PCR based Detection of Food Borne Pathogens

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Abstract**—**Many high-risk pathogens that cause disease in humans are transmitted through various food items. Food-borne disease constitutes a major public health problem. Assessment of the quality and safety of foods is important in human health. Rapid and easy detection of pathogenic organisms will facilitate precautionary measures to maintain healthy food. The Polymerase Chain Reaction (PCR) is a handy tool for rapid detection of low numbers of bacteria. We have designed gene specific primers for most common food borne pathogens such as *Staphylococci*, *Salmonella* and *E.coli*. Bacteria were isolated from food samples of various food outlets and identified using gene specific PCRs. We identified *Staphylococci*, *Salmonella* and *E.coli* O157 using gene specific primers by rapid and direct PCR technique in various food samples. This study helps us in getting a complete picture of the various pathogens that threaten to cause and spread food borne diseases and it would also enable establishment of a routine procedure and methodology for rapid identification of food borne bacteria using the rapid technique of direct PCR. This study will also enable us to judge the efficiency of present food safety steps taken by food manufacturers and exporters.

Keywords**—**food borne pathogens, PCR, food safety, rapid detection.

I. Introduction

RAPID detection of pathogenic organisms that cause food-borne illness is needed to ensure food safety. Even with improved methods for detecting pathogens in foods and environmental samples, microbiologists often face a "needle-in-a-haystack" challenge [1]. It is very difficult to detect small numbers of food-borne pathogens amid large numbers of harmless background microflora in a complex sample matrix. Traditional culture techniques for direct isolation and identification of food-borne pathogens in food samples in poisoning outbreaks are time-consuming and laborious; therefore, efforts have been made to reduce the time required to identify these pathogens [2]. Moreover elaborate DNA extraction methods also need to be subverted. The aim of our investigation is to use direct PCR from the food samples as template and also single colonies isolated from the samples for the gene specific PCRs. Using the gene specific PCRs, we have been able to identify pathogens directly from food samples This is a rapid and effective method to look for pathogenic bacteria in food samples, and it also serves as a measure to assess the quality of food packaging and storage.

II. MATERIALS AND METHODS

Bacterial cultures isolation and purification:

Food samples were obtained from various supermarkets and hypermarkets, restaurants and cafeterias located in the UAE. The samples used in the present study were frozen green peas, chocolates, condensed milk, chicken puff, cottage cheese and chicken sandwich. The samples were all collected from the outlets within the time period of expiry. All samples were homogenized with a homogenizer in 5 ml sterile water. To prevent microbial contamination from one sample to the other, the homogenizer was rinsed with alcohol. The samples were treated with Triton X 100 buffer at 95 C for 30 mins, centrifuged and then the supernatant was used for PCR as well as serial dilutions for plating on growth media. Nomenclature of the isolates from the various samples is indicated in Table 1.

After 3 rounds of purification, 15 isolates were obtained as pure cultures. Glycerol stocks of these cultures were maintained. The isolates were named based on the food samples that served as the source.

Gene specific PCR

PCR was carried out using primers used in previous studies. The primer sequences and PCR conditions are summarized in Tables 2 and 3. Multiplex PCR was carried out initially with the food samples directly. Each amplification in sterile thin-walled PCR tubes comprised DNA template 50 ng/µl, 75 pmol of each primer, 50 µM (each) deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 10× buffer (Vivantis), 1.0 Unit of Taq DNA polymerase (Vivantis) and 2.5 mM $MgCl_2$. Sterile DNAse free water was added to a final volume of 50µl. Isolated single colonies from the various samples obtained after serial dilution and plating were then subjected to colony PCR (Techne 512 thermal cycler) for detection of each of the pathogens. Colonies were picked from pure culture plates and boiled in 100 µl of 1% Triton X-100 buffer at 95 C for 10 mins, centrifuged at 10,000 rpm for 5 mins and 10 µl of the supernatant served as the template [3]

TABLE I
NOMENCLATURE OF FOOD SAMPLES

S.No	Sample	Symbol	Colony Annotation
1	GREEN PEAS	GP	GPa1/GPb1,….GPa10/GPb10
2	CHOCOLATE	CH	CHa1/CHb1,...CHa10/CHb10
3	CONDENSED MILK	RM	RMa1/RMb1,…RMa10/RMb10
4	CHICKEN PUFF	CP	CPa1/CPb1,....CPa10/CPb10
5	PANEER	PA	PAa1/PAb1,…..PAa10/PAb10
6	CHICKEN SANDWICH	CS	CSa1/CSb1,…..CSa10/CSb10

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TABLE II
LIST OF PRIMERS USED IN THE STUDY

Gene	Genus	Primer	Sequence
Fimbriae [3]	Salmonella	fim FP	5' CCT TTC TCC ATC GTC CTG AA 3'
		fim RP	5' TGG TGT TAT CTG CCT GAC CA 3'
Afa [3]	Pathogenic E.coli	Afa FP	5' GCT GGG CAG CAA ACT GAT AAC TCT C 3'
		Afa RP	5' CAT CAA GCT GTT TGT TCG TCC GCC G 3'
Coagulase [4]	Staphylococcus aureus	Coa FP	5′ATA GAG ATG CTG GTA CAG G3′
		Coa RP	5′GCT TCC GAT TGT TCG ATG C3′
Methicillin Resistance [5]	Staphylococcus	Mec FP	5′AAAATCGATGGTAAAG GTTGGC 3'
		Mec RP	5′AGTTCTGCAGTACCGG ATTTGC 3'
Shiga toxin [6]	E.coli O157	Stx 1 FP	5'CAGTTAATGTGGTGGC GAAG 3'
		Stx 1 RP	5'CTGTCACAGTAACAAA CCGT 3'

TABLE III
PCR CONDITIONS FOR VARIOUS GENES

Gene	Denaturation	Annealing	Extension
Fimbriae	94 °C	56 °C	72 °C
	1min	30 sec	1min
afa	94 °C	65°C	72 °C
	1min	1 min	1min
Coagulase	94 °C	56 °C	72 °C
	1min	30 sec	1min
Methicillin	94 °C	56 °C	72 °C
resistance	1min	30 sec	1min
Shiga toxin	94 °C	65°C	72 °C
	1min	1 min	1min

Final extension was at 72°C for 4 mins, followed by cooling at 4°C for 30 cycles for all PCR reactions

III. RESULTS

Multiplex PCR from the food samples revealed the presence of pathogenic *Staphylococcus aureus*, *E.coli* O157 and *Salmonella* in most of the samples, showing a series of bands corresponding to the genes amplified (Fig 1). In order to characterize the bacteria in each of the samples, serial dilution and plating was done and individual colonies were used for PCR to detect the pathogens based on gene specific PCRs. It was found that chicken sandwich and chocolate samples predominantly contained *Staphylococcus aureus* as observed by the 500- 600 bp coagulase gene product (Fig 2). All the Staphylococcus strains also turned out to be methicillin resistant MRSA as seen by amplification of the mecA gene product of ~ 600 bp (Fig 3). Chicken sandwich and chicken puff also contained *Salmonella* as seen by the 120 bp fimbrial gene amplified from cultures isolated from these samples (Fig 4). Colonies isolated from green peas, chocolates and condensed milk answered positive for afa gene product of pathogenic *E.coli* showing a product of ~400 bp (Fig 5). These samples were then subjected to PCR for stx to confirm if they were *E.coli* O157. It was found that some of the pathogenic *E.coli* were positive for the shiga toxin gene product of ~480 bp (Fig 6).

Fig 1: Multiplex PCR of food samples.
Lane 1 : Marker – 100 bp ladder
Lane 2 : Chocolate
Lane 3 : Green peas
Lane 4 : Chicken sandwich
Lane 5: Condensed milk
Lane 6 : Chicken Puff
Lane 7 : Negative control

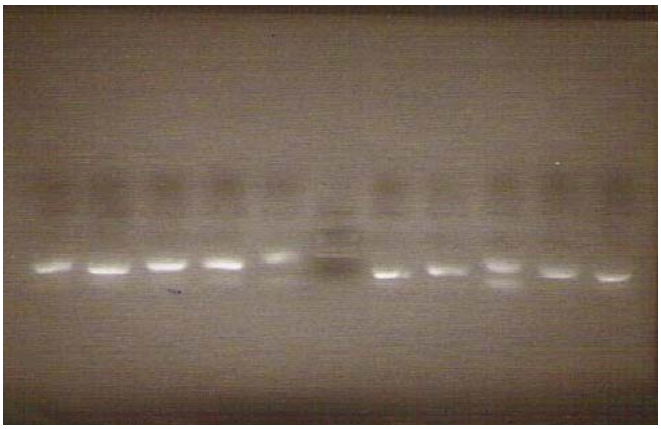

Fig 2: Coagulase PCR
Lane-1: CSa1
Lane-2: CSa2
Lane-3: CSa3
Lane-4: CSa4
Lane-5: CSa5
Lane-6: Marker -λEcoRI/HindIII
Lane-7: CH a1
Lane-8: CH a2
Lane-9: CH a4
Lane-10: CH a5
Lane -11: CH b5

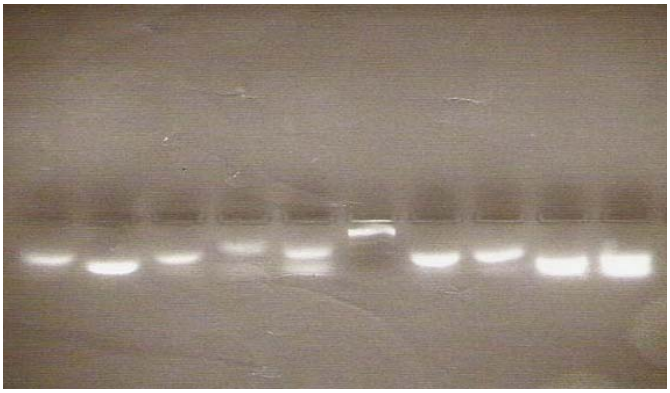

Fig 3:Coagulase positive strains tested for mecA

Lane-1: CSa1
Lane-2: CSa2
Lane-3: CSa3
Lane-4: CSa4
Lane-5: CSa5
Lane -11: CH b5
Lane-6: Marker -λEcoRI/HindIII
Lane-7: CH a1
Lane-8: CH a2
Lane-9: CH a4
Lane-10: CH a5

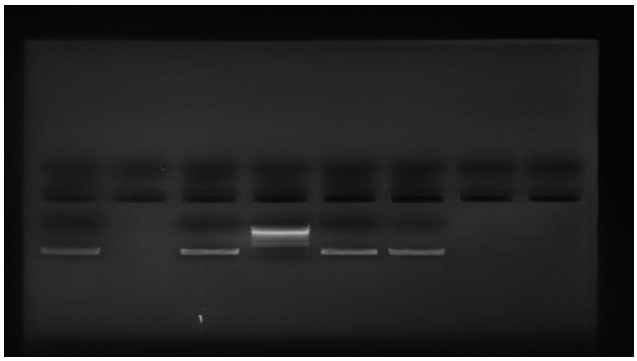

Fig 4: PCR for fim gene of Salmonella

Lane1: Cpa1
Lane2: Cpa3
Lane3: Cpa4
Lane 4: Marker – 100 bp ladder
Lane5: Cpa5
Lane6: Cpb6

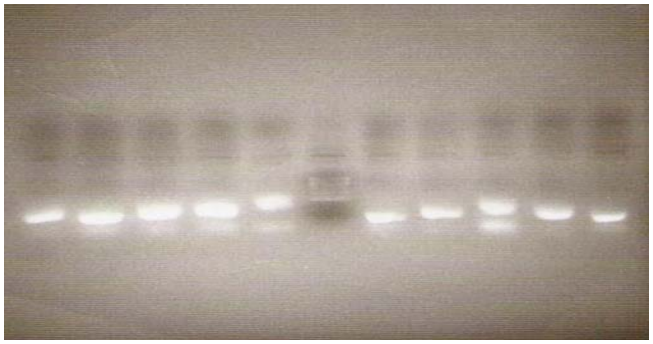

Fig 5 : PCR for afa gene of pathogenic E.coli

Lane-1: GPa1
Lane-2: GPa2
Lane-3: CHb6
Lane-4: CPa3
Lane-5: RMa2
Lane-6: Marker -λEcoRI/HindIII
Lane-7: PAa3
Lane-8: PAb4
Lane-9: PAb5
Lane-10: PAb6

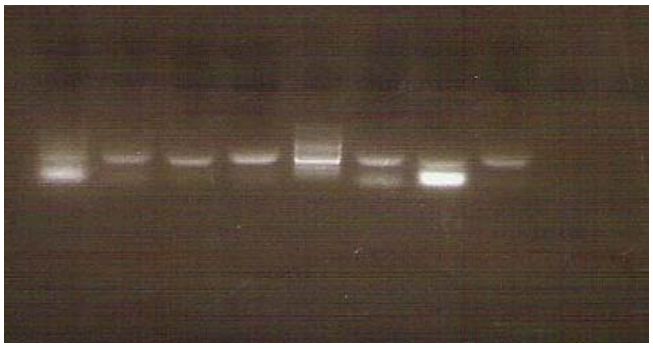

Fig 6: PCR to check if the afa positive E.coli were stx positive for E.coli O157

Lane-1: GPa1
Lane-2: GPa2
Lane-3: CHb6
Lane-4: CPa3
Lane-5: Marker-100 bp ladder
Lane-6: RMa2
Lane-7: PAa3
Lane-8: PAb4
Lane-9: PAb5

IV. DISCUSSION

The multiplex PCR conducted was the first step in directly indicating the presence of pathogenic bacteria in the food samples. The PCR showed amplification of the genus specific genes. Serial dilution and plating was done to further characterize the bacteria in each of the samples. It is striking to note that viable colonies were obtained despite treating the food samples with Triton X-100 at 95 C for 30 mins, indicating that these bacteria are quite robust. An important factor to note is that samples such as chicken puff, sandwich, condensed milk and chocolates are ready to eat food stuff that are generally not subjected to further cooking conditions. The fact that ready to eat food on being subjected to conditions as mentioned above still yielded viable pathogenic bacteria is definitely reason for concern. This study actually leads to giving a serious thought to the food safety measures in both packing as well as storage aspects, the important factor here being that all the food samples analyzed were well within the expiry date. This study also shows us how useful the direct PCR can be in rapid detection and identification of pathogenic bacteria. This technique avoids time consuming and cumbersome methods of DNA extraction and inspires a new outlook towards better food safety and quality assurance standards. Inclusion of the direct PCR as a baseline technique in food manufacturing and packaging units would definitely increase the standards of food safety.

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