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Exopolysaccharides production as affected by lactic acid bacteria and fermentation time

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Abstract

The aim of this work was to examine the ropiness of *Lactobacillus helveticus* BCRC14030, *L. helveticus* BCRC14076, and *Streptococcus thermophilus* BCRC14085, and evaluate the effect of fermentation time on exopolysaccharides (EPS) production by the ropy strain. Each of the three strains was inoculated in skim milk medium and incubated in a fermenter for 0–84 h at pH 5, 37 °C. The fermented samples, containing a net volume of 300 ml skim milk, were withdrawn at intervals of 0, 12, 16, 24, 32, 36, 48, 60, 72, and 84 h for determinations of ropy condition, EPS yield, and molecular mass. EPS with ropiness values of 11.3–21.0 mm, produced from *L. helveticus* BCRC14030 at 32–60 h demonstrated the ropy nature of the strain. Those EPS were composed of high molecular mass of 26,500 kDa. The highest EPS yield of 0.73 g l^{-1} from this strain was observed ($P < 0.05$) at less favourable fermentation condition of 60 h. In addition, a relationship between the presence of high molecular mass and the ropiness of EPS from *L. helveticus* BCRC14030 was revealed.

Keywords: Exopolysaccharides; Lactic acid bacteria; Fermentation time

1. Introduction

Lactic acid bacteria (LAB) producing exopolysaccharides (EPS) have gained considerable attention in the fermented dairy industry because of their potential application as viscosifiers, texturizers, and emulsifying agents (Grobben, Smith, Sikkema, & de Bont, 1996). They also possess antitumoral (Ebina, Ogata, & Murata, 1995; Oda, Hasegawa, Komatsu, Kambe, & Tsuchiya, 1983), immunostimulatory (Hosono et al., 1997), and macrophage (Nishimura-Uemura et al., 2003) and lymphocyte (Kitazawa et al., 1998) activating activities. EPS produced by those food-grade microorganisms with GRAS (Generally Recognized as Safe) status are an important source of natural alternatives to commercial additives of plant or animal origin. Most of those additives used are chemically modified to improve the rheological properties of the product (Roller & Dea, 1992) and hence are not allowed in most European Union countries (Gibson & Roberfroid, 1995). Since the popularity of natural food products without any additives has increased (Schellhaass & Morris, 1985), the use of EPS-producing LAB could result in a safe, natural, and healthy end-product with enhanced texture and improved stability, which may have an important impact on the development of novel products.

EPS produced by LAB are in a great variety, depending on the type of LAB strains, culture conditions, and medium composition (Looijesteijn & Hugenholtz, 1999). *Strep. thermophilus* ST 111 was observed to produce EPS in a milk medium composed of galactose and rhamnose with a molecular mass of more than 5000 kDa (Vaningelgem et al., 2004). While *Strep. thermophilus* LY 03 produce both high- and low-molecular-mass EPS, and CH101 produced

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only low-molecular-mass EPS, other LAB were reported to produce EPS with various molecular masses. Grobben et al. (1997) found that *L. bulgaricus* strain NCFB 2772, grown in chemically defined media containing glucose and fructose, produced two EPS fractions, composed of galactose, glucose and rhamnose, which possess molecular masses of 1700 and 40 kDa, respectively. Marshall, Cowie, and Moreton (1995) reported similar results with *L. lactis* subsp. *cremoris* LC 330.

Ropy cultures were able to synthesize EPS with high molecular mass which lead to an improvement in product viscosity. Guzel-Seydim, Sezgin, and Seydim (2005) observed that the viscosity of yogurts increased when made from milk inoculated with ropy EPS producing culture (B-3). Petry et al. (2003) demonstrated the presence of a high MW fraction of EPS produced by ropy *L. delbrueckii* subsp. *bulgaricus* strains, which exhibited a high intrinsic viscosity. In addition, De Vuyst et al. (2003) found that ropy *Strep. thermophilus* LY 03 produced large amounts of EPS with high molecular mass and displayed high apparent viscosity in fermented milk. However, correlation between viscosity and EPS production was not observed (Faber, Zoon, Kamerling, & Vliegenthart, 1998; Sebastiani & Zelger, 1998; Shihata & Shah, 2002; Wacher-Rodarte et al., 1993).

Fermentation time is one of the critical environmental parameters affecting content, molecular mass, and sugar composition of EPS. Pham, Dupont, Roy, Lapointe, and Cerning (2000) reported that the content of EPS produced by *L. rhamnosus* declined with prolonged fermentation time, due to the presence of various glycohydrolases. Goret, Maubois, Engasser, and Ghoul (2001) observed similar results with *Propionibacterium acidi-propionici*. Vaningelgem et al. (2004) found no change in molecular mass of EPS produced by *Strep. thermophilus* ST 111 in milk medium during the fermentation time, whereas Shu and Lung (2004) observed that the proportion of high molecular weight EPS produced by *Antrodia camphorate* declined with fermentation time, possibly due to the presence of an endo-amylase capable of partially hydrolyzing EPS (Catley, 1980), and this was consistent with results obtained by Cerning, Bouillane, Desmazeaud, and Landon (1988) who observed that high molecular mass EPS produced by *Strep. thermophilus* decreased after 48 h, probably due to the presence of either lactate and/or hydrolytic enzymes. While Bouzar, Cerning, and Desmazeaud (1996) reported that the sugar composition of EPS from *L. delbrueckii* subsp. *bulgaricus* CNRZ 1187 changed during the fermentation cycle, the sugar composition of *Strep. thermophilus* LY03 remained constant during the whole batch fermentation process (De Vuyst, Vanderveken, Van de Ven, & Degeest, 1998).

The objective of this study was to examine the ropiness of three lactic acid bacteria: *L. helveticus* BCRC14030, *L. helveticus* BCRC14076, and *Strep. thermophilus* BCRC14085, and evaluate the effect of fermentation time on EPS production by the ropy strain.

2. Materials and methods

2.1. Bacterial strains and growth conditions

L. helveticus (BCRC14030), *L. helveticus* (BCRC14076), and *Strep. thermophilus* (BCRC14085) obtained from Bioresource Collection and Research Centre (BCRC), Food Industrial Research Institute, Shin Chu, Taiwan, were subcultured twice under aerobic conditions at 37 °C for 24 h in MRS broth (Difco Lab., Detroit, MI, USA). One percent of the subcultures were then inoculated into 1000 ml MRS broth (v/v) and incubated toward the end of logarithmic phase at 37 °C. Fermentations were carried out in a 5 l fermenter (FB-6B, Firstek Scientific, UK). After filling with 5 l 10% (w/v) reconstituted skim milk, the fermenter was sterilized at 121 °C for 15 min, and an aliquot of 150 ml of the inoculum was introduced into the fermenter after the skim milk medium was cooled. Fermentation occurred at 37 °C for 24 h, and pH was kept at 5.0 by adding sterile 1 M NH_4OH or HCl under agitation at 100 rev min^{-1}.

2.2. Sampling

Fermentations were allowed to proceed for 84 h. The fermented skim milk samples containing a net volume of 300 ml skim milk were withdrawn at intervals of 0, 12, 16, 24, 32, 36, 48, 60, 72, and 84 h. The amounts of NH_4OH and HCl added were measured to determine the total volume of sampling at each fermentation time. After being withdrawn from the fermenter, the sample was immediately cooled to 4 °C, followed by isolation of exopolysaccharides (EPS) for detecting ropy condition, EPS yield, and molecular mass. Total viable counts of samples withdrawn from skim milk medium inoculated with ropy strain were also determined, by plating on MRS agar.

2.3. Isolation of exopolysaccharides

EPS was isolated from the fermented sample, according to a modified method of Yang, Huttunen, Staaf, Widmalm, and Tenhu (1999). Trichloroacetic acid solution was added to the fermented sample to give a final concentration of 4% (w/v), and the precipitated protein and bacteria were removed by centrifugation (22,000*g* for 35 min at 4 °C). The supernatant was then mixed with an equal volume of ethanol, stored at 4 °C for 24 h, and centrifuged, as described above, to collect the precipitated EPS. After 24 h of freezing at −80 °C, followed by 24 h of freeze-drying by lyophilisation at −18 °C, the dry weight of the precipitated EPS was determined.

2.4. Measurement of ropiness

The method described by Torino, Taranto, Sesma, and Font de Valdez (2001) was employed to measure the ropiness of the precipitated EPS. A spatula was placed on the surface of the precipitated EPS (0.15 g) and lifted slowly.

The length of thread formed was measured as the ropiness value and expressed in millimeters. The measurement for each precipitated sample was performed in three replications. The average length of three replicates between 0 and 6 mm was recorded as non-ropy and those higher than 6 mm as ropy.

2.5. Purification of exopolysaccharides

After being dissolved in water to give a final concentration of 1% (v/v), the precipitated EPS was ultracentrifuged (1290*g* for 1 h at 4 °C) using an an Amicon centrifugal filter unit fitted with a Centriplus-20 membrane of 5 kDa nominal molecular weight cutoff (Millipore, Bedford, MA, USA), followed by filtration through a 0.45 μm membrane filter for the analyses of molecular masses.

2.6. Molecular mass of EPS

Instrumentation used for the analysis was as follows: a HPLC system equipped with a TSKgel $GMPW_{XL}$ HPLC column (7.8 mm i.d. × 300 mm stainless steel; Tosoh Corp., Tokyo, Japan) with TSK guard column PW_{XL} (6.0 mm i.d. × 40 mm stainless steel; Tosoh Corp., Tokyo, Japan), a SFD RI 2000 refractive index detector (Schambeck SFD GmbH, Bad Honnef, Germany), and a Jasco PU-980 pump (Jasco Co., Tokyo, Japan). The EPS was eluted with HPLC grade water and operated isocratically at a flow rate of 0.6 ml min^{-1}. The column head pressure was maintained at 22 kg cm^{-2} at this flow rate. The injection volume was 50 μl. The column was calibrated with 1 g l^{-1} of Shodex Standard P-82 ($0.59–78.8 \times 10^4$ Da) and PSS WINGPC 6.2 (16.7×10^6 Da) standards (American Polymer Standards, Mentor, OH, USA). The molecular weight of EPS was determined by a SISC32 Chromatography Data Station, equipped with GPC data processing software (SISC, Taipei, Taiwan). The formula of molecular mass calculation was molecular mass $= \sum CiMi/Ci$, where Ci was the area of the peak at a specific time in the HPLC chromatogram and Mi was the molecular weight at a specific time.

2.7. Statistical analysis

Each treatment was performed in three replications. All data were subjected to general ANOVA and Duncan's multiple range test and critical ranges using STATISTICA (StatSoft, 1998) and a significance level of 0.05 was used.

3. Results and discussion

3.1. Ropy character of three strains tested

EPS with ropiness value of 11.3–21.0 mm produced from *L. helveticus* BCRC14030 at 32–60 h demonstrated the ropy nature of the strain (Table 1). The highest ropiness value of 21.0 mm was observed ($P < 0.05$) at 60 h of fermentation and the value decreased to 5.6–6.0 mm at 72–84 h. A similar trend was observed by Mårtensson, Dueñas-Chasco, Irastorza, Öste, and Holst (2003) who reported that the highest ropiness value of EPS produced by *Pediococcus damnosus* 2.6 was at 22 h of fermentation and the value decreased at 24 h. Since thread was not formed in the precipitated EPS produced from *L. helveticus* BCRC14076 and *Strep. thermophilus* BCRC14085 at any of the fermentation times, those two strains did not appear to be capable of producing EPS with ropy texture under the incubation conditions tested.

Table 1
Ropiness value of exopolysaccharides produced by *Lactobacillus helveticus* BCRC14030 at different fermentation times

Fermentation time (h)	Ropiness value (mm)
12	0[a]
16	0[a]
20	0[a]
24	0[a]
32	11.3[b]
36	12.6[b]
48	13.3[b]
60	21.0[c]
72	6.0[d]
84	5.6[d]

[a–d] Means in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

3.2. Total viable counts of Lactobacillus helveticus BCRC14030

A rapid increase in total viable counts from 2.2×10^6 CFU ml^{-1} at 0 h to 3.0×10^8 CFU ml^{-1} at 20 h was observed in *L. helveticus* BCRC14030 treatment (Fig. 1). Total viable counts decreased gradually after 20 h of fermentation and reached a minimum of 5.3×10^6 CFU ml^{-1} at 84 h of fermentation. Total viable counts in *L. helveticus* BCRC14076 and *Strep. thermophilus* BCRC14085 treatments were not determined due to the absence of the ropy nature.

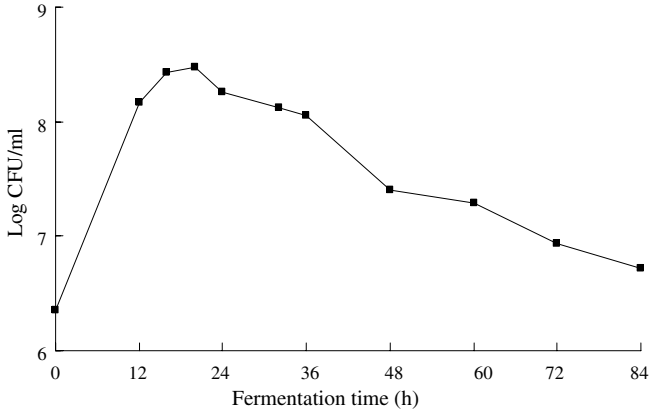

Fig. 1. Total viable counts of *Lactobacillus helveticus* BCRC14030 at different fermentation times.

3.3. Yields of exopolysaccharides

EPS yield of *L. helveticus* BCRC14030 between 32 and 84 h of fermentation time ranged from 0.25 to 0.73 g l^{-1} (Table 2). While the highest yield of 0.73 g l^{-1} was observed ($P < 0.05$) at 60 h of fermentation, the total viable count had decreased from 3.0×10^8 CFU ml^{-1} at 20 h to 1.9×10^7 CFU ml^{-1} at 60 h. Less favourable fermentation conditions for the growth of this strain at 60 h were probably the reason for the decrease in total visible count, which resulted in more EPS being produced for protecting the microbial cell itself (Sutherland, 1999). The yield decreased to 0.53 g l^{-1}at 84 h of fermentation probably due to the presence of glycohydrolases, capable of hydrolyzing EPS and liberating monomer. The result coincided with the decline in EPS yield of *L. rhamnosus* with prolonged fermentation time reported by Pham et al. (2000). EPS yield did not change ($P > 0.05$) with fermentation time in both non-ropy *L. helveticus* BCRC14076 and *Strep. thermophilus* BCRC14085 treatments, and the yields were in the range of 0.63–0.93 and 0.73–0.93 g l^{-1}, respectively.

3.4. Highest-molecular-mass EPS

One to four different EPS with molecular mass ranged from 3 to 26,500 kDa were produced by ropy *L. helveticus* BCRC14030 at different fermentation times, whereas EPS with molecular mass from 3 to 395 kDa were produced by non-ropy *L. helveticus* BCRC14076 and *Strep. thermophilus* BCRC14085. A sharp increase in the highest molecular mass from 26 kDa at 12–24 h of fermentation time to 26,500 kDa at 32–60 h was observed in *L. helveticus* BCRC14030 treatment (Table 3), which coincided with the increase in ropiness value from 0 to 11.3–21.0 mm at the same fermentation interval (Table 1). The ropiness value decreased to 5.6–6.0 mm at 72–84 h of fermentation with a decrease in the highest molecular mass to 2700 kDa, possibly due to enzymatic degradation (Catley, 1980). The decline in molecular mass of EPS with prolonged fermentation time was consistent with results obtained by Cerning et al. (1988) and Shu and Lung (2004). The results revealed a relationship between the presence of high-molecular-mass EPS and ropiness, which confirmed the report of Petry et al. (2003) who observed a high fraction of high-molecular-mass EPS produced from ropy *L. delbrueckii* subsp. *bulgaricus* in skim milk medium. The highest molecular mass of EPS produced by non-ropy *L. helveticus* BCRC14076 and *Strep. thermophilus* BCRC14085 were 346 and 136–395 kDa, respectively, smaller than those from *L. helveticus* BCRC14030 at 32–84 h of fermentation. Ropiness was not observed in EPS produced by those strains, possibly due to the absence of EPS of higher molecular mass. The fraction of highest-molecular-mass EPS from *L. helveticus* BCRC14030 ranged from 19% to 52% at 32–84 h (Table 3). Ropiness value of EPS with 52% of 26,500 kDa at 36 h of fermentation was lower than that of EPS with 34% of the same molecular mass at 60 h. This observation established that factors other than molecular mass ratio of EPS also contributed to the ropiness, such as the constituent sugar residues, the linkages between the residues, and the presence of side groups in the EPS (Tuinier et al., 2001).

Table 2
Yields of exopolysaccharides produced by three lactic acid bacteria

Fermentation time (h)	Yield (dry weight, g l^{-1})		
	L. helveticus BCRC14030	*L. helveticus* BCRC14076	*Strep. thermophilus* BCRC14085
32	0.25[a]	0.63[a]	0.73[a]
36	0.25[a]	0.80[a]	0.73[a]
48	0.48[b]	0.80[a]	0.80[a]
60	0.73[c]	0.93[a]	0.93[a]
72	0.54[b]	0.83[a]	0.93[a]
84	0.53[b]	0.67[a]	0.83[a]

[a–c] Means in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

Table 3
Highest-molecular-mass exopolysaccharides produced by three lactic acid bacteria and mass fraction of total EPS

Fermentation time (h)	Highest molecular mass (kDa)		
	L. helveticus BCRC14030	*L. helveticus* BCRC14076	*Strep. thermophilus* BCRC14085
12	26 (100)[a]	346 (82)	395 (71)
16	26 (68)	346 (53)	136 (3)
20	26 (77)	346 (72)	136 (28)
24	26 (100)	346 (55)	284 (41)
32	26,500 (20)	346 (59)	284 (60)
36	26,500 (52)	346 (57)	395 (77)
48	26,500 (19)	346 (74)	197 (47)
60	26,500 (34)	346 (75)	197 (19)
72	2,700 (20)	346 (93)	136 (93)
84	2,700 (23)	346 (82)	395 (67)

[a] Value in parentheses is fraction (%) of total EPS.

4. Conclusions

EPS with ropiness values of 11.3–21.0 mm, produced from *L. helveticus* BCRC14030 at 32–60 h, demonstrated the ropy nature of the strain. The highest EPS yield of 0.73 g l^{-1} was observed at the less favourable fermentation condition of 60 h with a total viable count, which had decreased from 3.0×10^8 CFU ml^{-1} at 20 h to 1.9×10^7 CFU ml^{-1}. In addition, the ropiness value increased along with highest molecular mass as fermentation extended from 12–24 to 32–60 h, and decreased as fermentation further extended to 72–84 h in *L. helveticus* BCRC14030, which revealed a relationship between the presence of high molecular mass and the ropiness of EPS. Further investigation on improving EPS yield and ropiness of the ropy strain, including optimizing fermentation conditions and using immobilization techniques, is needed.

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References

Bouzar, F., Cerning, J., & Desmazeaud, M. (1996). Exopolysaccharide production in milk by *Lactobacillus delbrueckii* subsp. *bulgaricus* CNRZ1187 and by two colonial variants. *Journal of Dairy Science, 79*, 205–211.

Catley, B. J. (1980). The extracellular polysaccharide, pullulan, produced by *Aureobasidium pulluans*: a relationship between elaboration rate and morphology. *Journal of General Microbiology, 120*, 265–268.

Cerning, J., Bouillane, C., Desmazeaud, C., & Landon, M. (1988). Exocellular polysaccharide production by *Streptococcus thermophilus*. *Biotechnology Letters, 10*, 255–260.

De Vuyst, L., Vanderveken, F., Van de Ven, S., & Degeest, S. B. (1998). Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *Journal of Applied Microbiology, 84*, 1059–1068.

De Vuyst, L., Zamfir, M., Mozzi, F., Adriany, T., Marshall, V., Degeest, B., et al. (2003). Exopolysaccharide-producing *Streptococcus thermophilus* strains as functional starter cultures in the production of fermented milks. *International Dairy Journal, 13*, 707–717.

Ebina, T., Ogata, N., & Murata, K. (1995). Antitumor effect of *Lactobacillus bulgaricus* 878R. *Biotherapy, 9*, 65–70.

Faber, E. J., Zoon, P., Kamerling, J. P., & Vliegenthart, J. F. G. (1998). The exopolysaccharides produced by *Streptococcus thermophilus* Rs and Sts have the same repeating unit but differ in viscosity of their cultures. *Carbohydrate Research, 310*, 269–276.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition, 124*, 1401–1412.

Gorret, N., Maubois, J. L., Engasser, J. M., & Ghoul, M. (2001). Study of the effects of temperature, pH and yeast extract on growth and exopolysacccharides production by *Propionibacterium acidi-propionici* on milk microfiltrate using a response surface methodology. *Journal of Applied Microbiology, 90*, 788–796.

Grobben, G. J., Smith, M. R., Sikkema, J., & de Bont, J. A. M. (1996). Influence of fructose and glucose on the production of exopolysaccharide and the activities of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. *Applied Microbiology and Biotechnology, 46*, 279–284.

Grobben, G. J., van Casteren, W. H. M., Schols, H. A., Oosterveld, A., Sala, G., Smith, M. R., et al. (1997). Analysis of the exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture on glucose and fructose. *Applied Microbiology and Biotechnology, 48*, 516–521.

Guzel-Seydim, Z. B., Sezgin, E., & Seydim, A. C. (2005). Influences of exopolysaccharide producing cultures on the quality of plain set type yogurt. *Food Control, 16*, 205–209.

Hosono, J., Lee, J., Amenati, A., Natsume, M., Hirayama, M., Adachi, T., et al. (1997). Characterization of a water-soluble polysaccharide fraction with immunopotentiating activity from *Bifidobacterium adolescentis* M101-4. *Bioscience, Biotechnology and Biochemistry, 61*, 312–316.

Kitazawa, H., Ishii, Y., Uemura, J., Kawai, Y., Saito, T., Kaneko, T., et al. (1998). Phosphate group requirement for mitogenic activation of lymphocytes by an extracellular phosphopolysaccharide from *Lactobacillus delbrueckii* subsp. *bulgaricus*. *International Journal of Food Microbiology, 40*, 169–175.

Looijesteijn, P. J., & Hugenholtz, J. (1999). Uncoupling of growth and exopolysaccharide production by *Lactobacillus lactis* subsp. *cremoris* NIZO B40 and optimization of its synthesis. *Journal of Bioscience and Bioengineering, 88*, 178–182.

Marshall, V. M., Cowie, E. N., & Moreton, R. S. (1995). Analysis and production of two exopolysaccharides from *Lactococcus lactis* subsp. *cremoris* LC330. *Journal of Dairy Research, 62*, 621–628.

Mårtensson, O., Dueñas-Chasco, M., Irastorza, A., Öste, R., & Holst, O. (2003). Comparison of growth characteristics and exopolysaccharide formation of two lactic acid bacteria strains, *Pediococcus damnosus* 2.6 and *Lactobacillus brevis* G-77, in an oat-based, non-dairy medium. *Lebensm.-Wiss. U.-Technology, 36*, 353–357.

Nishimura-Uemura, J., Kitazawa, H., Kawai, Y., Itoh, T., Oda, M., & Saito, T. (2003). Functional alteration of murine macrophages stimulated with extracellular polysaccharides from *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1. *Food Microbiology, 20*, 267–273.

Oda, M., Hasegawa, H., Komatsu, S., Kambe, M., & Tsuchiya, F. (1983). Anti-tumor polysaccharide from *Lactobacillus* sp.. *Agricultural and Biological Chemistry, 47*, 1623–1625.

Petry, S., Furlan, S., Waghorne, E., Saulnier, L., Cerning, J., & Maguin, E. (2003). Comparison of the thickening properties of four *Lactobacillus delbrueckii* subsp. *bulgaricus* strains and physicochemical characterization of their exopolysaccharides. *FEMS Microbiology Letter, 221*, 285–291.

Pham, P. L., Dupont, I., Roy, G., Lapointe, G., & Cerning, J. (2000). Production of exopolysaccharide by *Lactobacillus rhamnosus* R and analysis of its enzymatic degradation during prolonged fermentation. *Applied Environmental Microbiology, 66*, 2302–2310.

Roller, S., & Dea, I. C. M. (1992). Biotechnology in the production and modification of biopolymers for foods. *Critical Reviews in Biotechnology, 121*, 261–277.

Schellhaass, S. M., & Morris, H. A. (1985). Rheological and scanning microscopic examination of skim milk gels obtained by fermenting with ropy and non-ropy strains of lactic acid bacteria. *Food Microstructure, 4*, 279–287.

Sebastiani, H., & Zelger, G. (1998). Texture formation by thermophilic lactic acid bacteria. *Milchwissenschaft, 53*, 15–20.

Shihata, A., & Shah, N. P. (2002). Influence of addition of proteolytic strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* to commercial ABT starter cultures on texture of yoghurt, exopolysaccharide production and survival of bacteria. *International Dairy Journal, 12*, 765–772.

Shu, C.-H., & Lung, M.-Y. (2004). Effect of pH production and molecular weight distribution of exopolysaccharide by *Antrodia camphorate* in batch cultures. *Process Biochemistry, 39*, 931–937.

StatSoft Inc. (1998). STATISTICA for Windows. In *Computer program electronic manual*. Tulsa, Okla.

Sutherland, I. W. (1999). Polysaccharides for microbial exopolysaccharides. *Carbohydrate Polymer, 38*, 319–328.

Torino, M. I., Taranto, M. P., Sesma, F., & Font de Valdez, G. (2001). Heterofermentative pattern and exopolysaccharide production by *Lactobacillus helveticus* ATCC 15807 in response to environmental pH. *Journal of Applied Microbiology, 91*, 846–852.

Tuinier, R., van Casteren, W. H. M., Looijesteijn, P. J., Schols, H. A., Voragen, A. G. J., & Zoon, P. (2001). Effects of structural modifications on some physical characteristics of exopolysaccharides from *Lactobacillus lactis*. *Biopolymers, 59*, 160–166.

Wacher-Rodarte, C., Galvan, M. V., Farres, A., Gallardo, F., Marshall, V. M. E., & Garcia-Garibay, M. (1993). Yoghurts production from reconstituted skim milk powder using different polymer and non-polymer forming starter cultures. *Journal of Dairy Research, 60*, 247–254.

Vaningelgem, F., Van der Meulen, R., Zamfir, M., Adriany, T., Laws, A. P., & De Vuyst, L. (2004). *Streptococcus thermophilus* ST 111 produces a stable high-molecular-mass exopolysaccharide in milk-based medium. *International Dairy Journal, 14*, 857–864.

Yang, Z., Huttunen, E., Staaf, M., Widmalm, G., & Tenhu, H. (1999). Separation, purification and characterization of extracellular polysaccharide produced by slime-forming *Lactococcus* subsp. *cremoris* strains. *International Dairy Journal, 9*, 631–638.