi and MacDonald 2009).

Yogurt, one of the most popular fermented milk products in South Asia is prepared by lactic acid fermentation of milk through the action of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. It is more nutritive than milk in terms of digestibility and vitamins and it is a source of calcium and phosphorus and helps in curing gastrointestinal disorder (Adolfsson et al. 2004).

Yogurt is an autodigestive source of lactose and contains free galactose (5 g/100 g) (Dewit et al. 1988). Ever since, yogurt is manufactured from Gal^- *S. thermophilus* and Gal^- *L. delbrueckii* subsp. *bulgaricus* and they are able to metabolize only glucose portion of lactose and hence yogurt prepared from these strains contains free galactose. Therefore, yogurt consumption is associated with a higher risk for galactosemia patients, regardless of the lactase activity of the population. Stang et al. (2005) reported that consumption of milk fat and galactose increases the risk of Seminoma (testicular cancer) in adolescence. In order to resolve these problems, an attempt was made to prepare low galactose yogurt using wild galactose positive *S. thermophilus* that were isolated in our previous study from diversified natural habitat (Umamaheswari et al. 2014) and later submitted to National Collection of Dairy Cultures (NCDC) (Karnal, Haryana, India). This study evaluated the galactose utilization ability of Gal^+ *S. thermophilus* in milk as single and mixed starter and examined the suitability of cultures for the preparation of low galactose yogurt.

Materials and methods

Bacterial strains used and culture conditions

Three thermophilic Gal^+ *S. thermophilus* dairy isolates viz., AJM, JM1 and KM3 submitted to National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, Haryana under culture collection No. NCDC 659 (AJM), NCDC 660 (JM1) and NCDC 661 (KM3) were taken for the study. Gal^- *S. thermophilus* NCDC 218 was used as reference strain. Gal^- *L. delbrueckii* subsp. *bulgaricus* NCDC 04 is used for the preparation of low galactose yogurt. Strains were stored at -20°C as frozen glycerol (15 %) stocks. Frozen Gal^+ *S. thermophilus* isolates were routinely revived and sub-cultured in J8 broth supplemented with 0.5 % (w/v) galactose and Gal^- strains with 0.5 % (w/v) lactose at 42°C . The authenticity of the strains was checked by polyphasic characterization and 16S rRNA gene sequencing. The identity of the obtained sequences was analyzed by BLAST (Basic local alignment search tool) and the obtained sequences were submitted to GenBank under following accession numbers JN561295, JN561296 and JN561299 for AJM, KM3 and JM1 respectively.

Assessing the ability of Gal^+ *S. thermophilus* strains in milk

The Gal^+ strains were grown in sterile reconstituted 10 % (w/v) Non Fat Dry Milk (NFDM) for 10 h at 42°C . Curdled milk was aseptically drawn and analyzed for titratable acidity, lactose and galactose content. Similarly, three Gal^+ strains were co-cultured individually with Gal^- *L. delbrueckii* subsp. *bulgaricus* NCDC 04 in the ratio of 1:1 for 8 h at 42°C . Curdled milk samples were aseptically drawn at 8 h and analyzed for titratable acidity, lactose and galactose content. Titratable acidity was determined by the procedure described in AOAC (1999). Acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator. Residual lactose and galactose in milk culture were determined using lactose and galactose quantification kit (Megazyme, Ireland).

Preparation of low galactose yogurt

All the three Gal^+ *S. thermophilus* strains were used for the preparation of low galactose yogurt by co-culturing with Gal^- *L. delbrueckii* subsp. *bulgaricus* NCDC 04. Fresh raw buffalo milk (containing 7–8 % fat) collected from Experimental Dairy at NDRI was standardized for fat and SNF. Standardization was done by adjusting fat % of buffalo and skim milk by Pearson Square method (De 1980). Standardized Buffalo Milk (fat 4.5 %, SNF 11.0 %) was used for the preparation of low galactose yogurt. The product was prepared according to the method described by De (1980) with suitable modifications in which standardized milk was heat

treated at 90–95 °C for 10 min and 100 ml of milk was distributed in 100 ml plastic containers. It was then cooled to 42–43 °C and inoculated with Gal⁺ *S. thermophilus* strains and Gal⁻ *L. delbrueckii* subsp. *bulgaricus* NCDC 04 in the ratio of 1:1 and incubated in water bath maintained at 42 °C for 4 h. Yogurt samples prepared with Gal⁻ *S. thermophilus* NCDC 218 and Gal⁻ *L. delbrueckii* subsp. *bulgaricus* NCDC 04 was used as control.

Analysis of low galactose yogurt

The yogurt samples were analyzed for their physicochemical properties and viable cell count. Periodically, samples were aseptically withdrawn at 4 h on 1st day, 7th day and 14th day and analyzed for viable cell count, titratable acidity, residual lactose and galactose in yogurt. Enumeration of *S. thermophilus* from yogurt was carried out by pour plating on M17 agar and Gal⁻ *L. delbrueckii* subsp. *bulgaricus* on MRS agar followed by incubation at 42 °C for 24 h. Titratable acidity and pH was determined (AOAC 1999). Residual lactose and galactose in culture supernatant fluids were determined using a lactose and galactose quantification kit (Megazyme, Ireland).

Statistical analysis

The results were expressed as mean±standard deviation for 3 individual experiments. Analysis of variance with post hoc test (Bonferroni) was done to compare the mean of different isolates with reference culture. The data was statistically analyzed with the application of SYSTAT 6.0.1 (Statistical Software Package, SPSS, USA).

Results

Fermentation of milk with Gal⁺ *S. thermophilus* strains

Titratable acidity, pH and residual sugar content of fermented milk produced using single culture, Gal⁺ *S. thermophilus* isolates were analyzed after 10 h of

incubation (Table 1). The Gal⁺ isolate NCDC 659 released minimum amount of galactose (0.27 %) followed by NCDC 661 (0.3 %) and NCDC 660 (0.45 %) respectively, which was significantly lower than reference Gal⁻ NCDC 218 (0.85 %). At the same time, titratable acidity increased from initial value of 0.162±0.01 to 0.86±0.02, 0.76±0.03, 0.68±0.008 for NCDC 659, NCDC 661 and NCDC 660 respectively which did not differ significantly from that of galactose negative NCDC 218 (0.78±0.02).

In mixed culture of each Gal⁺ isolate with Gal⁻ *L. delbrueckii* subsp. *bulgaricus* NCDC 04, the release of galactose was found to be 0.49±0.02 % for NCDC 659, 0.51±0.02 % for NCDC 661 and 0.60±0.01 % for NCDC 660. The standard control, Gal⁻ *S. thermophilus* NCDC 218 released more galactose (0.79±0.06 %) after 8 h of incubation. Titratable acidity of mixed culture ranged from 0.79±0.04 to 0.94±0.04 (Table 2).

Preparation and analysis of low galactose yogurt using galactose fermenting *S. thermophilus*

Standardized buffalo milk (fat 4.5 %, SNF 11.0 %) was used for preparation of yogurt. Three Gal⁺ strains viz., *S. thermophilus* NCDC 659, NCDC 660, NCDC 661, Gal⁻ reference strain NCDC 218 in combination with Gal⁻ *L. delbrueckii* subsp. *bulgaricus* NCDC 04 respectively were used as yogurt inoculum and fermentation was carried at 42 °C for 4 h. The clean set yogurt samples were stored at 4 °C and analyzed for lactose, galactose, titratable acidity and viable count of the yogurt bacteria at 4 h on 1st, 7th and 14th day. The results are represented in Table 3.

Titratable acidity and pH

Percent titratable acidity of yogurt was recorded as 1.47±0.09, 1.2±0.1, 1.25±0.002 for NCDC 659, NCDC 660 and NCDC 661 respectively, whereas standard Gal⁻ NCDC 218 showed 1.02±0.02. The pH value of yogurt

Table 1 Fermentation of milk by different Gal⁺ strains as single culture

S.No.	Parameters analyzed	Incubation (10 h)				
		Uninoculated control	NCDC 659	NCDC 660	NCDC 661	NCDC 218
1	Lactose (%)	4.22±0.17 ^a	3.31±0.14 ^b	3.88±0.1 ^{cf}	3.88±0.17 ^{abd}	3.40±0.06 ^{acb}
2	Galactose (%)	0.044±0.003 ^a	0.27±0.01 ^b	0.45±0.03 ^{cd}	0.3±0.02 ^d	0.85±0.045 ^f
3	pH	6.5±0.01 ^a	4.15±0.02 ^{bc}	4.5±0.01 ^{cd}	4.25±0.02 ^d	4.2±0.03 ^{cef}
4	Titratable acidity (%)	0.162±0.01 ^a	0.86±0.02 ^{bc}	0.68±0.008 ^{cd}	0.76±0.03 ^{db}	0.78±0.02 ^{bcd}

Values are presented as mean±standard deviation of three individual experiments (n=3). Means bearing different superscripts differed significantly by Bonferroni test at p<0.05

Table 2 Fermentation of milk by different Gal⁺ isolates in co-culture with *L. bulgaricus* NCDC 04

S.No.	Cultures	Incubation time	Parameters analyzed			
			Lactose (%)	Galactose (%)	pH	Titrateable acidity (%)
1	<i>S. thermophilus</i> NCDC 659+ <i>L. bulgaricus</i> NCDC 04	8 h	2.33±0.10	0.49±0.02	4.0±0.05	0.94±0.04
2	<i>S. thermophilus</i> NCDC 660+ <i>L. bulgaricus</i> NCDC 04	8 h	2.49±0.42	0.60±0.01	4.06±0.09	0.86±0.07
3	<i>S. thermophilus</i> NCDC 661+ <i>L. bulgaricus</i> NCDC 04	8 h	2.48±0.13	0.51±0.02	4.21±0.02	0.82±0.02
4	<i>S. thermophilus</i> NCDC 218+ <i>L. bulgaricus</i> NCDC 04	8 h	2.1±0.04	0.79±0.06	4.32±0.11	0.79±0.04

Values are presented as mean±standard deviation of three individual experiments (n=3)

ranged from 4.27 to 4.63 (Table 3). Lower pH was recorded in Gal⁺ strains compared to Gal⁻ strains. The difference in pH at day 1 was significant ($p<0.05$) as compared to 7 and 14 days of storage. However, there was no significant difference between 7 and 14 days.

Residual sugars of yogurt after fermentation and during storage

Quantitative changes in sugar content of yogurt during fermentation are mentioned in Table 3. Initial lactose content of

Table 3 Development of low galactose yogurt and evaluating physicochemical changes and viable cell count occurring during fermentation and storage

S.No.	Isolates	Parameters	Fresh yogurt	Days of storage			
			4 h	1	7	14	
1	<i>S. thermophilus</i> NCDC 659 + <i>L. bulgaricus</i> NCDC 04	Lactose (%)	5.16±0.05 ^a	4.90±0.10 ^a	4.61±0.07 ^a	4.11±0.46	
		Galactose (%)	0.37±0.05 ^a	0.40±0.03 ^{ac}	0.42±0.01 ^a	0.68±0.01	
		pH	4.27±0.03 ^{ac}	4.2±0.05 ^a	4.18±0.04 ^a	4.10±0.2	
		Acidity (%)	1.47±0.09 ^a	1.55±0.06 ^a	1.51±0.01 ^a	1.51±0.008	
		CFU/ml in M17 agar	8.86±0.02 ^a	9.18±0.02 ^{ab}	9.27±0.01 ^a	8.76±0.08	
		CFU/ml in MRS+pH 5.4	9.13±0.02 ^a	9.02±0.01 ^{ab}	8.74±0.08 ^b	7.95±0.02	
2	<i>S. thermophilus</i> NCDC 660 + <i>L. bulgaricus</i> NCDC 04	Lactose (%)	5.07±0.16 ^a	4.54±0.20 ^{bd}	4.13±0.28 ^a	4.14±0.006	
		Galactose (%)	0.65±0.05 ^a	0.78±0.09 ^b	0.88±0.03 ^b	1.04±0.02	
		pH	4.63±0.08 ^b	4.30±0.008 ^a	4.22±0.04 ^a	4.10±0.01	
		Acidity (%)	1.2±0.01 ^b	1.46±0.01 ^{ab}	1.54±0.03 ^a	1.56±0.06	
		CFU/ml in M17 agar	8.85±0.05 ^{ba}	9.31±0.01 ^{bd}	8.74±0.02 ^a	7.60±0.02	
		CFU/ml in MRS+pH 5.4	8.57±0.01 ^b	9.25±0.02 ^b	8.80±0.01 ^{ab}	6.70±0.04	
3	<i>S. thermophilus</i> NCDC 661 + <i>L. bulgaricus</i> NCDC 04	Lactose (%)	5.02±0.20 ^a	4.66±0.06 ^{ac}	4.27±0.02 ^a	3.99±0.04	
		Galactose (%)	0.51±0.05 ^a	0.69±0.05 ^{bc}	0.77±0.01 ^{bc}	0.87±0.10	
		pH	4.59±0.002 ^{cd}	4.21±0.008 ^a	4.23±0.054 ^a	4.19±0.003	
		Acidity (%)	1.25±0.002 ^{ac}	1.38±0.046 ^{ac}	1.53±0.01 ^a	1.51±0.01	
		CFU/ml in M17 agar	8.80±0.06 ^a	9.00±0.07 ^{bc}	8.86±0.05 ^a	7.65±0.10	
		CFU/ml in MRS+pH 5.4	8.75±0.03 ^c	9.12±0.014 ^{ac}	8.94±0.005 ^c	7.57±0.09	
4	<i>S. thermophilus</i> NCDC 218 + <i>L. bulgaricus</i> NCDC 04	Lactose (%)	5.10±0.11 ^a	4.60±0.10 ^{cd}	4.18±0.03 ^a	4.02±0.06	
		Galactose (%)	0.98±0.06 ^b	1.01±0.02 ^{bd}	1.10±0.01 ^d	1.24±0.10	
		pH	4.49±0.03 ^d	4.30±0.006 ^a	4.25±0.01 ^a	4.25±0.01	
		Acidity (%)	1.02±0.02 ^d	1.12±0.05 ^d	1.2±0.04 ^b	1.3±0.005	
		CFU/ml in M17 agar	9.78±0.03 ^d	8.71±0.16 ^d	7.97±0.01 ^a	7.66±0.07	
		CFU/ml in MRS+pH 5.4	8.97±0.03 ^d	8.14±0.017 ^{cd}	6.69±0.05 ^d	6.54±0.03	

Values are presented as mean±standard deviation of three individual experiments (n=3). Means bearing different superscripts differed significantly by Bonferroni test at $p<0.05$

the milk used for yogurt was 7.46 % and it decreased progressively to 5.0 % after 4 h of fermentation at 42 °C for both Gal⁺ and Gal⁻ isolates. The galactose content of yogurt was found to be 0.37 %, 0.65 %, 0.5 % and 0.98 % for NCDC 659, NCDC 660, NCDC 661 and NCDC 218 respectively.

Microbial quality of yogurt during fermentation and storage

The changes in viable counts of different combinations of yogurt co-cultures are shown in Table 3. In the present study, viable count of *S. thermophilus* was significantly ($p<0.05$) different between the samples. At the end of fermentation, the viable count of *S. thermophilus* in yogurt were 8.86 log cfu/g⁻¹, 8.85 log cfu/g⁻¹, 8.80 log cfu/g⁻¹ and 9.78 log cfu/g⁻¹ for NCDC 659, NCDC 660, NCDC 661 and NCDC 218, respectively.

One day after fermentation, viable counts of Gal⁻ *L. delbrueckii* subsp. *bulgaricus* were similar ($p<0.05$) between different samples but the counts decreased with time. At the same time *S. thermophilus* counts showed a slight increase on day one and then it decreased by about 1–2 log cycle during 7–14 day storage period. The viable counts of *S. thermophilus* in yogurt, during storage varied from 9.3 log cfu/g⁻¹ on day one to 7.6 log cfu/g⁻¹ on day 15 ($p<0.05$). Gal⁻ *Lactobacillus delbrueckii* subsp. *bulgaricus* counts showed a gradual decrease in viable numbers starting from around 9.2 log cfu/g⁻¹ on day one to 8.7–8.8 log cfu/g⁻¹ on day 7 and it further came down to 7.9–6.6 log cfu/g⁻¹ on the 14th day of storage. The decrease in viable counts of Gal⁻ *L. delbrueckii* subsp. *bulgaricus* was significantly higher during storage of yogurt samples prepared from reference cultures as compared to yogurt prepared using Gal⁺ isolates of *S. thermophilus*. But, at the end of the storage period a significant difference in the final concentration of galactose was observed. At 14 days storage of yogurt, residual galactose was 0.68 % for NCDC 659, 0.87 % for NCDC 661 and 1.04 % for NCDC 660 that were significantly ($p<0.05$) lower than 1.24 % as observed in the control NCDC 218.

Discussion

The residual galactose present in yogurt may lead to galactosemia and it can be also used as a substrate for heterofermentative bacteria to produce detrimental end products. Hence, thermophilic starter cultures that ferment lactose without releasing galactose are in demand. In view of the above information, the present study analyzed wild Gal⁺ *S. thermophilus* strains (NCDC 659, NCDC 660, NCDC 661) for its galactose utilization in milk and then they were used as starter culture for the preparation of low galactose yogurt and the results were compared with a commercial

culture (NCDC 218). The galactose fermenting isolate released less galactose viz. NCDC 659 (0.27 %), NCDC 661 (0.3 %), NCDC 660 (0.45 %) than Gal⁻ isolate NCDC 218 after 10 h of fermentation in milk. Similarly, Hutkins et al (1986) reported that Gal⁺ strains KK1, KK2, KK3 and KK5 released less galactose (0.05 %) than the Gal⁻ KK4 strain (0.06–0.22 %) incubated in skim milk. Robitaille et al (2009) reported that, Gal⁺ extracellular polysaccharide (EPS) producing recombinant *S. thermophilus* RD-534-S1 released galactose 4.3 g/kg similar to parental strain RD-534 4.4 g/kg after 12 h fermentation of reconstituted skim milk at 40 °C. However, lactic acid/galactose molar ratio was more in recombinant strain than parental strain, which could be attributed to the utilization of galactose for metabolic process.

In case of low galactose yogurt, NCDC 659 (4.27 ± 0.03) showed lowest pH followed by NCDC 661 (4.59 ± 0.002) and NCDC 660 (4.63 ± 0.08) after 4 h of fermentation. Euber and Brunner (1979) reported that the pH of yogurt decreased during fermentation from 6.2 to 4.0 accompanied by decrease in lactose content from 6.5 to 2.5 % (w/v). Fermentation of lactose to lactic acid during storage could contribute to this significant decline in pH from day 1 to day 7 (Kosikowski 1982). Kailasapathy et al. (2008) reported that pH of the yogurt reached to 4.07 after two weeks of storage.

The enzymes from yogurt starter cultures including *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are active even at refrigeration temperatures and produce small amount of lactic acid by fermentation of lactose which results in noticeable decrease in pH (Shah et al. 1995; Dave and Shah 1996). In mean time, lactose was decreased from 7.46 % to 5.0 % after 4 h fermentation at 42 °C. In yogurt, glycolytic activity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* leads to accumulation of similar amount of lactic acid and galactose. Mouillet et al (1977) reported lactose in yogurt was reduced from 6.23 to 3.88 % after fermentation for 3 h. Similarly, Goodenough and Kleyn (1976) reported that after fermentation lactose in yogurt was reduced from 8.56 to 5.56 %. In skimmed and whole milk samples, glycolytic activity of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* leads to accumulation of similar amounts of lactic acid (1.32 and 1.25 g·100 g⁻¹) and galactose (1.23 and 1.18 g·100 g⁻¹). As the catabolic activity of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* mainly results in fermentation of the glucosyl moiety of lactose to produce lactic acid (Salminen and Wright 1998), the decrease in lactose concentration parallels the increase in lactic acid concentration which in turn equals the amount of unfermented galactose (De Noni et al. 2004). They also reported that the glycolytic activity in plain stirred yogurt seems not to involve galactose, the content of which approximates the level of lactic acid (De Noni et al. 2004).

The galactose content of yogurt was found to be 0.37 %, 0.65 %, 0.5 % and 0.98 % for NCDC 659, NCDC 660, NCDC

661 and NCDC 218 respectively. De Noni et al (2004) reported that the average galactose content of 9 out of 11 sweetened yogurts was 0.98 g/100 g, which differed significantly from plain yogurt (0.8 g/100 g). Moreover, lactic acid content in nine samples was higher than that of galactose by more than 0.2 g/100 g and they suggested that low level of galactose was achieved mainly through activation of galactokinase, and also galactose produced during Leloir pathway are used for the EPS synthesis.

Robitaille et al (2009) prepared fat free yogurt using recombinant Gal⁺ exopolysaccharide producing strain. Milk fermented with recombinant strain RD-534-S1 produced more galactose (4.3 g/kg) like parental strain RD-534 (4.4 g/kg), but lactic acid/galactose molar ratio in RD-534-S1 fermented milk was significantly greater than RD-534 parental strain. They reported that the recombinant strain used galactose more efficiently for metabolic purpose than the parental strain. Similar results were observed for the recombinant strain *S. thermophilus* SMQ-301 by Vaillancourt et al (2004) and for MR-AAC by Robitaille et al (2007).

In the present study, viable count of *S. thermophilus* was significantly different between the samples. One day after fermentation, viable counts of Gal⁻ *L. delbrueckii* subsp. *bulgaricus* were similar ($p < 0.05$) between different samples but the numbers showed a declining trend with time. There was significant decrease in the population of Gal⁻ *L. delbrueckii* subsp. *bulgaricus* during storage in all samples. This decrease has been observed previously Dave and Shah (1996). This decrease in counts of lactobacilli was due to the lower storage temperature and over acidification during storage of yogurt (Serra et al. 2009). *S. thermophilus* counts slowly increased up to first day of the storage and later decreased by about 1 log cycle. Similar results were reported by Birollo et al (2000) that counts of *S. thermophilus* slightly decrease during storage probably due to the gradual decrease in pH and the viable counts of *S. thermophilus* in yogurt during storage changed from 8.33 log cfug⁻¹ on day1 to 6.33 log cfug⁻¹ on day 15.

The yogurt base is essentially sterile since it is normally heated to about 90 °C before inoculation with levels of lactic acid bacteria in excess of 10⁷ CFU g⁻¹ to initiate the fermentation (Silvia et al. 2001). The yeasts are not involved in the fermentation process, mainly due to the high processing temperatures and, if correctly stored under refrigeration (5 °C), a product shelf life of 3 to 4 weeks may be expected. They usually appear as contaminants originating either from poor hygienic practices during package/processing operation or they originate from ingredients like fruits, nuts, sugar, honey etc. (Fleet 1990; Kroger 1976). However, when storage temperatures are abused, a rapid growth of yeast results in gas formation, off flavor development and discoloration can be observed (Davis 1970; Bennie et al. 2003). In our study no yeasty flavor, discoloration or swelling of yogurt was observed during the 14 day storage

period, suggesting lack of any yeast growth in the developed product. Moreover, the product studied was plain yogurt, in which ingredients like sugar, fruits, nuts were not added also helped to keep the yeast contamination at bay.

All the Gal⁺ cultures analyzed in this study proved to be good yogurt starters as their performance matched with reference culture NCDC 218. From the above results, it could be concluded that the Gal⁺ isolates used in this study can be successfully paired with Gal⁻ *L. delbrueckii* subsp. *bulgaricus* to prepare defined strain starters for low galactose yogurt. Further, the effect of low galactose yogurt on galactosemia needs to be explored through animal studies.

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References

- Adolfsson O, Meydani SN, Russell RM (2004) Yogurt and gut function. *Am J Clin Nutr* 80:245–256
- Association of Official Analytical Chemists (1999) In: Cuniff P (ed) Official methods of analysis of AOAC international, vol II, 16th edn. AOAC International 5th revision, Maryland
- Bennie CV, Analie LH, Bridget I, Gabor P (2003) Temperature abuse initiating yeast growth in yogurt. *Food Res Int* 36:193–197
- Berry GT, Hunter JV, Wang Z (2001) *In vivo* evidence of brain galactitol accumulation in an infant with galactosemia and encephalopathy. *J Pediatr* 138:260–262
- Birollo GA, Reinheimer JA, Vinderola CG (2000) Viability of lactic acid microflora in different types of yogurt. *Food Res Int* 33:799–805
- Dave RI, Shah NP (1996) Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and bifidobacteria. *J Dairy Sci* 79:1529–1536
- Davis JG (1970) Laboratory control of yogurt. *Dairy Ind* 35:139–144
- De SK (1980) Indian dairy products. In: Outlines of dairy technology. Oxford University Press, New Delhi, pp 404–410
- De Noni I, Pellegrino L, Masotti F (2004) Survey of selected chemical and microbiological characteristics of (plain or sweetened) natural yogurts from the Italian market. *Lait* 84:421–433
- Dewit O, Pochart P, Desjeux JF (1988) Breath hydrogen concentration and plasma glucose, insulin and free fatty acid levels after lactose, milk, fresh or heated yogurt ingestion by healthy young adults with or without lactose malabsorption. *Nutrition* 4:131–135
- Euber JR, Brunner JR (1979) Determination of lactose in milk products by high-performance liquid chromatography. *J Dairy Sci* 62:685–690
- Fleet GH (1990) Yeasts in dairy products. A review. *J Appl Bacteriol* 68: 199–211
- Goodenough ER, Kleyn DH (1976) Qualitative and quantitative changes in carbohydrates during the manufacture of yogurt. *J Dairy Sci* 59: 45–47
- Goodman MT, Wu AH, Tung K, MuDuffie K, Cramer DW, Wilkens LR, Terada K, Reichardt JKV, Ng WG (2002) Association of galactose-1-phosphate uridylyltransferase activity and N314D genotype with the risk of ovarian cancer. *Am J Epidemiol* 156:693–701
- Hutkins R, Halameck SM, Morris HA (1986) Use of galactose-fermenting *Streptococcus thermophilus* in the manufacture of Swiss, Mozzarella, and short-method Cheddar cheese. *J Dairy Sci* 69:1–8

- Kailasapathy K, Harmstorf I, Phillips M (2008) Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. *Food Sci Technol* 41:1317–1322
- Kindstedt PS, Fox P (1993) Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of Mozzarella cheese. *Crit Rev Food Sci Nutr* 33:167–187
- Kosikowski (1982) Cheese and fermented milk foods. *J Food Microbiol* 45:200–305
- Kroger M (1976) Quality of yogurt. *J Dairy Sci* 59:344–350
- Mouillet L, Luquet FM, Boudier JF, Degros A (1977) Détermination des sucres par chromatographie en phase gazeuse. Applications à la mesure de l'activité de la bêta galactosidase dans les ultra-filtrats de lactosérum et à l'étude de l'évolution du lactose dans les yaourts nature. *Lait* 57:37–54
- Novelli G, Reichardt JKV (2000) Molecular basis of disorders of human galactose metabolism: past, present, and future. *Mol Genet Metab* 71:62–65
- Portnoi PA, MacDonald A (2009) Determination of the lactose and galactose content of cheese for use in the galactosaemia diet. *J Hum Nutr Diet* 22:400–408
- Robitaille G, Moineau S, St-Gelais D, Vadeboncoeur C, Britten M (2007) Galactose metabolism and capsule formation in a recombinant strain of *Streptococcus thermophilus* with a galactose fermenting phenotype. *J Dairy Sci* 90:4051–4057
- Robitaille G, Tremblay A, Moineau S, St-Gelais D, Vadeboncoeur C, Britten M (2009) Fat-free yogurt made using a galactose-positive exopolysaccharide-producing recombinant strain of *Streptococcus thermophilus*. *J Dairy Sci* 92:477–482
- Salminen S, von Wright A (1998) Lactic acid bacteria, microbiology and functional aspects. Marcel Dekker, New York
- Serra M, Trujillo JA, Guamis B, Ferragut V (2009) Flavour profiles and survival of starter cultures of yogurt produced from high-pressure homogenized milk. *Int Dairy J* 19:100–106
- Shah N, Lankaputhra WEV, Britz M, Kyle WSA (1995) Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* commercial yogurt during refrigerated storage. *Int Dairy J* 5: 515–522
- Silvia RM, Rosane FS, Eliana PDC, Alan EW (2001) Isolation and identification of yeasts and filamentous fungi from yogurts in Brazil. *Braz J Microbiol* 32:117–122
- Stang A, Ahrens W, Baumgardt-Elms C, Bromen K, Stegmaier C, Jöckel KH (2005) Carpenters, cabinet makers, and risk of testicular germ cell cancer. *J Occup Environ Med* 47:299–305
- Thomas TD, Crow VL (1984) Selection of galactose-fermenting *Streptococcus thermophilus* in lactose-limited chemostat cultures. *Appl Environ Microbiol* 48:186–191
- Thomas TD, Turner KW, Crow VL (1980) Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris*: pathways, products, and regulation. *J Bacteriol* 144:672–682
- Tinson W, Hillier A, Jago G (1982) Metabolism of *Streptococcus thermophilus* 1. Utilization of lactose, glucose and galactose. *Aust J Dairy Technol* 37:8–13
- Umamaheswari T, Anbukkarasi K, Singh P, Tomar SK, Singh R (2014) *Streptococcus thermophilus* strains of plant origin as dairy starters: isolation and characterisation. *Int J Dairy Technol* 67: 117–122
- Vaillancourt K, LeMay JD, Lamoureux M, Frenette M, Moineau S, Vadeboncoeur C (2004) Characterization of a galactokinase-positive recombinant strain of *Streptococcus thermophilus*. *Appl Environ Microbiol* 70:4596–4603