J. Food and Dairy Sci., Mansoura Univ., Vol. 2 (4): 175 - 182, 2011

ISOLATION OF EXOPOLYSACCHARIDE – PRODUCING LACTIC ACID BACTERIA FROM TRADITIONAL EGYPTIAN DAIRY PRODUCTS

El-Awady, Asmaa A.*; M. M. Zien El-Din and W.M. El-Sharoud***

*** Food Safety and Microbial Physiology Laboratory, Dairy Department, Faculty of Agriculture, Mansoura University**

**** Faculty of Tourism and Hotels, Mansoura University.**

ABSTRACT

The present study aimed to isolate and characterize strains of exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) from traditional Egyptian dairy products. A total of 388 suspected LAB isolates were recovered from 100 samples of traditional Egyptian dairy products including Kariesh cheese, Zabady, Laban Rayeb and Ras cheese. Out of these suspected LAB cultures, 123 and 27 isolates were identified as potential LAB cocci and lactobacilli, respectively. These 150 LAB isolates were further examined for their ability to produce exopolysaccharides (EPS) in milk. Only 7 LAB isolates were found to produce EPS. These isolates were cultured form Laban Rayeb (3 isolates) and Zabady (4 isolates). A PCR identification procedure was carried out to identify these EPS-producing isolates to the species level. All the 7 isolates were found to belong to *Streptococcus thermophilus*. These results generally suggest the possibility of using Egyptian dairy products as a source of EPS-producing LAB.

Keywords: lactic acid bacteria, Exopolysaccharides, *Streptococcus thermophilus*

INTRODUCTION

Bacterial strains are able to produce extracellular polysaccharides (exopolysaccharides) that bind to the cell surface as capsules or diffuse as slime in the growth environment (Cerning 1995). This has been shown to be associated with the ability of some pathogenic bacteria to cause human diseases, such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Nisseria gonorrhoeae* and *Klebsiella pneumoniae*. However, the ability of lactic acid bacteria (LAB) strains to produce EPS has been reported to improve the quality of fermented dairy products. LAB strains can be categorized into three groups based on their ability to produce EPS: a) LAB that do not produce EPS, b) LAB producing EPS in the form of cellular capsules, c) LAB producing EPS in the form of cellular capsules and slime in the growth medium (Hassan 2008). LAB strains also differ in the type of chemical composition of EPS they produce. For example, *Leuconostoc mesenteroides* strains can produce dextrans and glucans (homopolysaccharides), but most other industrial LAB strains including those of *Streptococcus thermophilus*, *Lactococcus lactis* and *Lactobacillus* spp. produce heteropolysaccharides (De Vuyst *et al*. 2001).

The incorporation of EPS-producing LAB in dairy products was found to provide viscosity, stability and water-binding functions that improve the taste and texture of fermented dairy products (De Vuyst and Degeest 1999). Therefore, EPS-producing LAB starters have been utilized to control syneresis or "wheying off" in yoghurt (Cerning 1995) and sour cream (Adapa

and Schmidt 1998). These starters have been also employed to improve the texture and functionality of cheese (Petersen *et al*. 2000). They have particularly proved useful for the preparation of low-fat cheese given the massive ability of EPS to bind water and cause its retention within the protein matrix, which confers softness and improves the taste of low-fat cheese (Broadbent *et al*. 2003). EPS-producing LAB were also applied to the preparation of frozen yoghurt, which was associated with increasing apparent viscosity, overrun, and resistance to heat shock (Abd El-Rahman *et al*. 1999).

European dairy manufacturers have been employing EPS-producing LAB starters for many years to produce a variety of fermented milks with unique properties (Cerning 1995). The nature of these starters have not been revealed until progress in dairy sciences and technologies have been made over the recent decades. Still, the production of EPS-producing LAB starters is mainly based in Western countries such as Denmark, France, and USA, from which large amounts of dairy starters are annually purchased and imported to Egypt. This represents an actual burden on the development and independence of dairy industries in Egypt (El-Sharoud and Ayad 2008). The present study was therefore designed to isolate and characterize strains of EPS-producing LAB from traditional Egyptian dairy products. This was in an attempt to make use of these locally available dairy products as a source for EPS-producing LAB strains.

MATERIALS AND METHODS

Collection of Dairy Product Samples

A total of 100 samples of dairy products were randomly collected from local markets in Mansoura city and villages in its vicinity. These samples included 39 samples of Kariesh cheese, 30 samples of Zabady, 13 samples of Laban Rayeb and 18 samples of Ras cheese.

Isolation of Lactic Acid Bacteria from Dairy Products

Dairy product samples were serially diluted in sterilized saline solution (0.85% NaCl). Resultant dilutions were plated onto Elliker agar (BD, New Jersey, USA) and MRS agar (Oxoid, Basingstoke, UK), followed by incubation at 37°C for 48 h. Suspected colonies were picked and maintained on MRS agar till further examinations.

Identification of LAB Isolates:

Suspected LAB isolates were subjected to Gram staining and the following physiological examinations:

Catalase Activity

Suitable amount of growth from one discrete colony of LAB isolate was transferred into a clean glass slide, followed by the addition of 1 drop of H_2O_2 (30%). Immediate bubbling (gas formation) was taken as a positive result (Macfaddin 1977).

Growth at 45°C and 10°C

An overnight culture of LAB isolate grown in MRS broth (Oxoid, Basingstoke, UK) was inoculated at 1% (v/v) into MRS broth, followed by incubation at 45°C for 48 h and 10°C for 2 weeks. LAB growth was observed within these incubation times (Sharpe 1979).

Growth at in 4% and 6.5% NaCl

An overnight culture of LAB isolate grown in MRS broth was inoculated at 1% (v/v) into MRS broth containing 4% or 6.5% NaCl, followed by incubation at a temperature optimum for the growth of the examined isolate (Abd El-Malek and Gibson 1948). Cell growth was observed after 48 h of incubation.

Growth at pH 9.6

An overnight culture of LAB isolate grown in MRS broth was inoculated at 1% (v/v) into MRS broth adjusted to pH 9.6, followed by incubation at a temperature optimum for the growth of the examined isolate (Sharpe 1979). Cell growth was observed after 48 h of incubation.

Growth on BEA agar medium

One hundred microliters of an overnight culture of LAB isolate grown in MRS broth were spread onto bile esculin agar (BEA) (BD, New Jersey, USA), followed by incubation for 24 h at a temperature optimum for the growth of the examined isolate.

Growth in SF medium

An overnight culture of LAB isolate grown in MRS broth was inoculated at 1% (v/v) into Streptococcus faecalis (SF) broth (BD, New Jersey, USA) followed by incubation for 48 h at a temperature optimum for the growth of the examined isolate. Inoculated SF broth tubes were observed for the growth and formation of the yellow color, which indicated acid formation due to sugar fermentation by the examined isolate (Sharpe 1979).

PCR Identification of potential *S. thermophilus* isolates

Potential *S. thermophilus* isolates were grown overnight at 37°C on M17 agar (Oxoid, Basingstoke, UK) containing lactose at a final concentration of 1.0%. One discrete bacterial colony from each M17 agar plate was suspended in 50 µl of TES (10 mM Tris-HCl, 1 mM EDTA, 25% sucrose), and DNA was extracted by cell lysis at a high temperature of 95°C for 10 min, followed by cooling at 4°C for 15 min using the Primus 25 advanced thermocycler (peQLab, Wilmington, Delware, USA). A PCR reaction mixture of 50 µl was formulated using 5 µl bacterial DNA, 25 µl mater mix (peqGOLD PCR-MasterMix, peQLab, Wilmington, Delware, USA), 2 µl of forward primer (GGTCCAAGAAGAAGTAATTGA), 2 µl reverse primer (GACCTTATACAAATCTGGTT), and 16 µl water. Primers were designed to target the *serB* gene encoding phosphoserine phosphatase in *S. thermophilus,* and were synthesized by Applied Biosystems (Applied Biosystems, Foster City, CA, USA). PCR reactions were conducted in the Primus 25 advanced thermocycler using the following cycling parameters: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min. Resultant PCR amplicons were separated by gel electrophoresis using 1% agarose gel in TBE buffer (AppliChem, Darmstadt, Germany). Gel was visualized using UV transiluminator (Vilber lourmat, France) and photo was captured using a gel documentation system.

Assessment of Exopolysaccharide (EPS) Production

LAB isolates were grown overnight at 37°C in sterilized reconstituted skim milk (RSM) containing 10% total solids (w/v). Resultant cultures were then used to inoculate fresh amounts of sterilized RSM at 2% (v/v), followed

by incubation at 37°C for 24 h. LAB coagulated cultures were gently stirred for 5-7 times with a spoon, which was finally used to withdraw samples of coagulated milk. The formation of stick, continued threads of milk during the withdrawal of samples was taken as an indicator of EPS production.

RESULTS AND DISCUSSIONS

Isolation of Lactic Acid Bacteria (LAB) from Traditional Egyptian Dairy Products

One hundred samples of traditional Egyptian dairy products were randomly collected from local markets in Mansoura city and villages in its vicinity. These samples included 18 samples of Ras cheese, 30 samples of Zabady, 13 samples of Laban Rayeb and 39 samples of Kariesh (Table 4.1). Samples were serially diluted and plated onto Elliker agar and MRS agar. A total of 388 suspected LAB isolates were recovered from these samples and subjected to preliminary identification involving Gram-staining, catalase test and milk coagulation test.

Table 1 shows the varieties and numbers of examined dairy product samples and the numbers of suspected LAB isolates recovered from each product. It also shows the results of a preliminary identification of these isolates. Isolates that were Gram-positive and catalase-negative and could coagulate milks were considered as potential LAB. Out of 388 suspected LAB cultures, 123 and 27 isolates were identified as potential LAB cocci and lactobacilli, respectively (Table 1). Potential LAB cocci were isolated from all varieties of dairy products examined. While potential LAB lactobacilli isolates were recovered from both Zabady and Laban Rayeb samples, they could not be isolated from Ras cheese and Kariesh cheese. These results could reflect the composition of natural or industrial dairy starters used for the preparation of these products.

Table 1: Isolation and Preliminary Identification of Lactic Acid Bacteria from Traditional Egyptian Dairy Products

Samples	No. of Samples	No. of Suspected LAB Isolates	Potential LAB Isolates	
			Cocci	Lactobacilli
Ras cheese	18	23	13	0
Kariesh cheese	39	195	30	0
Zabady	30	71	54	17
Laban Rayeb	13	99	26	10
Total	100	388	123	27

Potential LAB isolates were subjected to further examinations as shown in table 2. All the 27 potential lactobacilli and 43 cocci could not grow at 10°C, pH 9.6 or in the presence of 6.5% NaCl, but could grow at 45°C. However, 80 potential cocci could grow at 10°C, 45°C, pH 9.6 and in the presence of 6.5% NaCl. This suggested that these 80 cocci could belong to the *Enterococcus* species (Sharpe 1979 and Hardie & Whiley 1995). Further examinations of the 123 LAB cocci showed the ability of these 80 isolates to grow in the SF broth and on the BEA medium producing black growth. The other 43 cocci could not grow in the SF broth or on the BEA medium. This suggested their belonging to the *Streptococcus* species.

Table 2: Further Identification of Potential LAB Isolates Recovered from Traditional Egyptian Dairy Products.

Potential LAB Isolates (No. of Isolates)	Growth at 10°C	Growth at 45°C	Growth at pH 9.6	Growth in 6.5% NaCl	Growth in SF broth	Growth on BEA medium	Results of Identification (No. of Isolates)
Lactobacilli (27)	-	+	-	-	NE*	NE	*Lactobacillus* spp. (27)
Cocci (123)	-	+	-	-	-	-	*Streptococcus* spp. (43)
	+	+	+	+	+	+	*Enterococcus* spp. (80)

*** NE: Not Examined**

The above results show that diverse LAB isolates belonging to different species could exist in traditional Egyptian dairy products, which was also reported in previous studies. For instance, Ayad *et al*. (2004 & 2006) isolated LAB strains of the genera *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Pediococcus* from Domiatti cheese, Ras cheese, Mish, Zabady and Laban Rayeb. However, the present results show that *Enterococcus* isolates were the most frequently isolated LAB from traditional Egyptian dairy products. This is consistent with Süßmuth (1995) who could isolate 300 LAB strains from Domiatti cheese and Ras cheese. The majority of these strains belonged to *Enterococcus* with only 3.5% of the strains belonging to *Lactococcus* and 1.5% belonging to *S*. *thermophilus* and *S. bovis*. This could be attributed to the ability of *Enterococcus* strains to tolerate adverse environmental conditions of low pH and high salt levels existing in traditional Egyptian dairy products (Jokovic *et al.* 2008).

Exopolysaccharide (EPS) production by Lactic Acid Bacteria (LAB) isolated from Traditional Egyptian Dairy Products

The ability of the 150 LAB isolates recovered from traditional Egyptian dairy products to produce exopolysaccharides (EPS) in milk was examined. Overnight LAB cultures were used to inoculate sterilized reconstituted skim milk (RSM), followed by incubation at 37°C for 24 h. Resultant coagulated cultures were then gently stirred for 5-7 times with a spoon, which was finally used to withdraw threads of coagulated milk, if any. The formation of stick, continued threads of milk coagulated by a LAB culture was taken as a positive result. Out of 150 examined LAB isolates, only 7 *Streptococcus* spp. cultures were found to produce EPS. These isolates were cultured from Laban Rayeb (3 isolates) and Zabady (4 isolates).

Table 3: Exopolysaccharide (EPS) Production by LAB Recovered from Traditional Egyptian Dairy Products

Isolates	EPS Production	
	+	-
Lactobacillus spp.	0	27
Streptococcus spp.	7	36
Enterococcus spp.	0	80
Total	7	143

PCR Identification of EPS-producing *Streptococcus* spp. Isolated from Traditional Egyptian Dairy Products

A PCR identification testing was carried out to identify the EPS-producing *Streptococcus* isolates to the species level. Since the results of the physiological characterization of these isolates, shown in table 2 above suggested their belonging to *Streptococcus thermophilus*, a primer pair targeting the *serB* gene in *S. thermophilus* has been applied. Figure 1 shows a photo of agarose gel containing samples of PCR amplicons resulted from amplifying the DNA of 7 potential *Streptococcus* isolates. As shown in this gel photo, lane 1 contains DNA ladder, lanes 4 through 6 contain PCR amplicons of *Streptococcus* isolates recovered from Laban Rayeb, and lanes 7 through 10 contain PCR amplicons of *Streptococcus* isolates recovered from Zabady. All amplicons contained a DNA fragment of the size 570 bp, which indicated the presence of the *serB* gene in examined isolates. This confirmed the belonging of the examined 7 *Streptococcus* isolates to the *Streptococcus thermophilus*. Previous studies have also showed that *Streptococcus thermophilus* strains are among the most frequently EPS-producing LAB. For instance, Mora *et al*. (2002) found that 50% of 44 *Streptococcus thermophilus* strains isolated from dairy products in Europe were able to produce exopolysaccharides. Vaningelgem *et al*. (2004) could also isolate 26 LAB from European dairy products and characterized strains of *S. thermophilus* that were able to produce EPS. However, the present results generally show that it is possible to use Egyptian dairy products as a source of EPS-producing LAB cultures that could be used in relevant local dairy industries.

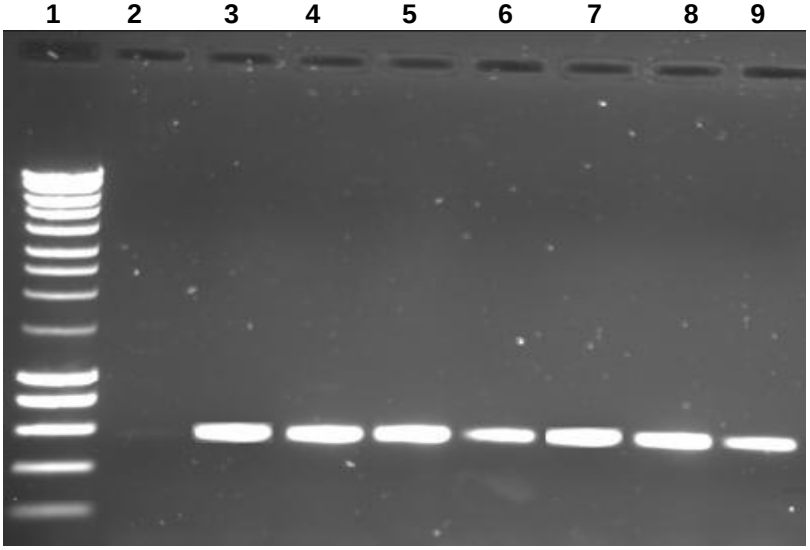

Figure 1: PCR Identification of EPS-Producing LAB Recovered from Traditional Dairy Products. Lane 1 contains DNA ladder, lanes 3 through 5 contain PCR amplicons of isolates from Laban Rayeb and lanes 6 through 9 contain PCR amplicons of isolates recovered from Zabady.

REFERENCES

Abd El-Rahman, A.M., Hassan, A.N. and Khear, E.E. (1999). Modification of low fat soft cheese structure by using capsule-forming nonropy lactic cultures. International Food Technologists Annual Meeting, Chicago, USA.

Abd-El-Malek, Y., and Gibson, T. (1948). Studies in the bacteriology of milk. J. Dairy Res.,15: 233-248.

Adapa, S., and Schmidt, K.A. (1998). Physical properties of low-fat sour cream containing exopolysacchride-producing lactic acid bacteria. J. Food Sci*.,* 63: 901-903.

Ayad, E.H.E., Nashat, S., El-Sadek, N., Metwaly, H., and El-Soda,M. (2004). Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiol., 21: 715-725.

Ayad, E.H.E., Omran, Nadia., and El-Soda, M. (2006). Characterisation of lactic acid bacteria isolated from artisanal Egyptian Ras cheese INRA, EDP Sciences Lait 86: 317-331.

Broadbent, J.R., McMahon, D.J., Welker, D.L., Oberg, C.J. and Moineau, S. (2003). Biochemistry, genetics and applications of exopolysaccharide production in *Streptococcus* thermophilus. J. Dairy Sci., 86: 407-423.

Cerning, J. (1995). Extracellular polysaccharides by lactic acid bacteria and dairy propionibacteria. Lait 75:463-472.

De Vuyst, L., and Degeest, B. (1999). Heteropolysaccharides from from lactic acid bacteria. FEMS Microbiol. Rev*.,* 23:153-177.

De Vuyst, L., De Vin, F., Vaningelgem, F. and Degeest, B. (2001). Recent developments in the biosynthesis and applications of heteropolysaccharie from lactic acid bacteria. Int. Dairy J., 11:687-707.

El-Sharoud, W.M., and Ayad K.M., (2008). Technological characteristics and antibacterial activities of *Lactococcus lactis* subsp. *lactis* isolates recovered from Laban Rayeb and Kariesh cheese. J. Agric. Sci. Mansoura Univ., 33 (7): 5153 – 5161.

Hardie, J. M., and Whiley, R. A. (1995). The genus streptococcus. In: The Genera of Lactic Acid Bacteria, Wood, B.J.B and and W. H. Holzapfel (Eds.), Chapman and Hall, London, pp. 55-124.

Hassan, A.N. (2008). Possibilities and Challenges of Exopolysaccharide-Producing Lactic Cultures in Dairy Foods. J. Dairy Sci., 91:1282 - 1298.

Jokovic,N., Nikolic.M., Begovic.J., Jovcic.B., Savic.D., Topisirovic.L., (2008). A survey of the lactic acid bacteria isolated fromSerbian artisanal dairy product kajmak. *Int. J. Food Microbiol,* 127: 305–311.

Macfaddin, J. (1977). Biochemical tests for identification of medical bacteria. The Williams and Wilkins, Baltimore, Md., U.S.A.

Mora, D., Fortina. M.G., Parini.C., Ricci.G., Gatti.M., Giraffa.G. and Manachini.P.L.,(2002).Genetic diversity and technological properties of *streptococcus thermophilus* strains isolated from dairy products. J.f Appl. Microbiol., 93: 278–287.

Petersen, B.L., Dave, R.I., McMahon, D.J., Oberg, C.J. and Broadbent, J.R. (2000). Influence of capsular and ropy exopolysaccharide-producing *Streptococcus thermophilus* on Mozzarella cheese and cheese whey. J. Dairy Sci., 83:1952-1956.

Sharpe, M. E. (1979). Identification of the lactic acid bacteria. In: Identification Methods for Microbiologists, 2nd ed, Skinner, F. A. and D. W. Lovelock (Eds.), Academic Press, London, pp:233-259.

Süßmuth, R. (1995). Selection and characterization of natural isolates of lactic acid bacteria in African cheeses with specific reference to salt tolerance, bacteriophage resistance and impact on product quality. Summary reports of European Commission supported STD-3 projects (1992-1995), published by *CTA1998.*

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. Int. Dairy J., 14, 857–864.

عزل بكتريا حامض اللاكتيك المنتجة للسكريات الخارجية العديدة من منتجات الألبان المصرية التقليدية.

أسماء عبد الله العوضي*، محمد محمد ابراهيم زين الدين و وليد محمود الشارود***

*** معمل أمان الأغذية وفسيولوجيا الميكروبات- قسم الألبان- كلية الزراعة- جامعة المنصورة.**

**** كلية السياحه و الفنادق – جامعة المنصورة**

استهدفت هذه الدراسة عزل وتعريف سلالات من بكتريا حامض اللاكتيك لديها القدرة علي إنتاج سكريات خارجية عديدة وذلك من منتجات الألبان المصرية التقليدية. حيث تم عزل ٣٨٨ مزرعة من بكتريا حامض الاكتيك من ١٠٠ عينة من منتجات الألبان المصرية مع تعريفها. وقد أمكن تعريف ١٢٣ عزلة علي أنها تنتمي لبكتريا حامض اللاكتيك الكروية (cocci) بينما تم تعريف ٢٧ عزلة علي أنها تنتمي لبكتريا حامض اللاكتيك العصوية (lactobacilli). ولقد تم اختبار قدرة جميع هذه العزلات ومجموعها ١٥٠ عزلة علي إنتاج السكريات الخارجية العديدة، ووُجد أن ٧ عزلات منها قد تمكنت من إنتاج هذه السكريات. وبتعريف هذه العزلات إلي مستوي النوع وذلك باستخدام طريقة "تفاعل السلسلة المتبلمر" (PCR)، وُجد أن جميعها ينتمي إلي النوع "استربتوكوكس ثرموفيلس" (*Streptococcus thermophilus*). وتشير هذه النتائج بصفة عامة إلي إمكانية استخدام المنتجات اللبنية المصرية كمصدر محلي لبكتريا حامض اللاكتيك المنتجة للسكريات الخارجية العديدة، والتي يؤدي استخدامها كبادئات إلي تحسين صفات الألبان المتخمرة.

قام بتحكيم البحث

أ.د / طه عبد الحليم نصيب	**كلية الزراعة – جامعة المنصورة**
أ.د / جمال الدين أحمد مهران	**كلية الزراعة – جامعة عين شمس**