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Exopolysaccharides Producing Lactic Acid Cultures for Indigenous Fermented Milk Products

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ABSTRACT

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 milk products. Fat removal has several undesirable effects on physical properties of fermented milks such as inferior flavour, texture and rheological properties that consequently hamper their acceptability. Several attempts were made including increase in milk solids and addition of stabilizers to tackle such problems (Rohm and Schimid 1993). However, these approaches did not address an increasing consumer demand for products with natural, low-cost and with as few food additives as possible. Furthermore, additives particularly, stabilizers are strictly prohibited in some fermented milk products like dahi in India and yoghurt in Norway by the stringent regulations. In this context, there is no alternative to use EPS producing lactic cultures, which offer a natural and usually acceptable way for making low-fat/fat-free fermented milks. Use of EPS producing cultures in indigenous fermented milk products such as dahi, lassi, cultured butter milk etc. has been reported recently. EPS producing cultures have been found to improve rheological and sensory properties of low-fat dahi and lassi (Behare *et al.*, 2009a; Behare *et al.*, 2010; Behare *et al.*, 2013). Apart from this EPS cultures improves yield, without need of additives and reduces cost of production of such fermented milk.

INTRODUCTION

Lactic acid bacteria (LAB) are widely used in the dairy and food industry since time immemorial. Apart from production of lactic acid, flavoring compounds and bacteriocin like substances, several strains of LAB secrete extracellular polysaccharide in favorable environment such as milk. The term exopolysaccharide (EPS) is used to describe extracellular polysaccharide either attached as capsule with bacterial cell wall or liberated into the medium as ropy polysaccharide (Sutherland 1972). The EPS plays an important role in the improvement of physical properties of fermented milks, which act like a food stabilizer, viscosifier, emulsifier or gelling agent providing a product with natural thickness (Ruas-Madiedo and Reyes-Gavilan 2005). However the *in situ* EPS production is better approach as compared to use of crude and/or purified EPS in the manufacture of a variety of cultured dairy products (Dolyeres *et al.*, 2005; Behare *et al.*, 2009a). The *in situ* EPS production is extensively used in the manufacture of yoghurt, drinking yoghurt, cheese, cultured cream and milk-based dessert.

EPS PRODUCING LAB

A large variety of LAB is reported to produce EPS. Most of them belong to the genera of *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Pediococcus*. The use of polysaccharide producing lactic culture strains in the fermented milk manufacture is not new. The EPS producing LAB have been traditionally used in the Scandinavian fermented milk products to impart desirable texture and rheological properties (Macura and Townsley 1984). The products made with ropy strains have smooth body, high viscosity and less syneresis than the products made with non-ropy strains (Wacher-Rodarte *et al* 1993). The EPS producing lactic bacteria are isolated from dairy and non-dairy environment using different media supplemented with one or more type of sugars (Table 1). The media used for isolation of EPS producing cultures are: liquid EPS selection medium (ESM) containing (g/l) 90 skim milk, 3.5 yeast extract, 3.5 peptone and 10 glucose (Van den Berg *et al* 1995), milk indicator agar and M17 lactose agar (Terzaghi and Sandine 1975), MRS with high concentration of sugars (100/g) (Van Geel-Schutten *et al* 1998) and milk agar (Mozzi *et al* 2001). Production of EPS greatly affected by various factors such as compositions of the medium (sugar and nitrogen source), temperature, pH etc. The EPS production also varies from strain to strain and production medium used (Table 2). Mostly milk and whey based media have been developed to improve EPS extraction.

Application of Eps Producing Lactic Cultures in Fermented Milk Products

The manufacturers have used texture promoting or ropy cultures for many years particularly where addition of stabilizer is prohibited (Marshall and Rawson 1999). These cultures may impart higher intensity to flavour to the yoghurt due to the carbohydrate masking the flavour, mouth feel and other attributes (Tamime and Robinson 1999). To reduce the amount of added milk solids, improve yoghurt viscosity, to enhance texture and mouth feel and to avoid syneresis during fermentation or upon storage of the fermented milk products, EPS producing functional starters are interesting (De Vuyst et al 2003). These cultures have been used to improve technological properties of various fermented milk products (Table 3).

The most important application of EPS producing lactic cultures is in the manufacture of low-fat/fat-free fermented milk products. Milk fat contributes to

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Table 1: Isolation of exopolysaccharides (EPS) producing lactic acid bacteria from different source

Source of isolation	Medium	EPS producing isolates*
Cheeses, dairy products	Liquid ESM	30(4.9%)
Non dairy fermented foods	ESM with 50 g/l glucose	11
Fermented foods	MRS with 100 g/l sucrose	60(33%)
Thai fermented foods	MRS with 20 g/l sucrose	7(6.7%)
Nigerian fermented foods	Modified ESM	25(16%)
Burkino faso fermentedmilk	MRS agar, Rogosa Agar, M17 Agar	13(26%)
Dahiand naturally soured milk	Milk agar with 7g/l glucose	92(>90%)
Human intestine	MRS with 0.25% Ł cysteine agar	362(17%)

*Values in parentheses are the total percent of lactic cultures isolated; ESM- EPS selection medium

Source: (Behare *et al.,* 2009a)

the body, texture and flavour development of dairy products. Fat reduction to satisfy consumers demand leads to textural and functional defects in low-fat yoghurt, cheeses, *dahi,* and kefir (Mistry 2001, Guven et al 2005). In low-fat yoghurt and *dahi,* lack of flavour, weak body and poor texture are the major problems. Mechanical breaking particularly in stirred yoghurt, strongly affects the rheology of the coagulum and favour syneresis since the network formed by the gel is broken (Duboc and Mollet 2001). Low-fat cheeses have poor moisture retention ability, which otherwise moisture present in the cheese partially overcomes the problem of firm, rubbery body and texture created by high casein content (Mistry, 2001). Low-fat Mozzarella cheeses have less tendency to melt and inferior baking characteristics. These defects can be reduced by the use of additives to some extent but may not find wide acceptance to produce wholesome product.

Table 2: EPS producing lactic strains and EPS production using different media

LAB strains	Culture media*	EPS, mg/
Strep. thermophilus SY	WM	152
Strep. thermophilus ChH YC-38	Milk	600
Strep. thermophilus CRL 804	Milk	166
L. lactis subsp. *cremoris* LC3	CDM	25
L. lactis subsp *cremoris* T5	SM	600
L. lactis subsp *cremoris* B891	SM	80
Lact. delbrueckii subsp. *bulgaricus* 2-11	Whole milk	540
Lact. delbrueckii subsp. *bulgaricus* CNRZ416	SM	170
Lact. delbrueckii subsp. *bulgaricus* CRL852	Milk	150
Lact. rhamnosus R	BMM	495
Lact. rhamnosus RW-9595M	WPM	2300
Lact. helveticus ATCC15807	SM	549
Lact. paracasei Type V	BMM	85
Lact. reuteri LB121	MRS-sucrose	9800
Lact. sakie 0-1	SDM	1580
Lact. fermentum MR6	MRS-glucose	713
Ped pentosaceus AP-3	MRS-sucrose	2500

*WM: Whey media, CDM: Chemically defined medium, MRS: De Man Rogosa, sharp medium, SDM: Semi-defined medium, SM: Skim milk, BMM: Basal minimal medium, WPM: Whey permeate medium, pptn: Precipitation

Yoghurt made with ropy cultures exhibited increased viscosity and shear stress values; however, differences attributed to the type of polysaccharide secretion (capsule or slime) were apparent. The presence of bacterial capsule may enhance some rheological properties such as viscosity, but may weaken the gel structure. This has caused lower shear stress value compared to slime producers, which produce a more stretchable gel structure (Hassan et al 1996).

Incorporation of EPS strains in the starter culture retains significant amount of moisture in a variety of low-fat cheeses that positively influence their functionality (Awad et al 2005, Zisu and Shah 2005). Perry et al (1997) investigated the influence of an EPS-producing starter pair *Strep. thermophilus* MR-1C and *Lact. delbrueckii* subsp. *bulgaricus* MR-1R on the moisture and melt properties of low-fat Mozzarella cheese. Cheese manufactured with MR-1C and MR-1R contained significantly ($p<0.05$) more moisture and had better melting properties than cheese made with an EPS negative commercial starter pair (*Strep. thermophilus*TA061 and *Lact. helveticus* LH 100). The water binding properties of *Strep. thermophilus* MR-1C was ascribed to its large capsule. Cheddar cheese made with EPS-producing *Lact. lactis* retained more moisture (45.8-47.2%) than control made with no EPS producing strains (44.9-45.8%). Also, EPS producing cultures improved textural, melting and sensory characteristics of reduced fat cheddar cheese (Awad et al 2005). Nauth and Hayashi (2004) patented the process of manufacture of fat-free cream cheese, added with ropy culture. The fat-free cream cheese has comparable firmness, consistency and flavour of a conventional higher fat cream cheese.

Besides yoghurt and cheese, the other fermented milk products in which EPS cultures have been shown to affect physical properties includes sour cream, kefir and European cultured dairy products. Use of slime producing *Strep. thermophilus* strain greatly improved rheological properties of cream turo and number of other Hungarian cultured milk and cultured cream products (Obert, 1984). Kefir is traditional self-carbonated slightly alcoholic fermented milk from Eastern Europe (Roginski 1999, Tamime and Robinson 1999). Kefir is prepared by inoculating kefir grains, which consists of homofermentative, heterofermentative LAB, yeasts and acetic acid bacteria. The cells are embedded in kefiran, a slimy polysaccharide, which also found to affect texture of kefir

(Micheli et al 1999). EPS produced by LAB during fermentation are known to affect texture properties of cultured milk products significantly. EPS increases the viscosity, smoothness, and creaminess of fermented milks (Folkenberg*et al.*, 2005). Yoghurt with the EPS-producing culture exhibited increased consistency coefficient, lower flow behaviour index, yield stress, viscoelastic moduli and phase angle values than yoghurt with the non EPS producing culture (Hassan *et al*., 1996).

Table 3: Use of EPS producing cultures in fermented milk products

LAB	Product	Effect	References
Strep. thermophilus (EPS^-) and *Lact. delbrueckii* subsp. *bulgaricus* (EPS^+)	Stirred yoghurt	Increased viscosity	Marshall and Rawson (1999)
Strep. thermophilus (EPS^+) and *Lact. delbrueckii* subsp. *bulgaricus* (EPS^+)	Yoghurt	Improved rheological and textural properties	Hassan et al (1996)
Strep. thermophilus (EPS^+) and *Lact. delbrueckii* subsp. *bulgaricus* (EPS^-)	Yoghurt	Ropiness, low serum separation higher viscosity	Folkenberg et al (2005)
Strep. thermophilus MR-1C (EPS^+) and *Lact. bulgaricus* MR-1R (EPS^+)	Mozzarella cheese	Retained moisture, better melting properties	Perry et al (1997)
Lact. Lactis	Cheddar cheese	Increased moisture retention, melt properties and springiness	Michael et al (2003)
L. lactis subsp. *cremoris* JFR1	Cheddar cheese	Increased moisture retention, improved sensory, textural and melting properties	Awad et al (2005)
Lact. Lactis	Cream cheese	Better firmness, consistency and flavour	Nauth and Hayashi (2004)
Strep. Thermophilus	Hungarian cultured milk	Improved rheological properties	Obert (1984)
Lact. Kefir	Kefir	Improved texture	Micheli et al (1999)
Lactococcus lactis subsp. *lactis* KT24	Fat-free dahi	Improved physical, rheological and sensory properties	Behare et al (2009)
Streptococcus thermophilus IG16	Reduced fat lassi	Improved physical, rheological and sensory properties	Behare et al (2010)
Lactobacillus fermentum V10	Reduced fat dahi	Improved physical, rheological and sensory properties	Behare et al (2013)

CONCLUSION

Among the wide variety of EPS producing bacteria, lactic acid bacteria have gained special attention due to the remarkable property of the polymers they synthesized which do not carry any health risk and have GRAS status. EPS from LAB have potential application in the improvement of the rheology, texture and mouthfeel of indigenous fermented milk products. Use of EPS producing cultures reduce the amount of total solids required to manufacture dahi, lassi and yoghurt and thus making the process cost effective.

REFERENCES

Awad S, Hassan N, Muthukumarappan K. 2005. Application of exopolysaccharide producing cultures in reduced fat cheddar cheese: Texture and melting properties. J Dairy Sci 88:4204-4213.

Behare PV, Singh R, Kumar M, Prajapati JB, Singh RP. 2009a. Exopolysaccharides of lactic acid bacteria: a review. *J of Food Sci and Tech* 46(1):1-11

Behare PV, Rameshwar Singh, Nagpal R and Rao KH. 2013. Exopolysaccharides producing *Lactobacillus fermentum* strain for enhancing rheological and sensory attributes of low-fat Dahi. *J. Fd Sci. & Technol.*, DOI: 10.1007/s13197-013-0999-6.

Behare PV, Rameshwar Singh and Singh RP. 2009b. Exopolysaccharide producing mesophilic lactic cultures for preparation of reduced fat dahi-An Indian fermented milk. *J. Dairy Res.* **76**: 90-97.

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

De Vuyst L, Zamfir M, Mozzi F, Adriany T, Marshall V, Degeest B, Vaningelgem F. 2003. Exopolysaccharide-producing *Strep-*

tococcus thermophilus strains as functional starter cultures in the production of fer-mented 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.

Duboc P, Mollet B. 2001. Applications of exopolysaccharides in dairy industry.Int Dairy J **11**:759-768.

Folkenberg DM, Dejmek P, Skriver A, IpsenR. 2005. Relation between sensory texture properties and exopolysaccharide distri-bution in set and in stirred yoghurts produced with different starter cultures. J Texture Stud **36**:174-189.

Guven M, Yasar K, Karaca OB, Hayaloglu AA. 2005. The effect of inulin as a fat replacer on the quality of set-type low-fat yoghurt manufacture. Int J Dairy Technol**58**:180-184.

Hassan AN, Frank JF, Schmidt KA, ShalabiSI 1996. Textural properties of yoghurt made with encapsulated non-ropy lactic cultures. J Dairy Sci **79**:2098-2103.

Macura D, Townsley PM. 1984. Scandinavian ropy milk: Identification and characterization of endogenous ropy lactic streptococci and their extracellular excretion. J Dairy Sci **67**:735-744.

Marshall VM, Rawson HL. 1999. Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt.Int J Food SciTechnol **34**:137-143.

Michael T, Diane VH and Edyth M. 2003, Rheology of low-fat cheddar cheese made with exopolysaccharide producing cultures. *Am Chem Soc* Paper Nr Agf cc **63** (Abstract).

Micheli L, Uccelletti D, Palleschi C and Creasenzi V. 1999. Isolation and characterization of a ropy *Lactobacillus* strains producing the exopolysaccharide kefiran. *Appl Microbiol Biotechnol* **53**:69-74.

Mistry VV 2001. Low-fat cheese technology. Int Dairy J **11**:413-422.

Mozzi F, Rollan G, Savoy de Giori G, Font de Valdez G. 2001.Effect of galactose and glucose on the exopolysaccharide production and the activities of biosynthetic enzymes in *Lactobacillus casei*CRL 87. J Appl Microbiol **91**:160-167.

Nauth RK and Hayashi D. 2004. Methods for manufacture of fat-free cream cheese. US Patent US 6,689,402.

Obert G. 1984. Manufacturing technology of cream turo with extended shelf-life, and isolation of a special strain of lactic acid bacteria which improves product consistency.Tejipar **33**:47-48.

Perry DB, McMahon DJ, Oberg CJ. 1997. Effect of exopolysaccharide producing cultures on moisture retention in low-fat Mozzarella cheese. J Dairy Sci **80**:799-805.

Roginski H. 1999. Fermented milks: Product from Northern Europe. In: Encyclopedia of food microbiology. Robinson RK (ed), Academic Press, San Diego. p 791-798.

Rohm H and Schimid W. 1993. Influence of dry matter fortification on flow properties of yoghurt.Evaluation of flow curves. Milchwissenschaft 48:556-560.

Ruas-Madiedo P, de los Reyes-Gavilan CG. 2005. Methods for the screening, isolation, and characterization of exopolysacch-arides produced by lactic acid bacteria. J Dairy Sci **88**:843-856.

Sutherland IW. 1972. Bacterial exopolysaccharides. Adv Microb Physiol **8**:143-212.

Tamime AY, Robinson RK. 1999. Yoghurt: Science and technology. CRC Press, Cambridge.

Terzaghi BE and Sandine WE. 1975. Improved medium for lactic Streptococci and their bacteriophages. Appl Environ Microbiol **29**:807-813.

Van den Berg DJC, Robijn GW, Janssen AC, Giuseppin MLF, Vreeker R, Kamerling JP, Vliegenthart JFG, Ledeboer AM, Verrips CT. 1995. Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide. Appl Environ Microbiol **61**:2840-2844.

Van Geel-Schuten GH, Flesch F, Brink Bten, Smith MR and Dijkhuizen L. 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, Gallardo F, Marshall VME and Garcia- GaribayM. 1993. Yoghurt production from recon-stituted skim milk powders using different polymer and non-polymer forming starter cultures. J Dairy Res 60:247-254.

Zisu B and Shah NP. 2005. Low-fat Mozzarella as influenced by microbial exopolysaccharides, preacidification and whey protein concentrate. J Dairy Sci **88**:1973-1985.