Detection of Pathogenic Bacteria in Milk and Its Products by Using PCR Technique

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Abstract: The detection of major pathogenic bacteria is an important measurement for providing health guarantee for milk and milk-related products. So, this research was directed to develop more simple, rapid and sensitive detection method in the field of food hygiene by using the PCR method. In this work three common pathogenic bacteria were detected and differentiated named (*Staphylococcus aureus*, *Salmonella typhemurium* and *Escherichia coli*) in 120 suspect isolates from milk and dairy products collected from farmer vendors and dairy shops in Al-Ahasa area. A primer targeting of the thermostable nuclease gene (*nuc* of *Staphlococcus, hil*A genes of *Salmonella* and A SLT *genes* of *E.coli*) were used in the PCR analysis. A DNA fragment of 279, 972 and 660 bp was amplified for *Staphylococcus aureus*, *Salmonella* and *Escherichia coli,* respectively. Result, showed that, the PCR method owned a higher detection rate than others. The detection rate of positive samples by using PCR was 51.6, 5.0 and 84.2 % for *Staphylococcus aureus*, *Salmonella* and *Escherichia coli,* respectively. The results confirmed that the rate of detection of pathogenic bacteria depended on the type of microbe, as well as the type of sample tested. The use of PCR technique in the detection was more accurate and sensitive than use the traditional method of detection, which sometimes gave results contrary to the obtained using the traditional method.The results also indicate that the *E.coli* was more pathogenic bacteria presence, followed by *Staphylococcus aureus* and *Salmonella typhemurium*, respectively.

Generally, using this technique a rapid, sensitive, and effective results for PCR detection of pathogenic bacteria in milk and milk products.

Keywords: pathogenic bacteria, detection, PCR, dairy products.

INTRODUCTION

Milk is a nutritious food for human beings, acting as a good medium for the growth of many microorganisms, especially bacterial pathogens (Chye *et al.*, 2004). An important application of bacterial genomics is to improve control of the microbiological safety and quality of food products. This is particularly relevant to dairy companies, which need to ensure that milk is free from pathogenic bacteria and that the concentration of contamination is as low as possible. Human pathogens that have been detected in raw milk include *Campylobacter jejuni*, enterohaemorrhagic *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, and *Yersinia enterocolitica* (Oliver, Jayarao, & Almeida, 2005; Schoeni & Wong, 2005). These pathogens have been linked to farms, feed, and storage conditions and are found in both pasteurized and unpasteurized milk (Goff & Griffiths, 2006; Oliver *et al.*, 2005).

The suitability of a detection method for routine diagnosis depends on several factors such as specificity, sensitivity, time required and applicability to large numbers of milk samples (Shet and Ferrieri 2004). PCR will be used to detect some common pathogenic bacteria in milk. Always one or two candidate genes will be selected from each bacterium for the PCR amplification. For example, (SLT) genes (Oberst *et al.*, 1998), *inv*A, *omp*C and *ori*C genes of *Salmonella* (Maciorowski *et al.*, 2005), and *mec*A, *nuc* and *ent*C genes of *staphylococcus* can be selected for the preliminary analysis (Leung *et al.*, 1999). PCR also provides valuable opportunities for quantifying bacteria in dairy environments. PCR is unmatched by other techniques in regard to its sensitivity and specificity (Glynn *et al.*, 2006).

Specific primers for PCR detection of *Staph. aureus* have been directed to the nuc gene, encoding the thermostable nuclease (Wilson *et al.*, 1991). Enterotoxigenic *Staph. aureus* cells could be detected in artificially contaminated dried skimmed milk samples at levels of lo5 CFU mL^{-1} within 8 h. The sensitivity of the PCR was low, the reason for which could be due to that the genomic DNA isolated from the dried skimmed milk contained some factors, which interfered with the reaction of PCR. Adesiyun *et al.* (1998) studied the prevalence of *Staph. aureus* in bulk milk collected from dairy farms. Mclauchlin *et al.* (2000) reported that the detection of *Staph. aureus* was less successful in three types of cheese and in cream because of food matrices factors. In this article a solvent extraction procedure was successfully modified for the extraction of *Staph. aureus* DNA, in which the interfering components including ions, protein, and fatty matter were eliminated.

The other major foodborne pathogen is *E.coli* O157:H7 are characterized by low infectious doses, 1-100 colony-forming units (Paton and Paton, 1998). Murinda *et al.* (2002b) reported the detection of *E. coli* O157:H7 from 8 of 30 (26.7%) dairy farms at different sampling times. *Salmonella enteric* Serotype typhimurium definitive type 104 (DT 104) is of particular concern to animal and public health agencies because of its multiple antibiotic resistance (Besser *et al.*, 1996). Six of 404 (1.5%) milk filters were positive for *Salmonella* spp.

It would be useful to have and establish a set of universal, sensitive and rapid PCR-based detection in

order to detect the major pathogenic bacteria in milk and its related dairy products. The aim of this study was developing a rapid, sensitive and reliable PCR-based method for specific detection of major pathogenic bacteria in milk and milk product samples.

MATERIALS AND METHODS

Bacterial strains and growth conditions:

120 isolated strains were used in this study. The strains were isolated from 35 dairy samples collected from farmer's vendors and dairy shops in Al-Ahasa area, KSA. Before they were subjected to PCR, strains were retrieved from frozen stock cultures and grown in adjusted media according to the strains i.e. Luria broth (LB) for *E.coli* and LB Baird-Parker+REF agar for *Staphylococcus* and brain heart infusion (BHI) broth (Difco Laboratories, Detroit) for *Sallmonella* at 37°C for 18 to 24 hrs. Stock cultures were maintained in 78 % glycerol at -20 °C during the experiment time.

Enrichment DNA preparation:

Dairy samples were collected from various dairy products i.e. samples of various cheeses: soft cheeses and sheep's cheese, sheep's milk, raw cow milk samples from various farms and other strains isolated from butter and fermented milk. A sample of 20 g or 10 ml of dairy samples was titrated and incubated in 225 ml of enrichment broth medium at elevated temperature for 24 and 48 h. For DNA isolation 1 ml of suspension after 24 hrs was necessary. Homogenate was centrifuged at 12.000 × *g* for 2 min and the supernatant was discarded. The pellet was resuspended in 100 µl of TE buffer (Sigma, Germany) and the whole process was repeated. Homogenate was incubated at 95 °C for 5-7 min. 2 µl of Proteinase K [20 mg/ml] (Promega, USA) were added to the homogenate after cooling and it was incubated at 55 °C for 2 h. Proteins were removed with a phenol-chloroform-isoamylalcohol [25:24:1] solution (Sigma, Germany). DNA was precipitated with ice-cold absolute ethanol at -20 °C for 4 h, centrifuged at 5.000 × *g* for 2 min. Pellet was dried and DNA was resuspended in 20 µl of sterile TE buffer.

DNA and Plasmid isolation:

Both total DNA and purified plasmid DNA were used in the PCR assays. Purified and plasmid DNA was prepared by using DNA isolation kit (QIAprep, Qiagen, Valencia,Calif) according to the manufacturer's instructions and bench protocol. Boiling methods was used to for DNA extraction as follows: ten milliliter of an overnight bacterial culture was centrifuged at 12,000 × *g* for 2 min (model Hermile Z233M-2, Germany). Pellets were resuspended in 100 µl of sterile distilled water, boiled for 10 min, and centrifuged as described above. A 5-µl aliquot was used as a template for PCR.

Specificity of PCR assays:

Oligonucleotide primers (Table 1) were designed in this study and synthesized by (Operon Biotecchnologies Cologne, Germany). Each 20-µl PCR mixture contained PCR master max (Qiagen-Germany), primers (0.25 µM each), and DNA template (5 µl, equivalent to approximately 10^7 CFU/ml). The final volume of the PCR mixture was adjusted to 20 µl with sterile distilled water. PCR were performed with DNA thermal cycler (myccler Thermal cycler, model 580BR11; Bio-Rad, USA) by using one cycle at 94°C for 4 min, followed by 30 cycles of 94°C forl min, 52°C for 1 min, and 72°C for 1.5 min and final extension at 72°C for 10 min for (*Staph. aureus*), by using one cycle at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec 60°C for 30 sec, and 74°C for 1 min and final extension at 74°C for 5 min (for *Salmonella*) and at 94°C for 1 min, followed by 30 cyclesv of 94°C for 1 sec 52°C for 30 sec, and 72°C for 1.5 min and final extension at 72°C for 10 min for (*E.coli*). In order to confirm the specificity of each primer set to its target gene, PCR amplifications were carried out as described above with the exception that only the primer set being tested was added to the reaction mix. The PCR amplicons were analyzed by gel electrophoresis on a 1% agarose (Promega, USA) gel in 1× TAE buffer (0.089 M Tris-acetat, 0.002 M EDTA; pH 8.0). The gel was stained with ethidium bromide and visualized with the Gel Doc System- Multi DOC-it-Imagaing system (UVP, LLC, UK).

Sensitivity of the PCR:

Trials were conduced to evaluate the lowest quantum of bacterial cells that would produce a visible signal by the PCR assay. The standard strains of (*Staphylococcus aureus* ATCC6538, *Salmonella typhemurium* ATCC 5007, *Eschrichia coli* ATCC10789 and *Ent. faecalis* ATCC29212) were used and adjusted to an approximate concentration 10^8 cells mL^{-1} based on spectrophotometer absorbance reading of 0.6 at 540nm and then ten fold serial dilution were made and subjected to PCR assay according to standardized protocol. The total viable count of the bacterial suspension was conformed by spread plate method.

Table (1): Oligonucleotide primers designed and used in this study

Strain	Primer	sequence	Product length, bp
Staphylococcus aureus	(*nuc*) Pri-1 Pri-2 (*nuc*)	5-GCG ATT GAT GGT GAT ACG GTT-3 5-AGC CAA GCC TTG ACG AAC TAA AGC-3	279
Salmonella typhemurium	HILA1 HILA2	5-CGA CGC GGA AGT TAA CGA AG-3 5-TCC TCC AAC TGA CCA GCC AT-3	972
Eschrichia coli	E.coli1 E.coli2	5-GCT TGA CAC TGA ACA TTG AG-3 5-CCA CTT ATC TCT TCC GCA TT-3	660

Comparison between the PCR and traditional detection methods of pathogenic bacteria in dairy products:

The methods of PCR and selective agar medium (Lb, Baird-Parker + RPF and BHI) were used to detect the 120 dairy products together [including 80 products of whole milk, 20 soft cheese products 10 products of butter and 10 products of labneh as fermented milk,which were purchased in the local supermarket. According to the results, the sensitivity rates of each were counted. The detection rate = the number of - positive sample/ (The number of positive samples + the number of negative samples).

RESULTS AND DISCUSSION

Primers specification:

The DNA of *Staph.aureus, Salmonella typhemurium* and *E.coli* could be amplified by using the specific primers, and other tested strains produced no reaction product (Fig. 1).Therefore, we could be proved that the primers were highly specific.

Detections by using PCR method:

In the tested samples, *Staph. aureus* could be detected by targeting the *nuc* gene (Fig.2a). But it was found that cheeses and butter had difficult matrices to be assayed by PCR because of the high fat content, which could affect the DNA extraction and PCR amplification. The products amplified with this pair of primers were relatively few. The detection rate of *Staph. aureus* in tested samples were 51.6 % However the detection of *Salmonella typhemurium* and *E.coli* were 5.0 and 84.2 % respectively.

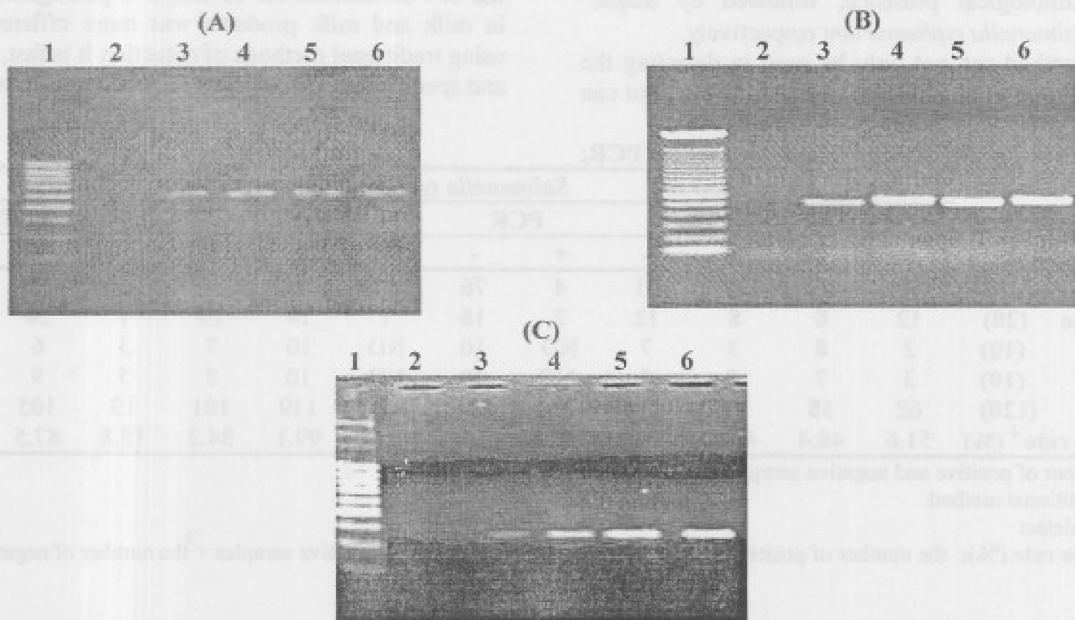

Fig. (1.a, b and c): Specificity of primer-based PCR assay for pathogenic bacteria in dairy product: gel electrophoresis of PCR products in 1% agarose in TBE buffer. (A) *Staph. aureus* strains; Lane 1, DNA ladder, lane 2, negative control *Ent. faecalis* ATCC2921, lane 3, positive control *Steaphylococus aureus* ATCC6538, lanes 4, 5 and 6, PCR products amplified from *Staph. aureus*- (279bp). (B) *Salmonella typhemurium* strains; Lane 1, DNA ladder; lane 2, negative control *Ent. faecalis* ATCC29212, lane 3, positive control *Salmonella typhemurium* ATCC5007, lanes 4, 5 and 6, PCR products amplified from *Salmonella typhemurium*- (972bp). (C) *E.coli* strains; Lane 1, DNA ladder, lane 2, negative control *Ent. faecalis* ATCC29212, lane 3, positive control *Eschrichia coli* ATCC10789), lanes 4, 5 and 6, PCR products amplified from *E.coli*- (660bp).

*This was only the choice of 6 reactions per strain for the display of total interactions.

Comparison between the PCR and traditional detection methods of pathogenic bacteria

The detection by using traditional method (selective medium) was also employed in the experiment, with parallel detection of pathogenic bacteria in dairy products samples. The sensitivity of the PCR was l00%. Data showed that, the detection rate for pathogenic bacteria depends on the type of microbial strain and type of dairy products. It could be concluded that PCR could detect the live cells and dead cells at the same time (Table 2). The PCR amplifying detection needed from 3-4 hrs; thus, it was rapid, easy to handle, sensitive, and specific.

DISCUSSION

Lantz *et al,* (1998) established a multiplex PCR assay to detect viable pathogenic strains of *Yersinia enterocolitica* in the samples and resulted even at low microbial count. This method used enrichment media to amplify cells. Jinneman *et al.* (1995) also employed multiplex PCR to identify *E.coli* 0157:H7 but before detection, a process of enriched strains was designed.

In this research work, the developed methodology of extracting DNA allows detection of pathogenic bacteria in dairy products in less than 4 h without strain enrichment. The detection and sensitivity of the PCR method is very high for *E.coli* 84.2, and 51.6 and 5.0 for *Staph. aureus* and *Salmonella typhemurium* respectively for the positive samples. However the rates of detection by using the traditional method was 45.8, 0.8 and 87.5 for *Staph.aureus*, *Salmonella typhemurium* and *E.coli* respectively. In some samples caused by the use of the PCR method gave the results of this conflicting with the results obtained using the traditional methods even results were negative or positive in terms of the presence of pathogenic bacteria (Table 2).

Confirmed test results by using the PCR and the traditional methods that the *E.coli* strain was more breeds pathological presence, followed by *Staph. aureus*, *Salmonella typhemurium* respectively.

This method can not only be used in detecting the contaminated dairy food by pathogenic bacteria, but can also be used to trace the pathogenic bacteria, before and after monitoring the critical control points during food processing in the factory. However, Publications on the application of PCR detection of pathogenic bacteria in food are very scarce. In fact, PCR detection is not of currently used in food microbiological analysis, probably because there is still a lack of standardized criteria for validation of PCR sample preparation methods, reaction components and assembly, as well as amplification conditions for pathogens and various food matrices. Following the development of PCR technology, the advantage will appear in practice.

Sensitivity and efficiency of the PCR test also adopted on the type of microbial strain, reaching the highest sensitivity to detect in the case of *E.coli*, while reached its lowest rate in both *Staph. aureus*, *Salmonella typhemurium* strains, respectively. Generally use of PCR method for detection of pathogenic bacteria in milk and milk products was more efficiently than using traditional methods of detection It is fast, sensitive and specific tool for microbiological applications.

Table (2): The sensitivity and detection rates of PCR:

Types of samples		*Staphylococcus.aureus*				*Salmonella typhemurium*				*E.coli*			
		PCR		TM[b]		PCR		TM		PCR		TM	
		+	-	+	-	+	-	+	-	+	-	+	-
Raw milk	**(80)**	45	35	39	41	4	76	ND[c]	80	70	10	70	10
Soft cheese	**(20)**	12	8	8	12	2	18	1	19	19	1	20	0
Butter	**(10)**	2	8	3	7	ND	10	ND	10	7	3	6	4
Labneh	**(10)**	3	7	5	5	ND	10	ND	10	5	5	9	1
Total	**(120)**	62	58	55	65	6	114	1	119	101	19	105	15
Detection rate[d] (%)		51.6	48.4	45.8	54.1	5.0	95	0.8	99.1	84.2	15.8	87.5	12.5

a: +,-: number of positive and negative samples
b: TM: traditional method
c: ND: not detect
d: Detection rate (%): the number of positive sample/ The number total samples (positive samples + the number of negative samples).

CONCLUSIONS

Genomics information provides an important asset to improve technologies for quantification, detection, and identification of bacteria in the dairy products. PCR has more advantages than other traditional bacteriological methods for the detection of pathogenic bacteria in terms of its rapidness, sensitivity and manipulation. Some factors existed in milk together with the PCR system should be considered to develop the rapid detection of pathogenic bacteria in dairy production.

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REFERENCES

Besser D., P. Verde, Y. Nagamine and F. Blasi (1996). Signal transduction and the uPA/uPAR system. Fibrinolysis, 10, 215-237.

Bickley J., J. K. Short, D. G. Macdoell and H. C. Parkes (1996). Polymerase chain reaction (PCR) detection of *Listeria monocytogenes* in diluted milk and reversal of PCR inhibition caused by calcium ions. Lett. Appl. Microbiol. 22, 153-158.

Brodeur B. R., M. Boyer, I. Charlebois, J. Hamel, F. Couture, C. R. Riouk and D. Martin (2000). Identification of group B streptococcal Sip protein, which elicits cross-protective immunity Infect. Immun. 68, 5610-5618.

Chye F. Y., A. Abdullah and M. K. Ayob (2004). Bacteriological quality and safety detection of foodborne *Salmonella* spp. in animal feeds. Critical endocarditis and a simple scheme to identify clumping factor-positive Environmental Microbiology. 64, 3389-96.

Ferretti A., C. Corradini and E. Serpagli (1998). The Silurian and Devonian sequence in SW Sardinia. In: Serpagli (Ed.), Guidebook, ECOS VII. Giornale di Geologia, 60, 57-61.

Forsman P., A. Tilsala and T. Alatossav (1997). Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S–23S rRNA spacer regions. Microbiology 143, 3491-3500.

Glaser P., C. Rusniok, C. Buchrieser, F. Chevalier, L. Frangeul, T. Msadek, M. Zouine, E. Couve, L. Lalioui and C. Poyart (2002). Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. Mol. Microbiol, 45, 1499-1513.

Glynn, B., S. Lahiff, M. Wernecke, T. Barry, T. J. Smith and M. Maher, M. (2006). Current and emerging molecular diagnostic technologies applicable to bacterial food safety. International Journal of Dairy Technology, 59, 126-139.

Goff, H. D. and M. W. Griffiths (2006). Major advances in fresh milk and milk products: Fluid milk products and frozen desserts. Journal of Dairy Science, 89, 1163-1173.

Jinneman K. C., P. A. Trost, W. E. Hill, S. D. Weagant, J. L. Bryant, C. A. Kaysner and M. M. Wekell (1995). Comparison of template preparation methods from foods for amplification of *Escherichia coli* 0,,, shiga-like toxins type I and 11 DNA by multiplex polymerase chain reaction. Journal of Food Protection, 58, 722-726.

Ke D.and M. G. Bergron (2001). Molecular methods for rapid detection of group *B streptococci*. Expert Rev.Mol.Diagn, 1, 175-181.

Lantz P. G, R. Knutsson, Y. Blixt, W. A. Al-Soud, E. Borch and P. Wstrom (1998). Detection of pathogenic *Yersiniu enterocolitica* in enrichment media and pork by a multiplex PCR: a study of sample preparation and PCR-inhibitory components. International Journal of Food Microbiology, 45, 93-105.

Leung M. J, N. Nuttall and M. Mazur (1999). Case of *Staphylococcus schleiferi* endocarditis and a simple scheme to identify clumping factor-positive staphylococci. Journal of Clinical Microbiology, 37, 3353-3356.

Lindahl G., M. Stalhammar-Carlemalm and T. Areschoug (2005). Surface proteins of *Streptococcus agalactiae* and related proteins in other bacterial pathogens. Clin.Microbiol.Rev. 18, 102-127.

Maciorowski K. G, S. D. Pillai and F. T. Jones (2005). Identifying *Streptococcus suis* using PCR. Vet.Med, 61, 522-525.

Mclauchlin J, G. L. Narayanan, V. Mithani and G. O'Neill (2000). The detection of enterotoxins and toxic shocsyndrome toxin genes in *Staphylococcus aureus* by polymerase chain reaction. Journal of Food Protection, 63, 479-488.

Murinda, S. E., L. T. Nguyen, S. J. Ivey, B. E. Gillespie, R. A. Almeida, F. A. Draughon and S. P. Oliver (2002*b*). Prevalence and molecular characterization of Escherichia coli O157:H7 in bulk tank milk and fecal samples from cull cows: a 12-month survey of dairy farms in east Tennessee. J. Food Prot. 65, 752-759.

Oberst R. D., M. P. Hays and L. K. Bohra (1998). PCR-based DNA amplification of raw milk in Malaysia. Food Microbial. 21, 535-541.

Oliver, S. P., B. M. Jayarao and R. A. Almeida (2005). Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathogens and Disease, 2, 15-129.

Paton A. W. and J. C. Paton (1998). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. Journal of Clinical Microbiology. 2, 598 - 602.

Schoeni, J. L. and A. C. Wong (2005). *Bacillus cereus* food poisoning and its toxins. Journal of Food Protection, 68, 636-648.

Shet A. and P. Ferrieri (2004). Neonatal group B streptococcal infections: clinical, microbiological, therapeutic and preventative aspects. The Indian Journal of Medical Research. 9, 141-150.

Tettelin H., Nelson., I. T. Paulsen, J. A. Eisen, T. D. Read, S. Petrson, J. Hidelberg, R. T. Deboy, D. H. Hafat, R. J. Dodson, A. S. Durrkin and C. M. Fraser (2001).Complete genome sequence of a virulent isolate of *Streptococcus pneumoniae*. Science, 293, 498–506.

Tettelin H., V. Masignani, M. J. Cieslewicz, J. A. Eisen, S. Petrson, M. R. Wessels, V. Masignani, M. J. Cieslewicz and C. M. Fraser (2002).Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V *Streptococcus agalactiae*. Proc. Nat.Acad.Sci.USA, 99, 12391-12396.

Wilson I. G., J. E. Cooper and A. Gilmour (1991). Detection of enterotoxigeic *Staphylococcus aureus* in dried skimed milk: Use of the polymerase chain reaction for amplication and detection of staphlococcal enterotoxin genes *ent B* and *entC* and the thermonuclease gene nuc. Applied and Environmental Microbiology, 57, 1793-1798.

الكشف عن البكتريا الممرضة فى الحليب ومنتجاته باستخدام تقنية الـ PCR

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الكشف عن البكتريا المرضية الملوثة للبن ومنتجاته تعتبر من أهم المقاييس المستخدمة في الكشف عن سلامة وجودة اللبن ومنتجاته. ومن أهم الاتجاهات البحثية الحديثة هو محاوله إيجاد طريقه بسيطة، سريعة ودقيقة للكشف عن صحة وسلامة الأغذية وذلك باستخدام تقنية تفاعل البلمرة المتسلسل الـ PCR. في البحث تم استخدام طريقه الـ PCR وفى وجود بريمر عام وأخر متخصص في الكشف عن البكتريا الممرضة المتوقع وجودها في اللبن ومنتجاته. أكدت النتائج المتحصل عليها من فحص ١٢٠ عينة من اللبن الخام وبعض المنتجات اللبنية الأخرى التي جمعت من منطقه الإحساء إن هناك ثلاثة سلالات رئيسيه مسئولة عن تلوث اللبن ومنتجاته وهى:(*Staphylococcus aureus*, *Salmonella typhemurium* and *Escherichia coli*). تم استخدام بريمر متخصص في تفاعل البلمرة المتسلسل واستخدام ثلاث جينات محددة في الكشف عن السلالات الثلاثة. وكان حجم ناتج تفاعل الـ PCR المتحصل عليه نتيجة عملية التضاعف هو ٢٧٩ ، ٩٧٢ و ٦٦٠ بازبار (bp)، للسلالات الثلاث على التوالي.

أظهرت النتائج أن استخدام تفاعل البلمرة المتسلسل كان له أعلى معدل في عملية الكشف مقارنة بالطرق التقليدية وكانت نسبة العينات الموجبة الملوثة بالسلالات الثلاث هي ٥١,٦،٦٥,٠٠ و ٨٤,٢ % للسلالات الثلاث على التوالي (*Staphylococcus aureus*, *Salmonella typhemurium* and *Escherichia coli*). أكدت النتائج على إن معدل الكشف عن البكتريا الممرضة اعتمد على نوع الميكروب وكذلك نوع العينة المختبرة.استخدام تقنيه الـ PCR في الكشف كان أكثر دقة وحساسية عن استخدام الطريقة التقليدية،حيث أعطت أحيانا نتائج مخالفة للمتحصل عليها باستخدام الطريقة التقليدية. كذلك أظهرت النتائج إن بكتريا *Escherichia coli* هي كانت أكثر البكتريا الممرضة تواجدا يليها بكتريا *Staphylococcus aureus* و أخيرا *Salmonella typhemurium* على الترتيب.

عموما نوصى باستخدام طريقة تفاعل البلمرة المتسلسل في الكشف عن البكتريا الممرضة في اللبن ومنتجاته امتازت الطريقة بالسرعة والدقة والفعالية وذلك عند مقارنتها بالطرق التقليدية المستخدمة في الكشف عن تلوث اللبن ومنتجاته بالبكتريا الممرضة.