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Exopolysaccharide-producing mesophilic lactic cultures for preparation of fat-free Dahi – an Indian fermented milk

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Forty seven exopolysaccharide (EPS) producing mesophilic lactic acid bacteria have been isolated from Dahi and raw milk and selected cultures were evaluated for their influence on rheological and sensory properties of fat-free Dahi. Two isolates namely B-6 and KT-24 that showed promising technological attributes were identified as *Lc. lactis* subsp. *lactis* strains. B-6 produced 184 ± 2 mg/l EPS in deproteinized whey medium compared with 193 ± 1 mg/l by KT-24. EPS produced by B-6 was a heteropolysaccharide (consisting of glucose and mannose, 1 : 7·4) with molecular weight of $3{\cdot}0 \times 10^4$ Da whereas KT-24 EPS was a homopolysaccharide (rhamnose) having molecular weight of $4{\cdot}5 \times 10^4$ Da. Both EPS producing cultures showed significant changes in rheological and sensory properties of fat-free Dahi. Dahi prepared by these cultures was more viscous, adhesive, sticky, showed lower susceptibility to whey separation, and received higher sensory scores than Dahi prepared with non-EPS producing culture.

Keywords: Lactic acid bacteria, exopolysaccharides, EPS, *Lc. lactis* subsp. *lactis*, Dahi, rheological properties, sensory properties, fermented milks.

Mesophilic lactic acid bacteria (LAB) have been widely used in traditional fermented milks, industrial fermentation processes and as starter cultures in the dairy industries (Wood, 1997; Savadogo et al. 2004). Apart from the production of lactic acid, flavouring compounds and bacteriocin-like substances, several strains are able to secrete exopolysaccharides (EPS) (Sutherland, 1972; Cerning, 1995). EPS comprises capsular polysaccharides (CPS) that are tightly associated with bacterial cell surface and/or liberated into medium as ropy/slime polysaccharides (Cerning et al. 1992). Both capsular and ropy polysaccharides increase the viscosity of fermented milks but slime polysaccharides produce a stretchable structure unlike capsular polysaccharides (Hassan et al. 1996).

EPS produced by LAB have received much attention in recent years because of their contribution to the rheology and texture properties of food products (Cerning & Marshall, 1999; Ruas-Madiedo & Reyes-Gavilan, 2005). The amounts of EPS produced by lactococcal cultures vary considerably between LAB strains. The quantities reportedly ranged from 45 to 350 mg/l when bacteria are grown under non-optimized culture conditions whereas optimized culture conditions resulted in polysaccharide yield from 150 to 600 mg/l (Cerning, 1995; Cerning & Marshall, 1999; Ricciardi & Clementi, 2000). Although, some authors suppose a direct correlation between EPS concentrations and viscosity of product, no clear relation has been demonstrated (Wacher-Rodarte et al. 1993; Sebastiani & Zelger, 1998), except that if a given strain produces more EPS the viscosity of the fermented milk will increase (Sebastiani & Zelger, 1998). Other factors such as the molecular mass of the EPS and type of linkages especially, β-(1–4), present in the polysaccharide chain play important role in the ability of the polymer to increase viscocity (Tuinier et al. 1999a, b).

Dahi is a popular fermented milk product of India consumed in almost every household (Prajapati & Nair, 2003). It is prepared from buffalo milk (6–8% fat), cow milk (3·5–4·5% fat) or standardized milk (4·5% fat). Annual production of cultured dairy products in India has been estimated to be more than 60,000 MT with an annual growth rate of 20%. (Singh, 2007). Dahi accounts for around 90% of the total cultured milk products produced in India. Generally, Dahi comprises mesophilic (lactococci) and thermophilic (*Streptococcus thermophilus, Lactobacillus delbrueckii, Lb. helveticus* etc.) species of LAB, but lactococci are most commonly used (Behare & Prajapati, 2007). Health awareness among consumers generated more demands for fat-free Dahi in the Indian dairy market. However, milk fat contributes to the flavour, body and texture development of the dairy

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products, removal leads to flavour and textural defects (Haque & Ji, 2003; Guven et al. 2005). Well known technological approaches to improve the quality of product comprise an increase in milk solids (Rohm & Schimid, 1993) and addition of stabilizers. However, these approaches do not address increasing consumer demand for product with low cost and as few food additives as possible (Duboc & Mollet, 2000). In this context, EPS producing LAB as 'biothickeners' can offer natural and more acceptable solution and can only be the preferred approach to many additives (Chistiansen et al. 1999; De Vuyst et al. 2003). These cultures meet the consumer requirement for products with low levels of chemical additives (De Vuyst et al. 2001; Jolly et al. 2002), reduce the amount of total solids required without affecting the textural attributes (Wacher-Rodarte et al. 1993; De Vuyst et al. 2003) and improves sensory properties (Folkenberg et al. 2006).

The present study aimed at isolation and screening of EPS producing mesophilic LAB strains, characterizing EPS from selected cultures and utilizing these strains to improve textural and sensory properties of fat-free Dahi.

Materials and Methods

The study was carried out in Dairy Microbiology Division at National Dairy Research Institute, Karnal, Haryana, India.

Isolation of EPS-producing mesophilic LAB

A total number of 94 Dahi and raw milk (allowed for natural souring at 30 °C) samples were collected from villages and Karnal city of India. Isolation of bacterial cultures was performed by plating appropriate dilutions of sample on milk agar (Mozzi et al. 2001) supplemented with glucose (10 g/l) and yeast extracts (3·5 g/l) and incubating the plates at 30 °C for 72 h. Mucoid colonies formed on milk agar medium were randomly picked by sterile tooth pick and transferred to sterilized skim milk. Tubes in which clean lactic fermentations were observed were retained while those with undesirable fermentation (gassiness, hydrolysis of casein, separation of water) were discarded.

Identification of genera and screening for technological attributes

The EPS-producing LAB isolates were characterized by morphological and biochemical tests comprising catalase activity, growth at different temperatures, growth in 6·5% NaCl, arginine hydrolysis and growth on rogosa agar (Holzapfel & Schillinger, 1992; Cogan & Accolas, 1995) and screened for technological attributes such as titratable acidity, viscosity, ropiness, flavour and body and texture in 10% reconstituted skim milk.

Identification of species by biochemical and PCR methods

Based on technological properties promising EPS-producing cultures were selected and identified by testing for growth at 10, 15 and 45 °C, salt tolerance (2, 4, and 6·5%), growth at pH 9·2 and 9·6, sucrose and maltose fermentation. The taxonomic identity was further confirmed by species-specific PCR using primers for *gad* B gene (Nomura et al. 2002).

Capsule staining

Capsule formation by the cultures was examined by the method of Anthony (1931). Smear was prepared from skim milk culture followed by air drying without heat fixing. Few drops of crystal violet were added, kept for 2 mins and rinsed with 2·0% copper sulphate. The slides were air dried and examined under oil immersion, the capsules could be observed as unstained layer around the cell surface.

EPS production, isolation and purification

Selected EPS-producing isolates were cultured in deproteinized whey (DPW) for EPS production (Rimada & Abraham, 2003). EPS from the fermented DPW was isolated by repetitive ethanol precipitation (Van Geel-Schuten et al. 1998). The crude EPS was purified by DEAE-cellulose ion exchange chromatography and fractions collected were analysed for sugar content by the anthrone method (Southgate, 1991) and protein content by Lowry's method (Lowry et al. 1951).

Characterization of EPS

Purified EPS was hydrolyzed with HCl by adding 500 µl EPS fraction and an equal volume of 2 M-HCl in a glass ampoule which was then sealed and heated at 100 °C for 4 h. Hydrolyzed material was neutralized with 2 M-NaOH (500 µl). Determination of monosaccharide composition was performed by HPLC with sugar Pak I (300 × 6·5 mm) column and a refractive index detector using 100% water (HPLC grade) as mobile phase.

Molecular weight of the EPS was determined by gel filtration using Seralose-4B (Manca de Nadra et al. 1985). The column was calibrated using dextrans of known molecular weights (40,000, 70,000 & 500,000 Da) at a concentration of 5 mg/ml. The molecular weight of purified EPS was determined by graphic plot of the log molecular weight of the dextran against elution volume.

Preparation of fat-free Dahi

Two selected EPS producing lactococcal isolates and non-EPS producing (mixed Dahi culture NCDC 167 obtained from National Collection of Dairy Cultures, Karnal, India)

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Table 1. Characteristics of mesophilic LAB† genera

Characteristics	*Lactococcus*	*Leuconostoc*	*Lactobacillus*	*Enterococcus*
Morphology	Cocci	Oval cocci	Rods	Cocci
Catalase test	–	–	–	+
Gas from glucose	–	+	–	–
Growth at 10 °C	+	+	+/–	+
45 °C	–	–	+/–	+
Growth in 6·5% NaCl	–	–	+/–	+
NH3 from arginine	+/–	–	+/–	+
Growth on rogosa agar	–	–	+	–
Total number of Isolates	15	13	6	13

'+' positive reaction; '–' negative reaction; +/– positive or negative reaction; † LAB were isolated from dahi and raw milk

cultures were used to ferment milk. Fat-free Dahi was prepared from reconstituted (12%) skimmed milk heat treated at 90 °C for 10 min, cooled to 30 °C, inoculated with 2% starter culture and incubated at optimum growth temperature of the cultures. After setting of Dahi, containers were stored at 5 °C for 12 h.

Physico-chemical analysis

Titratable acidity of Dahi was determined by titration method (IS:1479, partI, 1960). Whey separation was determined by the method of Wacher-Rodarte et al. (1993). Thirty gram of Dahi was centrifuged at 1535 ***g*** for 20 min and the expelled whey was measured. Whey separation (%) was expressed as weight (g) of expelled whey per 100 g Dahi.

Rheological analysis

Viscosity measurement An arbitrary procedure was adopted to provide uniform samples for viscosity measurement. The gel was broken by stirring with a glass rod (10 times clockwise; 10 times anticlockwise). Rotational viscosity measurements were made using a Contraves Rheomat 108 E/R Coaxial Cylinder Viscometer (Metler-Toledo, Switzerland) with a suitable shear rate of 200/S and spindle (2'2') immersed to about one third of the spindle length.

Texture profile analysis (TPA) analysis was carried out by the method described by Kumar & Mishra (2003) using a TAXT2 *i* Texture Analyzer (Texture Technologies corp., UK, Model TA. XT2 *i*, version 05.16 equipped with 5 kg load cell). Experiments were performed by compression tests that generated plot of force (grams) vs time (S). A 25 mm diameter perplex cylindrical probe (P 25) was used to measure textural profile of set Dahi samples prepared in a 100 ml beaker at the temperature of 25±1 °C, performing three repetitions.

Sensory Analysis

A panel of seven trained judges performed the sensory evaluation. Dahi samples were served in 100 ml glass containers. Sensory parameters such as flavour, body and texture, colour and appearance as well as acidity were rated on a 9-point hedonic scale (like extremely 9; dislike extremely 1).

Statistical analysis

The data were analysed using SYSTAT software (version 6). The mean and standard error of the values were determined and one-way analysis of variance was used to test significance between the cultures.

Results

Isolation and identification of EPS-producing mesophilic LAB genera

Forty seven mesophilic LAB isolates were obtained from different *Dahi* and raw milk samples. Based on morphological and biochemical tests, the isolates were identified as *Lactococcus* sp., *Leuconostoc* sp., *Lactobacillus* sp. and *Enterococcus* sp. (Table 1).

Screening of isolates for technological attributes and species identification

The cultures screened for technological parameters, revealed that only two isolates from *Lactococcus* sp. namely B-6 and KT-24, showed optimal acid production as per Bureau of Indian Standards (0·8–1·0% lactic acid), high viscosity, least ropiness, low whey separation (observed visually), pleasant flavour and good body and texture (Table 2). Biochemical tests identified B-6 and KT-24 as *Lc. lactis* subsp. *lactis*. Both the isolates yielded a PCR amplicon of 602 bp with species specific primers for *Lc. lactis* subsp. *lactis* (Fig. 1).

EPS production and characterization

Lc. lactis subsp. *lactis* B-6 and KT-24 were able to show capsules by copper sulphate staining (Fig. 2a & 2b). KT-24 produced significantly ($P<0·05$) higher amounts of EPS in comparison with B-6 (Table 3) and also showed large thick

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Table 2. Technological properties of two selected EPS-producing *Lc. lactis* subsp. *lactis* strains and EPS-negative NCDC 167 mixed *Dahi* culture in 10% skim milk

Name of culture	Acidity (% lactic acid)	Viscosity (Pa.s)	Curd setting time (hr)	Curd characteristics
B-6	0·95	0·177	6·0	Pleasant flavour, good body & texture
KT-24	0·90	0·179	5·5	Pleasant flavour, good body & texture
NCDC 167	0·98	0·146	8	Pleasant flavour, good body & texture

Values are average of three trials

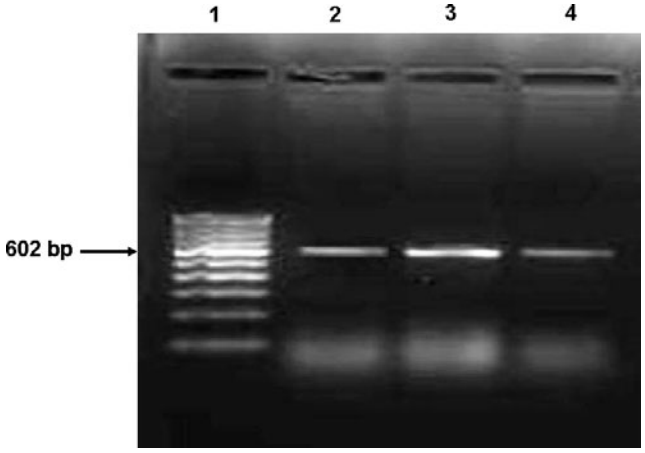

Fig. 1. PCR Products of B-6 and KT-24 cultures using species-specific primers for *Lc. lactis* subsp *lactis* **1.** Ladder 100 bp; **2.** *Lc. lactis* subsp *lactis* B-6; **3.** *Lc. lactis* subsp *lactis* KT-24; **4.** *Lc. lactis* subsp. *lactis* NCDC 91 (Reference strain).

capsule surrounding the cell surface. Repetitive ethanol precipitation steps followed for isolation of lactococcal EPS from DPW gave better yield of carbohydrates. Purification by DEAE-cellulose ion exchange led to pure EPS with greater than 99·5% carbohydrate with negligible amount of protein content (Table 3).

The exopolysaccharide produced by *Lc. lactis* subsp. *lactis* B-6 was heteropolysaccharide composed of glucose and mannose in a ratio of 1 : 7·4. On the other hand, *Lc. lactis* subsp. *lactis* KT-24 produced homopolysaccharide containing only rhamnose (Table 3).

Gel filtration analysis revealed that the approximate molecular weight of EPS from B-6 was comparatively lower than the molecular weight of KT-24 (Table 3).

Effect of EPS-producing cultures on fat-free Dahi

Physico-chemical characteristics The EPS producing cultures showed similar acidification profile to the control as titratable acidity of Dahi did not differ significantly between EPS^+ (EPS producer) and EPS^- (Non-EPS producer) cultures (Table 4). EPS production by the cultures had no bearing on titratable acidity. Susceptibility to whey separation

Fig. 2a. Capsule formation by *Lc. lactis* subsp. *lactis* B-6 as shown by negative staining using copper sulphate.

Fig. 2b. Capsule formation by *Lc. lactis* subsp. *lactis* KT-24.

decreased with use of EPS-producing cultures. Dahi made by *Lc. lactis* subsp. *lactis* KT-24 showed lesser whey separation. Whey separation for *Lc. lactis* subsp. *lactis* B-6 did not differ significantly from (and was midway between) EPS^- NCDC 167 and EPS^+ KT-24, which may be attributed to the comparatively lower EPS production by B-6.

Rheological characteristics

EPS^+ B-6 and KT-24 exhibited significantly higher viscosity ($P<0·05$) than EPS^- NCDC 167 (Table 4). EPS^+ B-6 and

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Table 3. Amount, recovery, molecular weight and monosaccharide composition of EPS from *Lc. lactis* subsp. *lactis* strains

Values are means ± SE for $n=3$

EPS from	EPS production (mg L^{-1})	EPS Recovery (%) before ion exchange	EPS Recovery (%) after ion exchange	Molecular weight (Da)	Monosaccharides
B-6	184 ± 2^{a}	81·41	99·49	$3{\cdot}0 \times 10^{4}$	Glc: Man
KT-24	193 ± 1^{b}	84·04	99·77	$4{\cdot}5 \times 10^{4}$	Rha

[a,b]values with different superscripts in a column differs significantly ($P<0{\cdot}05$); Glc- glucose; Man- Mannose; Rha- Rhamnose

Table 4. Effect of EPS-producing *Lc. lactis* subsp. *lactis* strains on physico-chemical, rheological and sensory properties of fat-free Dahi

Values are Mean ± SE for $n=3$

Parameters	Cultures NCDC167	B-6	KT-24	MSS Values
Physico-chemical				
Titratable Acidity (%)	$1{\cdot}03 \pm 0{\cdot}75^{a}$	$1{\cdot}02 \pm 0{\cdot}04^{a}$	$0{\cdot}89 \pm 0{\cdot}02^{a}$	$0{\cdot}018^{NS}$
Whey Separation (%)	$23{\cdot}14 \pm 0{\cdot}82^{a}$	$21{\cdot}82 \pm 0{\cdot}15^{ab}$	$20{\cdot}82 \pm 0{\cdot}11^{b}$	4·050*
Rheological				
Viscosity (Pa.Sec.)	$0{\cdot}174 \pm 0{\cdot}01^{a}$	$0{\cdot}187 \pm 0{\cdot}02^{b}$	$0{\cdot}199 \pm 0{\cdot}04^{c}$	0·001**
Firmness (g)	$112{\cdot}71 \pm 1{\cdot}11^{a}$	$98{\cdot}09 \pm 2{\cdot}0^{b}$	$93{\cdot}64 \pm 0{\cdot}86^{b}$	298·53**
Work of Adhesion (g.s)	$-34{\cdot}30 \pm 0{\cdot}73^{a}$	$-36{\cdot}24 \pm 2{\cdot}46^{a}$	$-42{\cdot}57 \pm 1{\cdot}02^{b}$	56·13*
Work of Shear (g.s)	$2046{\cdot}51 \pm 55{\cdot}36^{a}$	$1808{\cdot}24 \pm 20{\cdot}20^{b}$	$1764{\cdot}91 \pm 15{\cdot}12^{b}$	68977·14**
Stickiness (g)	$-24{\cdot}20 \pm 0{\cdot}58^{a}$	$-28{\cdot}12 \pm 0{\cdot}92^{b}$	$-30{\cdot}41 \pm 0{\cdot}23^{c}$	29·55**
Sensory				
Flavour	$5{\cdot}50 \pm 0{\cdot}29^{a}$	$6{\cdot}33 \pm 0{\cdot}17^{b}$	$7{\cdot}0 \pm 0{\cdot}12^{b}$	1·69**
Body and Texture	$6{\cdot}10 \pm 0{\cdot}21^{a}$	$7{\cdot}30 \pm 0{\cdot}15^{b}$	$7{\cdot}60 \pm 0{\cdot}06^{b}$	1·89**
Colour and Appearance	$7{\cdot}33 \pm 0{\cdot}09^{a}$	$7{\cdot}70 \pm 0{\cdot}06^{b}$	$7{\cdot}90 \pm 0{\cdot}06^{b}$	0·25**
Acidity	$7{\cdot}17 \pm 0{\cdot}09^{a}$	$7{\cdot}70 \pm 0{\cdot}06^{b}$	$7{\cdot}93 \pm 0{\cdot}03^{c}$	0·463**

**Significant at 1%; *Significant at 5%; [NS]Non-significant
[a,b,c]values with different superscripts in rows differs significantly ($P<0{\cdot}05$)
MSS- Mean sum square

KT-24 exhibited significantly higher viscosity ($P<0{\cdot}05$) than EPS^{-} NCDC 167. KT-24 showed highest viscosity of Dahi. Firmness of Dahi represents the strength of the coagulum and shows an inverse relation with EPS production. Dahi prepared from B-6 and KT-24 cultures showed lower firmness values. EPS producing strains made Dahi more adhesive, which would indicate a contribution of EPS to the tendency of the product to adhere to the surface of other materials. Shear force required for Dahi prepared using EPS^{-} NCDC 167 cultures was significantly higher ($P<0{\cdot}05$) than Dahi with EPS^{+} cultures. EPS-producing cultures also increased stickiness of Dahi which further increased for higher EPS producing culture KT-24.

Sensory characteristics

Sensory scores differed significantly ($P<0{\cdot}05$) between EPS^{+} and EPS^{-} cultures (Table 4). Flavour, body and texture, colour and appearance, as well as acidity scores of Dahi were increased for B-6 and KT-24 compared with NCDC 167. The scores for these attributes related directly to the ability of culture to produce more EPS.

Discussion

The carbon sources added to the screening media play an important role in the detection of EPS phenotypes in LAB and total amount of polysaccharides produced is strongly influenced by the sugar available in the medium (Ruas-Madiedo & Reyes-Gavilan, 2005). In the past, EPS-producing LAB have been isolated from dairy and non-dairy environments using different media; EPS Selection Medium, MRS, M17, Milk Indicator Agar and Milk Agar (Van Geel-Schuten et al. 1998; Smitinont et al. 1999; Sanni et al. 2002; Savadogo et al. 2004). These media contain high concentrations of sugar that facilitates easy differentiation of EPS strains from non-EPS by allowing them to

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form mucoid colonies on agar plates (Vescovo et al. 1989; Dierksen et al. 1997). In our study, we used milk agar supplemented with glucose to increase the probability of EPS producing phenotypes. However, limited numbers of EPS-producing mesophilic phenotypes could be obtained after screening a large number of Dahi and raw milk samples, which indicates low frequency of isolation of these cultures in agreement with other studies (Smitinont et al. 1999; Sanni et al. 2002).

Primary screening of EPS^+ LAB strains for technological attributes is important because not all EPS cultures produce product with desirable characteristics (De Vuyst et al. 2003). We selected only two *Lc. lactis* strains because most cultures showed poor flavour, weak curd and developed ropiness. Excessive ropiness produced in Dahi is undesirable. The ability to synthesize capsular polysaccharides (CPS) or produce ropy (R^+) polysaccharide by LAB is strain dependent and very few reports indicate LAB strains that produce both CPS and ropy polysaccharide (Knoshaug et al. 2000; Ruas-Madiedo et al. 2002; Hassan et al. 1995). Mozzi et al. (2006) reported that out of 201 EPS-producing LAB, only two thermophilic strains showed both CPS^+ and R^+ character, whereas six mesophilic LAB produced both capsular as well as ropy polysaccharides.

Analysis of monosaccharide composition revealed that the polymer of B-6 comprised glucose and mannose while that of KT-24 contained only rhamnose. The monosaccharides occurring most frequently in the various exopolysaccharides from mesophilic lactic acid bacteria are glucose (Cerning, 1995; Marshall et al. 1995), rhamnose (Nakajima et al. 1990; Mozzi et al. 2006) and mannose (Cerning et al. 1992; Savadogo et al. 2004). To our knowledge, this is the first report of a rhamnose homopolysaccharide produced by *Lc. lactis* subsp. *lactis*. Few lactobacilli and streptococci have been reported earlier to produce EPS containing rhamnose along with some other sugars, glucose or galactose (De Vuyst et al. 2003; Dolyeres et al. 2005, Savadogo et al. 2004).

The polysaccharide produced by KT-24 had a molecular weight of $4{\cdot}5 \times 10^4$ Da compared with 3×10^4 Da molecular weight of EPS produced by B-6. The molecular weight of a given polymer is related to the thickening effect of an EPS in aqueous solution (Tuinier et al. 1999a, b; Ruas-Madiedo et al. 2002).

Results of technological parameters for fat-free Dahi demonstrate that, EPS producing lactococcal cultures had significant effect on physico-chemical, rheological and sensory properties. Whey separation was reduced in Dahi made by *in situ* EPS producing cultures due to the ability of EPS to bind significant amount of water. Previous studies also report the water binding ability of EPS in yoghurt (Wacher-Rodarte et al. 1993; Marshall & Rawson, 1999; Doleyres et al. 2005).

Viscosity, adhesiveness and stickiness of fat-free Dahi increased when EPS-producing cultures were used indicating that EPS contributed to the rheological properties (Rawson & Marshall, 1997; Marshall & Rawson, 1999; Dolyeres et al. 2005; Folkenberg et al. 2005). Adhesiveness is an important factor for the description of mouth feel for a given food material. Dahi made from EPS negative culture was firmer and required higher shearing force, due to the formation of strong protein-protein interactions as a result of the fermentation process (Hassan et al. 1996; Bouzar et al. 1997; Marshall & Rawson, 1999). The contribution of the EPS producing strains to the textural properties is said to be a result of the secretion of extracellular polysaccharides and the ability of the polysaccharide to form strands which connect the bacteria to the casein micelles (Tamime et al. 1984).

Sensory scores such as flavour, body and texture, colour and appearance and acidity of Dahi were also improved by use of EPS-producing cultures. Folkenberg et al. (2005) reported that yoghurt fermented with EPS-producing cultures showed increased mouth thickness, shininess and tended to be creamier than yoghurt without these cultures.

The water holding capacity, viscosity, adhesiveness and stickiness of Dahi increased, when culture produced more EPS and high molecular weight EPS. However, it was difficult to say whether it was the effect of more EPS or high molecular weight because KT-24 showed higher EPS production as well as produced high molecular weight compound than B-6.

To conclude, two new EPS producing *Lc. lactis* subsp. *lactis* strains have been isolated and these cultures improved rheological and sensory properties of fat-free Dahi; showed lower susceptibility to whey separation, higher viscosity, adhesiveness, stickiness and sensory attributes.

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