African Journal of Biotechnology Vol. 3 (10), pp. 512-518, October 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Effect of ropy and capsular exopolysaccharides producing strain of *Lactobacillus plantarum* 162RM on characteristics and functionality of fermented milk and soft Kareish type cheese

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Accepted 14 July, 2004

The contribution of selected ropy and capsular *Lactobacillus plantarum 162RM* on texture of fermented milk as well as on the functionality of kareish cheese was established in this study. The cell suspension of this strain was used in combination with commercial starter cultures MY900 (*Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus)* and MM100 (*Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. diacetyllactis)* obtained from Rhodia Food to manufacture fermented milk and Kareish cheese respectively. The final pH of fermented milks manufactured with the combination of starter MY900 and different concentration of strain *L. plantarum* 162RM were not significantly different. Fermented milk hardness, consistency, and adhesiveness increased significantly when 8 % (V/V) of strain *Lactobacillus plantarum* 162RM was used. This strain produces exopolysaccharides (EPS), which by attaching to the casein matrix increases and improves the texture characteristics of fermented milk. The Experimental Kareish Cheese (EKC), made by pairing commercial starter MM100 with 8% (v/v) of *L. plantarum* 162RM were also compared to Control Kareich Cheese (CKC) in terms of their moisture content and textural properties. The EKC showed the greatest moisture retention and the use of ropy and capsular strain of *L. plantarum* 162RM affects significantly some textural properties of EKC cheese, relative to the control. The CKC samples were gummier and more chewy than the EKC fresh samples. It is therefore evident that, used in appropriate amount, this strain can increase moisture content in low fat Kareish cheese leading to improvement of textural properties.

Key words: Fermented milks, kareish cheese, ropy and capsular strain, moisture, textural properties.

INTRODUCTION

Several gram negative and gram-positive bacteria, including lactic acid bacteria, produce exocellular polysaccharides. Exopolysaccharides (EPS) are either excreted in the growth medium (slime), or remain attached to the bacteria cell wall (capsule) (Sutherland, 1972). In nature, bacteria EPS fulfils a variety of diverse functions including cell protection and adhesion cell-cell interactions. So, incorporation of EPS or EPS-producing (EPS+) cultures in dairy foods can provide viscosifying, stabilizing and water binding functions (Cerning, 1990; Broadbent et al., 2003). The ropy nature of milk fermented has been investigated with mesophilic and thermophilic lactic acid bacteria (Hassan et al., 1996; Laws and Marshall, 2001; Patricia et al., 2002). It is

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Table 1. Some chemical characteristics of milk used for the manufacturing of fermented milk and low fat kareish cheese.

	Acidity (°D)	pH	Fat (%)	Casein (%)
Pasteurised Standardised Milk (PSM): (2/3 cow milk enriched with cow milk's cream + 1/3 buffalo milk) for fermented milk.	16	6.55	4.6	5.6
Pasteurised Skim Cow Milk (PSCM) for cheese-making	16	6.74	0.2	3.4

generally accepted that the ropiness produced by these bacteria is related to synthesis and excretion of exopolymers (Cerning et al., 1992; Hassan et al., 1996). The use of cultures producing EPS increases resistance of yogurt coagulum to thermal and physical shock (Broadbent et al., 2003). The presence of viscous extracellular materials produced by some culture bacteria were also demonstrated to play an important role in achieving satisfactory firmness and apparent viscosity of yoghurt (Hassan et al., 1996; De Vuyst and Degeest, 1999; Patricia et al., 2002). Besides yogurt, other dairy product quality improved with EPS producing strain includes reduced fat cheese. Infact in many countries, several cheese varieties are made with reduced fat content to meet a general wish to decrease fat intake among population (Ardo, 1997). The kareish cheese is an acid coagulated fresh cheese, made from skim milk with soft composition, white curd and slightly salty. It is the most popular type cheese in Egypt and Arabian countries (Abou-Donia, 1991), mainly manufactured by smallholders and sold at local markets. As with other varieties of low/reduced fat cheeses, its consumption is still low because of poor perception of product, based on inadequate taste and texture. In fact, the low fat cheese in general, has a low intensity of typical flavour bitter taste and hard rubbery dry grainy texture (Ardo, 1997). Therefore, the challenge in development of low fat cheese is to improve both flavour attributes and texture of product to produce a cheese comparable to its full fat counter part (Ardo, 1997). Amongst the alternative, many authors have suggested that the EPS+ lactic acid bacteria (LAB) could enhance functionality properties of cheese (Low et al., 1998; Broadbent et al. 2001; Katsiari et al., 2002).

This study was carried out to determine the contribution of ropy and capsular EPS producing strain of *Lb. plantarum* (162RM) to the textural characteristics of fermented milk as well as on the functionality of kareish cheese.

MATERIAL AND METHODS

Culture and growth conditions

The strain *Lb. plantarum* 162RM used in this study was isolated from traditional fermented milk in the Laboratory of the Biochemistry of Dairy microorganisms, Alexandria University (Egypt). It was identified as *Lb. plantarum* 162RM on the basis of morphological and biochemical characteristics and the SDS-PAGE of whole cell protein (submitted for publication). The criteria for selection of this strain were based on its exopolysaccharide production (slime and capsule). It was grown in MRS (Biolife©) until it reached late exponential growth phase. The cells were harvested by centrifugation at 10,000 g for 10 mn at 4°C. The pellet was then washed twice with 0.01 M potassium phosphate buffer pH 7.0. The resultant pellet was suspended in 100ml sterile potassium phosphate buffer 0.01 M and stored at –20°C until used. The viable cell counts of this suspension was determine by enumeration on MRS plate agar and incubation at 30°C for 48 h in anaerobic condition (De Man et al., 1960). This suspension contained ≈ 10^8 ctu/ml.

In addition, two commercially available starter cultures named MY900 and MM100 were used for the manufacture of fermented milk and Kareish cheese, respectively. The MY900 culture consisted of *Lactobacillus delbrueckii spp bulgaricus* and *Streptococcus thermophilus,* while the MM100 contained *Lactococcus lactis ssp.lactis, Lactococcus lactis ssp cremoris* and *Lactococcus lactis ssp diacetyllactis*. The starter cultures MY900 and MM100 were obtained from Rhodia Food (Saint Romain, France). They were then inoculated and propagated in sterile skim milk to produce starter culture inoculum.

Manufacture of fermented milks

Fresh cow and Buffalo milks were obtained from the University Agricultural farm of Alexandria. Milk was pasteurised at 74°C for 15 s. The chemical composition of the milk is presented in Table I. 1% dry skim milk powder was added to pasteurised standardized milk and stirred. The mixture was heated at 80°C and cooled to 42°C before inoculation with the combination of starter culture MY900 and ropy strain of *Lactobacillus plantarum* 162RM. After inoculation of milk with starter MY900, the stirred inoculated milk was immediately divided in four portions. Each portion was respectively inoculated with 0%, 4%, 6% and 8% (V/V) cell suspension of strain *Lactobacillus plantarum* 162RM. The different portions were each mixed, filled out in sterile, labelled plastic cups, sealed with sterile plastic cup lids and incubated at 42°C for 5 to 6 h, until coagulation. After coagulation, the cups were stored at 4-5°C in the cold refrigerator before assessments. Two replicate of fermented milk trials were undertaken on two separate days.

Manufacture of Kareish cheese

Twenty litters of cow's milk was defatted and pasteurised at 74°C for 15s cooled to 32°C. The chemical composition of milk is given in Table 1.

Two vats of cheese (five liters each) were made on one day from skim cow milk. For each trial ten litters of pasteurised skim milk were inoculated with mesophlic starter culture named MM100 and stirred. After stirring, inoculated milk was divided in two batches (five liters each). One of the batches was inoculated with 8% V/V of cell suspension of adjunct culture of *Lb. plantarum* 162RM. The inoculated milks were incubated at 30°C overnight until coagulation. After coagulation (approximately 16h incubation), cheese curds were whey off in cheese clotche and salted (8g/kg). The cheeses were designated as: Experimental kareish Cheese (EKC),

Table 2. Evolution of pH during the manufacturing process of fermented milk using different combinations of commercial starter culture MY900 and ropy strain of *Lb. plantarum* 162RM.

Fermented milk Samples	pH at different incubation time at 42°C*					
	0 h	2 h	4 h	5 h	6 h	24 h after refrigeration
Starter	6.53 ± 0.01	6.38 ± 0.01	5.57 ± 0.01	4.82 ± 0.05	4.72 ± 0.03	4.56 ± 0.05
Starter + 4%162RM	6.54 ± 0.01	6.38 ± 0.02	5.77± 0.03	4.97 ± 0.04	4.75 ± 0.09	4.69 ± 0.08
Starter + 6%162RM	6.53 ± 0.01	6.38± 0.01	5.83 ± 0.04	4.91 ± 0.06	4.78 ± 0.02	4.59 ± 0.03
Starter + 8%162RM	6.53 ± 0.01	6.33± 0.01	5.53± 0.06	4.90 ± 0.04	4.70 ± 0.08	4.57 ± 0.04

*Mean value in each row did not differ significantly (P>0.05)

made by supplementing starter MM100 with the ropy culture of *Lactobacillus plantarum* 162RM at level 8% (V/V); Control kareish Cheese (CKC), made without ropy culture of *Lactobacillus plantarum* 162RM.

Samples from each cheese were taken, zero time, 7 and 15 days after manufacture for assessment of composition. The values reported are the means of the two cheesemaking trials.

Chemical analyses

Milk was analysed for fat content by Gerber method (BSI, 1955) and casein (IDF, 1964). The pH was determined with pH meter (Microcomputer pH-vision, model 05669-20) and the titratable acidity by the Dormic method. In addition, the decrease of the pH during the manufacture of fermented milk was continuously monitored using the pH meter above. All cheese were analysed for fat (BSI, 1955), moisture (moisture analyser) and pH.

Texture analysis

The texture properties of fermented milk and fresh Kareish cheese samples were evaluated using texture analyzer (CNS/FARNELL LFRA, Borehamwoad, Hertfordshimre, England).

For the texture analysis of fermented milk, samples were removed from the refrigerator (4°C) and centrally positioned beneath the probe within the container in which they were packed. Tests were conducted at ambient temperature (approximately 20°C). The probe used was TA4, (ϕ=38.1mm) perpex cylinder. Data were collected on computer and the following parameters texture profile parameters were calculated from LFRA texture analyzer and computer interface: hardness (maximum force that is exerted on the sample), consistency (Total Positive Area (TPA), and adhesiveness (Total Negative Area (TNA) and Adhesive Force).

Fresh Kareish cheese samples size was 30 mm of diameter and 20 mm of height. Speed was 1 mm/s and 10 mm was the distance of penetration. Samples were allowed to stand at ambient temperature for at least 20 min prior testing. The probe used was TA15-45°C perpex cone. Data were collected on computer and the texture profile parameters were calculated from LFRA texture analyzer and computer interface. The following texture profile parameters were obtained or calculated as describe by Bourne (1978). (i) The force (g) require to fracture the cheese, as a measure of fracturability. (ii) The compressive force (g) recorded at maximum compression during the first bite as a measure of hardness. (iii) The ratio of the positive force area under the curve during the second compression (bite) to that during the first compression (A_2/A_1) as a measure of cohesiveness. (iv) the height (mm) to which the sample recovered during the time that ellapsed between the end of the first bite and the start of the second bite, as a measure of springiness (elasticity). (v) The product of hardness x cohesiveness (g), as a measure of gumminess. (vi) The product of gumminess x springiness (g.mm), as a measure of chewiness. (vii) The modulus (the slope of force, representative of sample rigidity). Three measurements were made for each sample and the average values ± standard errors (SE), for the two trials are reported.

Sensory evaluation of fermented milk

A ten member's panel of sensory judges consisting of research assistants in the Laboratory of Microbial Biochemistry, University of Alexandria, familiar with the quality attributes of fermented milk and kareish cheese was constituted. The *panellists* were asked to rate the samples for appearance, flavour, aroma and texture.

The results obtained were subjected to statistical analysis to determine if or not any significant differences existed between the different types of fermented milk or cheese.

RESULTS AND DISCUSSION

A measurement of the pH values during the manufacture of fermented milks is given in Table 2. The fermented milks manufactured with different strain combinations reached pH 4.8 within 5-6 h of incubation at 42°C. No significant differences in pH values at the end of the fermentation process were observed inspite of the use of ropy strain of *Lb. plantarum* in the test samples. The acidification of milk within 5 h is essentially due to the fermentation of lactose by the starter MY900 that, according to the manufacturer is able to produce a variation in pH of 1 unit within 3 h. In addition, our previous study have shown that, *Lb. plantarum* has very slow acidification property. Infact, it has been shown that, during 5 h incubation, the variation in pH produce by this strain is less than 0.2 unit (submitted for publication).

The panelists comments indicated that the experimental fermented milk and the control were similar in appearance but the texture of the experimental fermented milk manufactured with the combination of starter MY900 and 4% or 8% of strain *Lb. plantarum* 162RM were the best. Thus, according to panellists observations, it is possible to improve the appearance and texture of fermented milk using 8% of strain L. plantarum 162RM.

The results of objective evaluation of fermented milk texture are given in Table 3. There were significant differences between the control and the fermented milks

Table 3. Texture parameters of fermented milk manufactured with different combination of commercial starter MY900 and ropy strain of *Lactobacillus plantarum* 162RM.

Texture parameters	Starter MY900	MY900 + 4%162RM	MY900 + 6%162RM	MY900 + 8%162RM
Hardness (g)	61 ± 0.7^{a}	65 ± 1.41^{b}	64 ± 2.12^{c}	72 ± 1.41^{abc}
TPA(g/s) (Consistency)	374 ± 11.31^{ab}	455 ± 8.69^{ab}	430.0 ± 16.97^{bc}	572.95 ± 20.01^{abc}
Adhesive force(g) (Adhesiveness)	-8.5 ± 2.12	-8.5 ± 3.53	-12.5 ± 2.12	-13 ± 5.65
TNA (g/s) (Resistance to flow)	-15.2 ± 7.49^{a}	-15.8 ± 10.32	-22.7 ± 9.89	-32.85 ± 9.68^{a}
Break load (Fracturability)	56 ± 8.48^{a}	56.5 ± 2.12^{b}	55 ± 0.7^{c}	68.5 ± 6.36^{abc}

[a,b,c] Means in each line bearing a common superscript differed significantly (P<0.05)
Mean in the same line without superscript did not differ significantly (P>0.05)

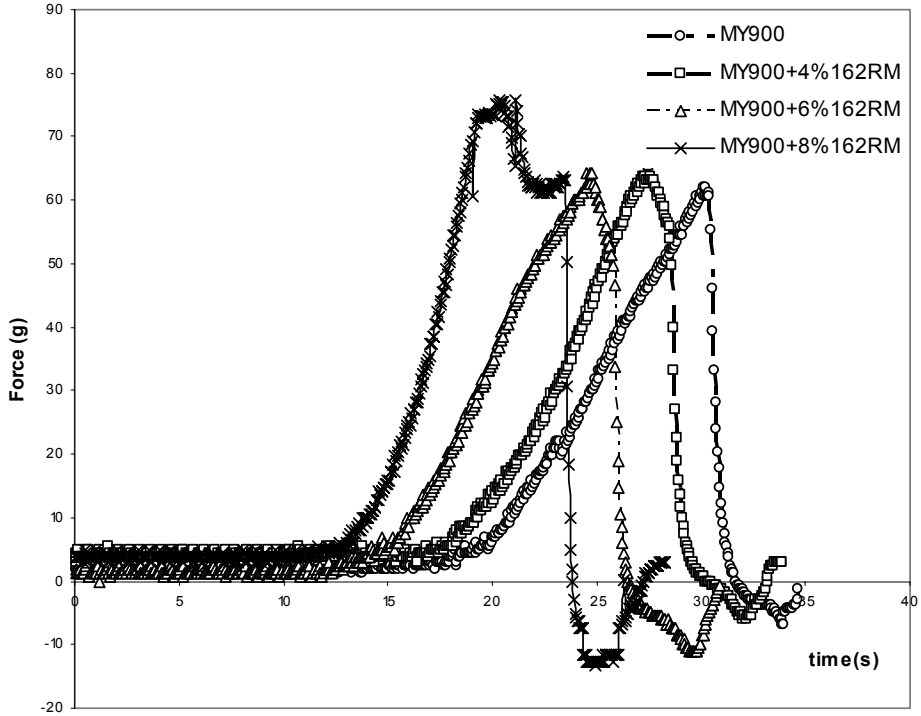

Figure 1. Texture profiles analysis of fermented milk samples using CNS/Farnell LFRA texture anylszer computer interface.

manufactured with the combination of starter MY900 and strain *Lb. plantarum* 162RM in hardness, consistency and adhesiveness. As shown in Figure 1, the fermented milk manufactured with the combination of starter and 8% of strain 162RM is thicker with more of gelled texture compared to the smooth flowing consistency of control fermented milk manufactured with the starter MY900 alone. The higher initial force and the total area under both positive and negative regions of the profile curves justify these observations. Infact, the positive and negative areas beneath the curve measure the resistance encountered by the plunger withdrawal, which indicates the flowing characteristic of the fermented milk influenced by its consistency or viscosity. Thus, the fermented milk manufactured with combination of starter MY900 and 8% of strain *Lb. plantarum* 162RM is thicker and consistent, since these values measured are greater. The first peak observed in the texture profile of the fermented milk sample manufactured with 8% (v/v) of strain 162RM highlights the gel breaking point which is clearly absent for the other fermented milk samples, due to their smooth flowing nature. The gel building in fermented milk system manufactured with starter MY900 alone depends only on casein proteins in the milk, which is the principal factor influencing the texture. It might also be due to the ropy nature of the starter MY900 as indicated by the manufacturer. In addition, the use of strain *Lb. plantarum* 162RM has strengthened the protein network, resulting in

Table 4. Contents of moisture, fat and pH of Kariesh cheeses manufactured with or without ropy strain of *Lb. plantarum* 162RM, during ripening*.

Starter and ropy strain combination	Ripening period (days)	Fat (%)	Moisture (%)	pH
Starter MM100	0	0.5	72.7[a]	4.58
	7	0.5	72.4[a]	4.55
	15	0.5	72.1[a]	4.51
Starter MM100 + *Lb. plantarum* 162 RM	0	0.5	75.2[ab]	4.62
	7	0.5	75[ab]	4.58
	15	0.5	74.3[ab]	4.50

*Mean values of the two trials
[a]Means in each row, without a superscript or bearing a common superscript did not differ significantly (p>0.05)
[b]In this row, at all ages the means of the two cheese type differ significantly (p<0.05)

Table 5. Textural characteristics of fresh kariesh cheese manufactured with and without ropy strain of *Lb. plantarum* 162RM*.

Texture parameters	Kariesh cheese samples	
	Starter MM100 + 8% *Lb. plantarum* 162RM (EKC)	Starter MM100 (CKC)
Force to fracture (Fracturability) (g)	26 ± 4.2	30 ± 1.4
Hardness (g)	111± 20	167 ± 31
Cohesiveness	1.61 ± 0.03[a]	1.78 ± 0.07
Springiness (elasticity) (mm)	7.11 ± 1.16	6.33 ± 0.28
Gumminess (g)	188 ± 37	299 ± 68
Chewiness (g mm)	1320 ± 450[a]	1886 ± 347
Relaxation	38.75 ± 0.21[a]	34.55 ± 1.9
Total Negative Area (Adhesiveness)	-35 ± 0.28[a]	-46.25 ± 4.31
Adhesive Force (g) (Resistance to flow)	-27.5 ± 3.5	-32 ± 4.2
Total positive area (g/s) (Consistency)	1168 ± 160	1544 ± 219
Modulus (Rigidity)	6.25 ± 0.63[a]	8.7 ± 0.84

* Mean values ± s.e of the two trials
[a] Means in the same line bearing superscript differed significantly (P<0.05)
Means in the same line without superscript did not differ significantly (P>0.05)
EKC: Experimental Kariesh Cheese, made with combination of starter MM100 and strain *Lb. plantarum* 162RM
CKC: Control Kareish Cheese made with starter MM100 alone.

firmer fermented milk with a better water binding properties. These results are in agreement with those of previous authors which demonstrated that lactic acid bacteria producing exopolysaccharides are often used to increase viscosity of stirred fermented milks, such as yoghurt and decrease susceptibility to syneresis (Cerning, 1995; Hassan et al., 1996; De Vuyst and Degeest, 1999; Patricia et al., 2002).

If used in appropriate amounts in combination with other starter culture, strain Lb. plantarum 162RM can improve smoothness, firmness, consistency and viscosity of fermented milk.

Characteristics of kareish cheese

The mean for the composition of kareish cheese is given in Table (4). There were significant (p<0.05) differences in moisture, between the experimental Kareish cheese (EKC) and the control Kariesh cheese (CKC) at all ages. Fat and pH values were similar in the cheeses at all ages. The average moisture content of the mature EKC (15 -day -old) was 74.3%, higher than the value observed in the CKC (72.1%). This result proves that EPS produced by Lb. plantarum 162RM have excellent water binding properties and moisture retention, which is vital to functionality of kareish cheese.

The gross composition of the Kareish cheeses was not significantly different throughout aging, indicating that, addition of adjunct culture to the cheese milk did not affect the composition of resultant cheese. These results are in agreement with those reported for different varieties of low fat cheese by others investigators (Bhowmik, et al., 1990; El -Soda et al., 1991; Katsiari et al., 2002) who observed that the composition of experimental low fat cheeses was not affected by various adjunct cultures in cheesemaking.

The results of objective evaluation of fresh cheese texture are given in Table (5). The EKC and the CKC cheese were not significantly different in both the force

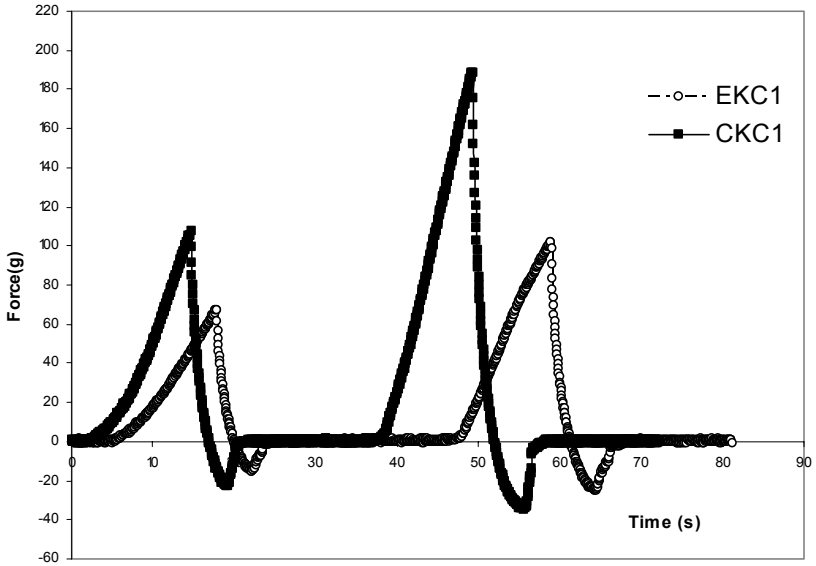

Figure 2. Texture profiles analysis of fresh kareish cheese samples using CNS/Farnell LFRA texture analyzer computer interface.

required to fracture and hardness. But the EKC has lesser hardness and force to fracture than the CKC. The values of adhesiveness, cohesiveness and chewiness, were significantly ($p<0.05$) higher in control cheese than those in the experimental cheese (Figure 2). We also found that,, the two cheeses type had similar values for springiness and gumminess, but the EKC was significantly ($p<0.05$) more relaxing than the CKC (Table 5) . These results are in agreement with those of Katsiari and Voutsimas (1994) for low fat high moisture Kefalograviera-type cheese. The decrease in values of some textural parameters of the EKC (Table 5) is in agreement with the results of previous investigators who suggested that positive results for flavour and texture development in reduced or low-fat cheeses depend strongly on the strain of adjunct culture used (Simard, 1991; Ardo, 1997; El-Soda, 1997; El-Soda et al., 2000). The pliable and soft texture of the EKC may be due to the excellent water binding properties of EPS producing by *Lb. plantarum* 162RM, which improve the hard rubbery texture of kareish cheese.

The results of the present study indicated that less rubbery and the most soft kareish cheese could be made with the combination of starter MM100 and ropy strain of *Lb. plantarum* 162RM.

ACKNOWLEDGMENTS

This study was performed in the pilot plant of the Laboratory of the Biochemistry of Dairy Microorganisms at the Faculty of Agriculture, Alexandria University, in the framework of the Nestle Nutrition scholarship program. The authors thank Nestle Company for their funding contribution. They would also like to thank the technicians of this laboratory for their helpful assistance.

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