J Food Sci Technol (November–December 2013) 50(6):1228–1232
DOI 10.1007/s13197-013-0999-6

SHORT COMMUNICATION

Exopolysaccharides producing *Lactobacillus fermentum* strain for enhancing rheological and sensory attributes of low-fat dahi

Pradip V. Behare · Rameshwar Singh · Ravinder Nagpal · K. H. Rao

Revised: 25 October 2012 / Accepted: 8 April 2013 / Published online: 17 April 2013

Abstract *Lactobacillus fermentum* V10 was able to show large capsules surrounding the cell surface and produced 247.37±0.76 mg/L polysaccharides in fermentation medium. The effect on technological properties of low-fat dahi found to be significantly ($P<0.01$) improved for *Lactobacillus fermentum* V10 than control dahi made by EPS$^+$ *Lb. delbrueckii* subsp. *bulgaricus* NCDC 285 and EPS$^-$ *Lb. delbrueckii* subsp. *bulgaricus* 09 cultures. Low fat dahi prepared by exopolysaccharides producing *Lactobacillus fermentum* V10 exhibited optimum acid production, lesser whey separation, higher viscosity, increased adhesiveness and stickiness whereas decreased firmness and work of shear as compared to control dahi.

Keywords Dahi · Exopolysaccharides · Lactobacillus · Low-fat dairy products

Introduction

Lactic Acid Bacteria (LAB) producing exopolysaccharides (EPS) have received much attention of scientific community, in recent years, due to their useful role in improvement of physical, rheological and sensory properties of fermented milks such as dahi, yoghurt, lassi, cultured buttermilk, etc. (Behare et al. 2009a, b, 2010). Besides acting as biothickening agents, some EPS produced by LAB have showed beneficial effects on human health (Chabot et al. 2001). Production of EPS varies from strain to strain and depends on various factors like composition of the medium, age of the cell, pH and temperature (De Vuyst et al. 2003; Ruas-Madiedo and de los Reyes-Gavilan 2005). Bacterial cell produces capsular polysaccharides associated with the cell surface and/or ropy polysaccharides in the fermentation medium (Sutherland 1972). Some studies have proved the additive effect of in situ EPS production on technological properties of fermented milk products than incorporating EPS powder externally (Behare et al. 2009c; Doleyres et al. 2005).

Dahi is a popular fermented milk product of India resembling yoghurt (Behare and Prajapati 2007). Most commonly used strains of *Lactobacillus* species for dahi making includes *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. lactis,* and *Lb. casei*. However, ability to produce extremely high level of acidity, their slow fermentation profile, lengthy and time consuming incubation period hampers their use as commercial fermentation agents than other lactic cultures. Nevertheless, several strains of lactobacilli are able to produce exopolysaccharides find suitable for dahi making. Lot of work has been done on development of EPS producing cultures and some of the commercial companies have introduced EPS cultures for western products. However, in India due to lack of supported research data and unavailability of EPS cultures of Indian origin, the commercial exploitation has not been possible. Moreover, the EPS producing strains used in western countries for preparation of fermented milks are specifically developed for particular product; their behavior may change with the product. This suggests search of new EPS strains of Indian origin that are made easily available for the commercial

P. V. Behare (✉) · K. H. Rao
Dairy Microbiology, National Dairy Research Institute Karnal, Haryana, Karnal 132001 Haryana, India
e-mail: pradip_behare@yahoo.com

K. H. Rao
e-mail: khrao@naarm.ernet.in

R. Singh
Dairy Microbiology Division, National Dairy Research Institute, Karnal 132001 Haryana, India
e-mail: rsndri@gmail.com

R. Nagpal
Department of Biotechnology, JMIT Institute of Engg. & Tech., Radaur 135133 Haryana, India

exploitation. Consumers demand for natural, healthy and low-calorie food has increased interest in the dairy industries for development and manufacture of low-fat/fat-free fermented milks (Behare et al. 2009a, b). However, these products develop certain undesirable textural, rheological and sensory defects that have been attempted to control by incorporation of additives like stabilizer and use of extra milk solids. But these approaches does not support the consumer's view of product with natural, low-cost and food with as few additives as possible. Alternative to above additives, the use of natural biothickening cultures particularly producing EPS may find suitable options. Hence, in present investigation, an effort was made to isolate indigenous biothickening lactic culture in order to improve textural, rheological and sensory properties of low-fat dahi. The diverse microflora from indigenous fermented milks prepared in rural and urban areas using traditional methods may be a potential reservoir for isolation and exploitation of such EPS producing cultures.

Materials and methods

EPS cultures and their maintenance

Exopolysaccharides producing *Lactobacillus fermentum* V10, previously isolated from traditionally made dahi samples is used in this study. Two commercial *Lactobacillus* strains EPS$^+$ *Lb. delbrueckii* subsp. *bulgaricus* NCDC285 and EPS$^-$ *Lb. delbrueckii* subsp. *bulgaricus* NCDC 09 (collected from National Collection of Dairy Cultures, NCDC, Karnal, Haryana, India) are used as control. Cultures were maintained in MRS broth and 10 % sterile reconstituted skim milk.

Capsule staining

Capsule formation by the culture was examined by the method of Anthony (1931). Smear was prepared from skim milk culture followed by air drying without heat fixing. Few drop of crystal violet were added, kept 2 mins and rinsed with 20 % (w/v) copper sulphate solution. The slides were air dried and examined under oil immersion, the capsules could be observed layer around the cell surface.

EPS production, isolation and purification

Lactobacillus fermentum V10 and control EPS-producing *Lb. delbrueckii* subsp. *bulgaricus* NCDC 285 were cultured in deproteinized whey (DPW) for EPS production (Rimada and 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 analyzed 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) (Behare et al. 2009a). Determination of monosaccharide composition was performed by HPCL with sugar Pak I (300×6.5 mm) column and a refractive index detector using 100 % water (HPLC grade as mobile phase (Behare et al. 2009a).

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 and 500,000 Da) at a concentration of 5 mg ml^{-1}. The molecular weight of purified EPS was determined by graphic plot of the log molecular weight of the dextran against elution volume.

Preparation of low-fat dahi

Lactobacillus fermentum V10 and two commercial *Lactobacillus* strains (EPS$^+$ *Lb. delbrueckii* subsp. *bulgaricus* NCDC285 and EPS$^-$ *Lb. delbrueckii* subsp. *bulgaricus* NCDC 09) were used for fermentation of milk. Low-fat dahi was prepared from reconstituting (12 % w/v) skimmed milk heat treated at 90 °C for 10 mins, cooled to optimum growth temperature (42 °C) of the cultures, inoculated with 2 % (v/v) starter culture and incubated at same temperature. 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 (Indian Standards 1960). Whey separation was determined by the method of Wacher-Rodarte et al. (1993) and Behare et al. (2009a). Whey separation was expressed as weight (g) of expelled whey per 100 g dahi.

Rheological analysis

Viscosity was measured by arbitrary procedure using Contraves Rheomat 108 E/R Coaxial Cylinder Viscometer (Metler-Toledo, Switzerland) as per the method described by Behare et al. (2009a). Texture Profile Analysis (TPA) was carried out by the method described by Behare et al. (2009a) and Kumar and Mishra (2003).

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 flavor, 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 analyses

The experiments were carried out in triplicates. The data were analyzed 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 and discussions

EPS production and characterization

EPS producing *Lactobacillus fermentum* V10 and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC 285 were able to show large capsules surrounding the cell surface by copper sulphate staining (Fig. 1a and b) and produced extremely higher quantity of polysaccharides in deproteinized whey (DPW). *Lactobacillus fermentum* V10 showed highest amount of exopolysaccharides in fermentation medium (Table 1). Repetitive ethanol precipitation steps followed for isolation of *Lactobacillus* EPS from DPW gave better yield of carbohydrates. Purification procedure followed by DEAE-cellulose ion exchange chromatography led to pure EPS with greater than 99 % carbohydrate with less than 1 % of protein content.

The EPS produced by isolate V10 and NCDC 285 was heteropolysaccharides. The compositions of *Lactobacillus fermentum* V10 and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC 285 were glucose: rhamnose : galactose in a proportion of 1 : 13 : 1.5 and rhamnose : galactose in a proportion of 1:6.8 respectively (Table 1). The monosaccharides most frequently occurring in the various LAB strains are glucose (Cerning 1995), rhamnose (Mozzi et al. 2006; Nakajima et al. 1990) and galactose (De Vuyst et al. 2003; Doleyres et al. 2005).

Gel filtration analysis revealed that the approximate molecular weight of EPS from isolate V10 that was comparatively higher than the molecular weight of EPS from NCDC 285 (Table 1). The molecular weight of given polymer is related to the thickening effect of an EPS in an aqueous solution (Ruas-Madiedo et al. 2002; Tuinier et al. 1999a, b), higher the molecular weight, greater will be the viscofying ability of the polymer.

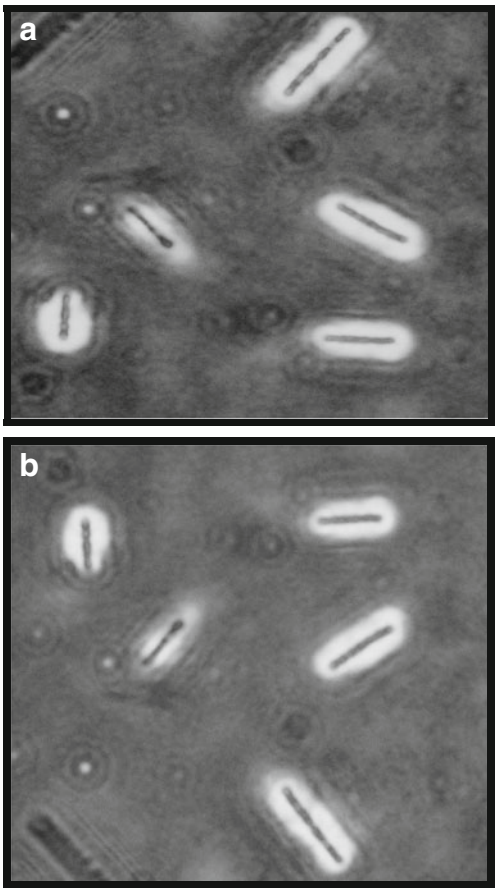

Fig. 1 Capsule formation by **a** *Lactobacillus fermentum* V10 and **b** *Lactobacillus delbrueckii subsp. bulgaricus* NCDC 285

Effect of *Lactobacillus* cultures on low-fat dahi

Physico-chemical characteristics The EPS producing cultures showed similar acidification profile to the control as titratable acidity did not differ significantly between the cultures either EPS-producer or non-EPS producer (Table 2). EPS production by the cultures had no effect on titratable acidity. Susceptibility to whey separation in low-fat dahi decreased with use of EPS-producing cultures and between EPS-producing cultures dahi made by isolate V10 exhibited lesser whey separation than NCDC 285. Thus Higher EPS producing culture showed maximum effect on control of syneresis (Table 2). Previous studies also reported the water binding ability of EPS in low-fat yoghurt, dahi and lassi (Behare et al. 2009a, 2010; Doleyres et al. 2005; Marshall and Rawson 1999; Wacher-Rodarte et al. 1993).

Rheological characteristics Lactobacillus fermentum V10 exhibited significantly ($P<0.01$) higher viscosity of low-fat dahi than EPS-producing *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC 285 and non-EPS producing *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC 09

Table 1 Amount, recovery, molecular weight and monosaccharide composition of EPS from V10 and NCDC 285 strains (Values are means ± SE for $n=3$)

EPS from	EPS production (mg/L)	EPS recovery (%)		Molecular weight (Da)	Monosaccharides
		Before ion exchange	After ion exchange		
NCDC 285	219.6±2.34 [a]	89.0	99.1	4.7×10^5	Rha : Gal (1:6.8)
V10 isolate	247.4±0.76 [b]	89.4	99.4	5.7×10^5	Glu: Rha: Gal (1:13:1.5)

a, b, values with different superscripts in column differs significantly ($p<0.01$); *glu* glucose; *rha* rhamnose; *ga* galactose, *EPS* exopolysaccharides., *SE* Standard Error

(Table 2). Firmness of dahi represents the strength of the coagulum and shows an inverse relation with EPS production. Dahi prepared by both EPS producing cultures showed lower firmness values. EPS producing cultures 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 EPS non-producing NCDC 09 culture was significantly higher than that required for EPS producing cultures. EPS producing cultures also increased stickiness of dahi which further increased for higher EPS producing cultures. Adhesiveness is an important factor, correlated with mouthfeel for a given food material. 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 polysaccharides to form strands, which connect bacteria to the casein micelles (Tamime et al. 1984).

Sensory characteristics Sensory parameters such as flavour, colour and appearance and acidity differ significantly for EPS^+ and EPS^- cultures, were higher for both EPS producing cultures. Body and texture scores were highly significant ($P<0.01$) for EPS producing V10 and NCDC 285. However, highest values could be observed for dahi made by *Lactobacillus fermentum* V10, indicating EPS culture increased mouth thickness, shininess and tended to be creamier than dahi without EPS culture. Behare et al. (2009a) and Folkenberg et al. (2005) found similar sensory observations respectively for dahi and yoghurt.

Conclusion

In present investigation, a new EPS producing *Lactobacillus fermentum* V10 of Indian origin has been isolated, which improved phsico-chemical, rheological and sensory properties of low-fat dahi; showed lesser whey separation, higher viscosity, adhesiveness, stickiness and sensory attributes. Incorporation of *Lactobacillus fermentum* V10 as dahi culture could reduce amount of total solids required and avoid use of additives. This could also be recommended for commercial preparation of fat-free, reduced fat and low-fat dahi.

Table 2 Effect of EPS-producing *Lactobacillus* cultures on physico-chemical, rheological and sensory properties of low-fat dahi (Values are means ± SE for $n=3$)

Parameters	Cultures			MSS values
	NCDC 09	NCDC 285	Isolate V10	
Physico-chemical				
Titratable acidity (% LA)	1.01±0.03 [a]	0.96±0.02 [a]	0.93±0.03 [a]	0.023[NS]
Whey separation (%)	26.9±0.25 [a]	16.5±0.30 [b]	14.7±0.10 [b]	130.810**
Rheological				
Viscosity (Pa. Sec.)	0.173±0.01 [a]	0.413±0.02 [b]	0.518±0.03 [c]	0.375**
Firmness (g)	161.6±17.92 [a]	114.6±12.08 [b]	103.6±9.60 [c]	11359.29**
Work of adhesion (g.s.)	−38.7±4.37 [a]	−80.4±8.76 [b]	−89.3±9.68 [c]	8737.90**
Work of shear (g.s.)	2691.3±224.44 [a]	1903.9±157.56 [b]	1615.9±119.84 [c]	3718518.83**
Stickiness (g)	−30.4±3.76 [a]	−72.4±8.26 [b]	−76.5±9.26 [c]	7810.96**
Sensory				
Flavor	5.5±0.13 [a]	6.5±0.12 [b]	6.9±0.12 [c]	6.09**
Body and texture	5.9±0.15 [a]	7.1±0.20 [b]	7.6±0.23 [c]	9.16**
Colour and appearance	6.4±0.08 [a]	6.8±0.08 [b]	7.0±0.08 [c]	1.09**
Acidity	5.9±0.11 [a]	6.6±0.10 [b]	6.9±0.12 [c]	2.86**

** Significant at 1 %; a, b, c, values with different superscripts in rows differs significantly ($P<0.01$)

MSS Mean Sum Square, *EPS* exopolysaccharides., *SE* Standard Error

Acknowledgments The authors wish to express gratitude to the ICAR, New Delhi, and NDRI, Karnal, for providing financial assistant and necessary facilities to carry out the research work.

References

Anthony EE (1931) A note on capsule staining. Science 73:319–320

Behare PV, Prajapati JB (2007) Thermization as a method for enhancing the shelf-life of cultured buttermilk. Ind J Dairy Sci 60:86–93

Behare PV, Singh R, Singh RP (2009a) Exopolysaccharide-producing mesophilic lactic cultures for preparation of fat-free Dahi- an Indian fermented milk. J Dairy Res 76:90–97

Behare PV, Singh R, Kumar M, Prajapati JB (2009b) Exopolysaccharide of lactic acid bacteria-a review. J Food Sci Technol 47:1–13

Behare PV, Singh R, Nagpal R, Kumar M, Tomar SK, Prajapati JB (2009c) Comparative effect of exopolysaccharides produced in situ or added as bioingredients on dahi properties. Milchwissenschaft 64:369–400

Behare PV, Singh R, Tomar SK, Nagpal R, Kumar M, Mohania D (2010) Effect of exopolysaccharide producing strains of *Streptococcus thermophilus* on technological properties attributes of lassi. J Dairy Sci 93:2874–2879

Cerning J (1995) Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. Lait 75:463–472

Chabot S, Yu HL, De LL, Cloutier D, van Calsteren MR et al (2001) Exopolysaccharides from *Lactobacillus rhamnosus* RQ-595M stimulate TNF, IL-6 and IL-12 in human and mouse cultured immune-competent cells, and IFN-g in mouse splenocytes. Lait 81:683–697

De Vuyst L, Zamfir M, Mozzi F et al (2003) Exopolysaccharides producing *Streptococcus thermophilus* strains as functional starter cultures in the production of fermented milks. Int Dairy J 13:707–717

Doleyres Y, Schaub L, Lacroix C (2005) Comparison of the functionality of exopolysaccharides produced in situ or added as bioingredients on yoghurt properties. J Dairy Sci 88:4146–4156

Folkenberg DM, Deijmek P, Skriver A, Ipsen R (2005) Relation between sensory texture properties and exopolysaccharide distribution in set and in stirred yoghurts produced with different starter cultures. J Texture Stud 36:174–189

Indian Standards (1960) Methods of testing for dairy industry: part-I. Rapid examination of milk. Indian Standards Institution, New Delhi

Kumar P, Mishra HN (2003) Optimization of mango soy fortified yoghurt formulation using response surface methodology. Int J Food Prop 6:499–517

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Manca de Nadra MC, Strasser de Saad AM, Pesce de Ruiz Holgado AA, Oliver G (1985) Extracellular polysaccharide production by *Lactobacillus bulgaricus* CRL. Milchwissenschaft 40:409–411

Marshall VM, Rawson HL (1999) Effects of exopolysaccharide producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. Int J Food Sci Technol 34:137–143

Mozzi F, Vaningelgem F, Hebert EM et al (2006) Diversity of heteropolysaccharide producing lactic acid bacterium strains and their biopolymers. Appl Environ Microbiol 72:4431–4435

Nakajima H, Toyoda S, Toba T et al (1990) A novel polysaccharide from slime forming *Lactococcus lactis* subsp. *cremoris* SBT 0495. J Dairy Sci 73:1472–1477

Rimada PS, Abraham AG (2003) Comparative study of different methodology to determine the exopolysaccharides produced by kefir grains in milk and whey. Lait 83:79–87

Ruas-Madiedo P, de los Reyes-Gavilan CG (2005) Invited review: methods for the screening, isolation and characterization of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci 88:843–856

Ruas-Madiedo P, Tuinier R, Kanning M, Zoon P (2002) Role of exopolysaccharides produced by *Lactococcus lactis* subsp. *cremoris* on the viscosity of fermented milks. Int Dairy J 12:689–695

Southgate DAT (1991) Selected methods in determination of food carbohydrates. Elsevier, London

Sutherland IW (1972) Bacterial exopolysaccharides. Adv Microb Physiol 8:143–212

Tamime AY, Kaloh M, Davies G (1984) Microstructure of set-style yoghurt manufactured from cows milk fortified by various methods. Food Microstruct 3:83–92

Tuinier R, Zoon P, Cohen-Stuart MA et al (1999a) Concentration and shear-rate dependence of an exocellur polysaccharide. Biopolymers 50:641–646

Tuinier R, Zoon P, Olieman C et al (1999b) Isolation and physical characterization of an exocellur polysaccharide. Biopolymers 49:1–9

Van Geel-Schuten GH, Flesch F, ten Brink B et al (1998) Screening and characterization of *Lactobacillus* strains producing large amounts of exopolysaccharides. Appl Microbiol Biotechnol 50:697–703

Wacher-Rodarte CM, Galvan MV, Farres A et al (1993) Yoghurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. J Dairy Res 60:247–254