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MEASURING THE CAPABILITY OF IMMUNOMAGNETIC SEPARATION-PCR (IMS-PCR) FOR THE DETECTION OF ONE CFU OF *ESCHERICHIA COLI* O:157 H:7 IN MILK AND SOFT CHEESE

(With 4 Tables and One Figure)

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(Received at 1/12/2004)

قياس قدرة الفصل المناعي المغناطيسي – سلسله تفاعل إنريم البلمره للكشف عن مستعمره واحده من ميكروب الإيشيريشيا كولاي 0157 H:7 في اللبن والجبن الطرى

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اجريت هذه الدراسة لعزل ميكروب الاشريشياكولاى والذي ينتمي إلىO:157 H:7 من اللبن الخام والجبن الأبيض الطرى وقياس مدى قدرة وحساسية طريقة الIMS-PCR على عزل هـذا المـيكروب منها حيث تم تجميع ١٠٠ عينة (٥٠عينة جبن ابيض طازج و ٥٠عينة لبن خام) من مناطق مختلفة من محافظة بنىسويف وتم عزل الميكروب من عينة واحدة فقط من اللبـن الخـام وعينتان من الجبن وتم حقن الميكروب المعزول فى عينات من اللبن والجبن الابيض الطرى بأعداد مختلفة وقياس مدى قدرة وحساسية طريقة الIMS-PCR على عزل هذا الميكروب.

SUMMARY

This study was conducted to determine the presence of *Escherichia coli* O:157 H:7 in raw milk and soft cheese and determination the ability and sensitivity of IMS-PCR method for detection one CFU of this micro-organism in the same products. A total of 100 samples of raw milk and fresh soft cheese (5o samples of each) were randomly collected from different localities in Bani-suef province and examined according to (Hitchins *et al.,* 2001). *Escherichia coli* O:157 H:7 was isolated from 1(2.0%) and 2(4.0%) of the examined raw milk and soft cheese samples, respectively. Milk and soft cheese samples were spiked with *E.coli* O157

at low levels. The samples were enriched in LB broth and incubated at 37°C. Aliquots of the enriched culture were analyzed either by PCR and IMS-PCR. This study showed that as few as one organism can be detected in 25 ml milk by IMS-PCR method. After 6 h of incubation IMS-PCR were able to detect *E.coli* from milk but not from cheese samples. After 8 hours of incubation PCR alone was not able to detect any of the samples tested, but IMS combined with PCR was able to detect *E.coli* in milk and cheeses at low inoculum level. The results indicated that the ability of the IMS to remove inhibiting factors from various food samples is different.

Key words: *PCR, E.coli O157: H7, milk, soft cheese*

INTRODUCTION

Raw milk has been described as a source of infections caused by shiga toxin-producing *Escherichia coli* serotype O157:H7 (Keene *et al.* 1997). The ability of *E. coli* to survive in fermented dairy products made from raw milk (Abdul-Raouf *et. al.,* 1996; McIngvale *et. al.,* 2000; Maher *et al.,* 2001) is of major concern because the consumption of such products has also led to infection in human.

Cultural methods for *E. coli* detection involve a non-selective pre-enrichment, followed by selective enrichment and plating on selective agars. Suspected colonies are confirmed biochemical and serologically; the complete test requires three to four days to obtain a negative result and 5-7 days to get confirmed positive result. A number of rapid methods for the detection of *E. coli* in foods have been developed, including electrical techniques, immunoassays, and nucleic acid probe (Meng *et. al.,* 1996). These assays utilize primers and probes that hybridize specifically to complementary sequences found in the verotoxigenic *E. coli* (VTEC) type including *E. coli* O157:H7. But in food analysis, rapid methods still lack sufficient sensitivity and specificity for direct testing; hence, food still in need to be culture-enriched before analysis (Feng, 1997). Although enrichment is a limitation in terms of assay speed, it provide essential benefits, such as diluting the effect of inhibitors, allowing the differentiation of viable from non-viable cells and allowing the repair of cell stress or injury that may be resulted during food processing.

The ability of PCR to amplify specific DNA reduces the need for the large quantities of test DNA required for the hybridization assays. In

theory one copy of the target gene is sufficient for successful amplification. In many ways, the extreme sensitivity of PCR can be compared with cultivation of bacteria on nonselective media, when a single live bacterium can be detected up on initiation of the colony (Olsvik *et. al.* 1994). However, certain disadvantages limit the technique. The sample volume traditionally used in PCR ranges from <1 to 20µl for several microbiological applications, such testing for *Salmonella* spp. In foods, requirements are often one cultivable organism per 100 g/ml of sample. Reduction of the sample to 1 to 20µl restrict the test sensitivity to a theoretical minimum of 5000 to 100,000 organism per ml (Olsvik and Stockbine 1993). An additional factor hindering the use of PCR directly from samples is the sensitivity of the Taq polymerase to inhibitor elements in food samples, thereby requiring extensive sample preparation to remove, dilute or inactivate inhibitors prior to PCR amplification (Fratamico *et. al.,* 2000).

Use of Immunomagnetic Separation (IMS) as a pre-PCR step appears to solve these problems. The bacteria in the sample are concentrated to a suitable volume of 1-100 µl, and specific Taq polymerase inhibitors are simultaneously removed. Adding the magnetic bead fraction to a growth medium for pre-cultivation can increase the number of target organisms for the PCR. Samples that have been frozen often contain nonviable cells, but these cells can still be extracted with IMS and identified by PCR. This method can also be of importance in identifying the origin of strains involved in food-borne outbreaks if only nonviable bacteria remain in the implicated food samples.

The potential low infective dose of *E.coli* necessitates the ability to detect low numbers in food. Therefore the goal of this study is to investigate the capability of IMS-PCR for detection of one CFU in milk and soft cheese.

MATERIALS and METHODS

One hundred random samples of milk and fresh soft cheese samples (50 of each) were collected from different localities in Bani-suef province, Egypt. All samples were examined for the presence of *Escherichia coli* O:157 H:7. according to (Hitchins *et al.,* 2001).

The obtained *Escherichia coli* O:157 H:7 strain was inoculated onto Luria-Bertani (LB) agar plates (Difco laboratories, Detroit, Mich.) and incubating the plates overnight at 37°C. LB broth inoculated with

reactions result in poor detection sensitivity and even complete reaction failure (false negative results).

Use of IMS as a pre-PCR step appears to solve several of these problems. Therefore we conclude that the IMS-PCR is a rapid, specific and an essential method for the detection of low numbers of *E. coli* in milk and dairy products.

Table 1: Incidence of *E. coli* O157: H7 in examined raw milk and fresh soft cheese samples:

Products	No.of examined samples	No.of positive samples	%
Raw milk	50	1	2.0
Fresh soft cheese	50	2	4.0

Table 2: Specificity of immunomagnetic separation for isolating *E.coli* O157:H7 in mixed culture

Samples	Mean CFU/ml before IMS *E.coli / Enterobacter cloacae / Salmonella*	Mean CFU /100 µl after IMS *E.coli / Enterobacter cloacae / Salmonella*
Milk	$8.4 X10^4$ / $5.0 X10^4$ / $1.5X10^4$	$8.3 X10^4$ / 0 / 0
Soft cheese	$8.4 X10^4$ / $5.0 X10^4$ / $1.5 X10^4$	$7.9 X10^4$ / 0 / 0

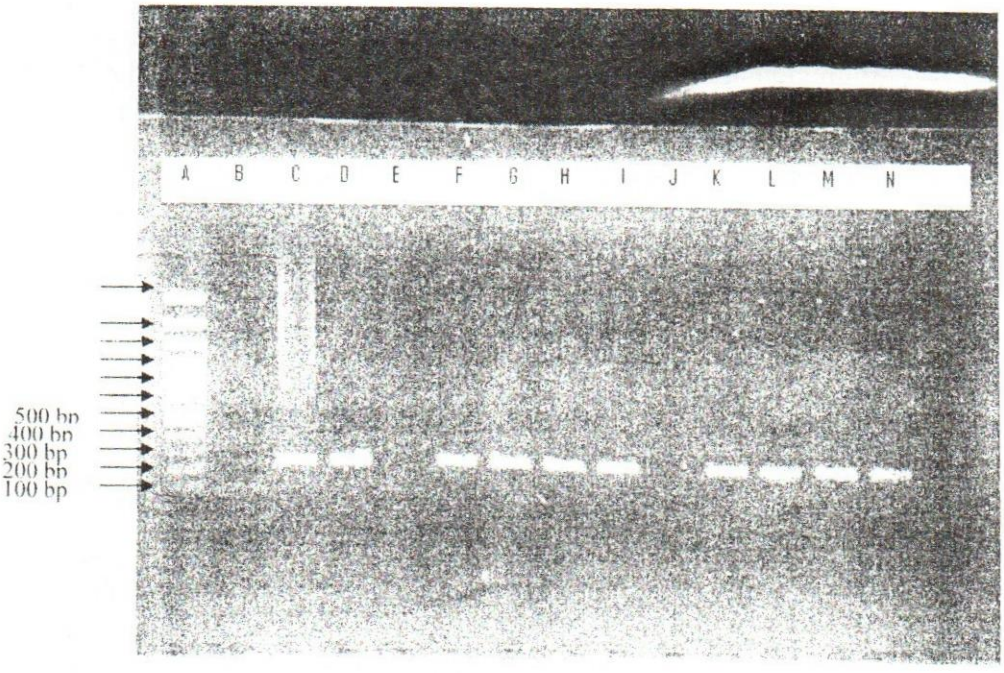
Table 3: Detection of *E. Coli* O157 at different incubation time using IMS-PCR

Incubation time	Milk		Soft cheese	
	Direct -PCR	IMS-PCR	Direct –PCR	IMS-PCR
0h	-	-	-	-
2h	-	-	-	-
4h	-	-	-	-
6h	-	+ (1 CFU)	-	+ (100 CFU)
8h	-	+ (1 CFU)	-	+ (10 CFU)
10h	-	+	-	+
12h	+	+	-	+
24h	+	+	+	+

Table 4: Sensitivity of IMS-PCR after enrichment for detecting *E.coli* O157 using different inoculation levels

Samples	CFU/25 gm/ml detected after 6 hours PCR (IMS-PCR)							CFU/25 gm/ml detected after 8 hours PCR (IMS-PCR)				
	10^4	10^3	10^2	10	1	0	10^4	10^3	10^2	10	1	0
Milk	- (+)	- (+)	- (+)	- (+)	- (+)	-(-)	-(+)	-(+)	-(+)	-(+)	-(-)	-
Cheese	- (+)	- (+)	- (+)	- (-)	- (-)	-(-)	-(+)	-(+)	-(+)	-(+)	-(-)	-

Figure 1: PCR profile of *E. coli* O157 strain 920333 from milk samples. A: molecular weight standard (100bp ladder plus); B, E, and J: non *E.coli* samples lanes; C,D,F,G,H,I, K,L,M and N: a band of 259bp (O157 antigen)



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