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PCR Primers Designed from the *UidA* Gene Sequence for the Detection of *Escherichia coli* O157:H7

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Abstract: One of the uniqueness of *Escherichia coli* O157:H7 is it inability to ferment sorbitol in 24 h and its negative test with the MUG assay, although this organism carried the *Uid*A gene which encodes for β-glucuronidase in its chromosome. Primers were designed based on the sequence of the β-glucuronidase gene and were evaluated in a polymerase chain reaction assays as a marker to detect *E. coli* O157:H7. Of the three pairs of primers tested, which produces the estimated product size of 352, 271 and 353 bp respectively, one primer pairs (UidA2F & UidA2R) was found to be more specific and useful as a marker in combination with a published primer for the H7 gene for the detection of *Escherichia coli* O157:H7.

Key words: *Escherichia coli* O157:H7, β-glucuronidase, polymerase chain reaction

Introduction

Escherichia coli O157:H7 is a serotype of enterohemorrhagic *Escherichia coli* (EHEC), which does not receive much attention in research until two major outbreaks of bloody diarrhea in human occur in Michigan and Oregon in the year 1982. *Escherichia coli* O157:H7, is verocytotoxin-producing microorganism because of its ability to produce toxic on the Vero cells of its host. Unlike other *E. coli*, *E. coli* O157:H7 isolates do not ferment sorbitol in 24 h (Farmer and Davis, 1985) and are tested negative for 4-methylumbelliferone-β-glucuronide (MUG) assay (Doyle and Schoeni, 1984; Thompson *et al.*, 1990), which measure for the β-glucuronidase activity in the *E. coli* (Feng and Hartmen, 1982). Therefore, *E. coli* O157:H7 isolates in the foods and clinical samples can be only identified by differential selection on the sorbitol-MacConkey agar (March and Ratnam, 1986), testing the resultant non-sorbitol fermenting colonies with the MUG assay and confirming the MUG-negative isolates with O157 and H7 antisera (Todd *et al.*, 1988; Padhye and Doyle, 1992) which usually consumed a lot of time. The objective of this study was to design primers based on the known sequence of the β-glucuronidase gene and to evaluate the primer pairs in PCR assays for the detection of *E. coli* O157:H7.

Materials and Methods

Bacterial Strains: The *Escherichia coli* O157:H7 and other bacterial strains used in this study were as listed in Table 1.

Primer Designing: The non-functional β-glucuronidase sequence was downloaded from http://www.ncbi.nlm.nih.gov. The sequence was then copied into Primer Premier version 5.0 (a primer designing software) by Premier Biosoft International to design the primers. The three pairs of primers designed for the purpose of this study were: UidA1F, 5'GTTGAACTGCGTGATGCG-3' and UidA1R, 5-TGCGAGGTACGGTAGGAG-3; UidA2F, 5'-TGTTGACTGGCAGGTGGTG-3' and UidA2R, 5'-TAAAGTAGAACGGTTTGTGG-3'; UidA3F, 5'GTGATGCGGATACCACGG-3' and UidA3R, 5'-CGTAAGGGTAATGCGAGG-3'. The estimated amplicon from these primer pairs were 352, 271 and 353 bp, respectively. Primer pair for the H7 gene (5'-GCGCTGTCGAGTTCTATCGAGC-3' and 5'-CAACGGTGACTTTATCGCCATTCC-3') was as described by Gannon *et al.* (1997).

Genomic DNA Extraction: Genomic DNA extraction was carried out following closely the method described by Ausubel *et al.* (1987). 1.5 ml of the overnight bacterial culture was poured into the 1.5 ml eppendorf tube and was spun at 10000 rpm for 1 min in a microcentrifuge (Eppendorf centrifuge 54150). The supernatant was then discarded and 700 μl of GET buffer (50 mM/l Glucose, 25 mM/l Tris-HCl and 10 mM/l EDTA) was added and was mixed well by using a vortex mixer. 20 μl of 10% SDS and 5 μl of proteinase K (Sigma) were added to the 1.5 ml tube and was incubated at 60°C in a water bath for 20 min. 500 μl of PCI (Phenol-Chloroform-Isoamyl) in the ratio of 25:24:1 respectively was then added and was spun at 12000 rpm for 1 min. 200 μl of the top layer was pipetted out and was transferred into a new 1.5 ml microfuge tube. 200 μl of 3M K-Ac and 400 μl of cold

Table 1: *Escherichia coli* O157:H7 and other bacterial strains used in this study

Bacterial strain	Amplification of the expected product using primer pairs of: UidA1F/R[a] (352 bp)	UidA2F/R[a] (271 bp)	UidA3F/R[a] (353 bp)	H7[b] (625 bp)
E. coli O157:H7 EDL933 (positive control)	+	+	+	+
EPEC strain I	-	-	-	-
E. coli O157 non-H7	+	-	+	-
Morganella morganii	-	-	-	-
Morganella catarrhalis	-	-	-	-
Shigella sonnei	-	-	-	-
Salmonella typhi	-	-	-	-
Serratia marcescens	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Proteus vulgaris	-	-	-	-
Escherichia coli O157:H7 (positive control)	+	+	+	+
Klebsiella pneumoniae	-	-	-	-
Vibrio cholerae	-	-	-	-
Enterococcus faecalis	-	-	-	-

[a] This study
[b] Primer pair for the H7 gene was as described by Gannon *et al.*(1997)

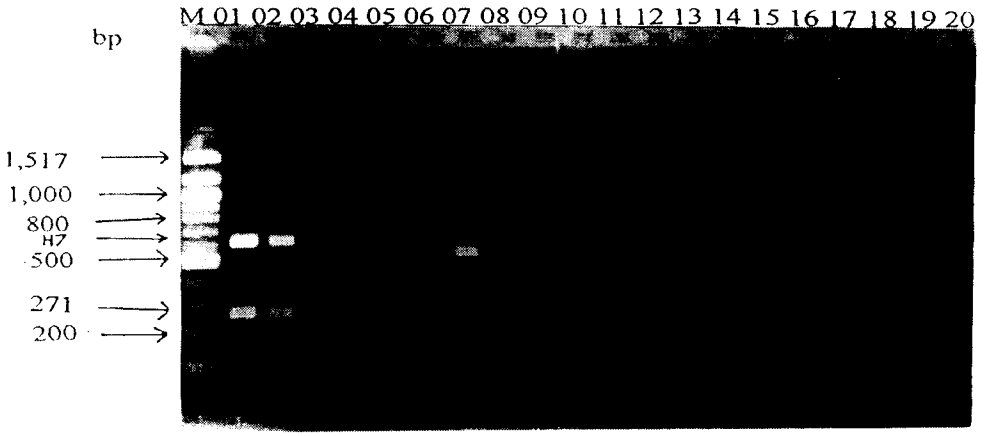

Fig.1: Specific PCR analysis using primer *UIDA* 2. Lanes 1, 2 and 14 = *E. coli* O157:H7; lanes 3, 4, 5, 6 and 17, *E. coli* non-O157:H7; lane 7, *Morganella morganii*; lane 8, *Shigella sonnei*; lane 9, *Morganella catarrhalis*; lane 10, *Salmonella* Typhi; lane 11, *Serratia marcescens*; lane 12, *Pseudomonas aeruginosa*; lane 13, *Proteus vulgaris*; lane 15, *Klebsiella pneumoniae*; lane 16, *E. coli* V517; lane 18, *Vibrio cholerae*; lane 19, *Enterococcus faecalis*; lane 20, negative control; and lane M, 100 bp marker

isopropanol was added and was spun at 12000 rpm for 7 min. After discarding the supernatant, 500 µl of 70% cold ethanol was added and spun at 12000 rpm for 5 min. The supernatant was discarded and the tube was left to dry before adding 30 µl of TE buffer. The concentration of the genomic DNA was then estimated by using a DNA marker in agarose gel.

Specific Polymerase Chain Reaction: Each 0.2 ml tube contained 16.2 µl of sdH_2O, 2.5 µl of 10x PCR buffer (Promega), 1.5 µl of $MgCl_2$ (Promega), 0.5 µl of dNTP's (Promega), 0.3 µl of *Taq* polymerase (Promega), 1.0 µl of both forward and reverse primer (Research Biolabs) respectively, and 2.0 µl (20-30 ng) of genomic DNA. The reaction tube was then put into Eppendorf Mastercycle PCR machine which has been programmed to preheat at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 1 min before a final extension at 72°C for 7 min. The PCR product was electrophoresed in

1.7 % agarose gel with a 100 bp DNA ladder (Research Biolabs) used as a molecular size marker. The gel was then stained with ethidium bromide and was then photographed under UV illumination.

Results and Discussion

In vitro amplification of nucleic acids using polymerase chain reaction (PCR) is now one of the most powerful diagnostic tool for the analysis of microbial infections as well as for the analysis of microorganisms in food or the environments. In this study, primer pairs designed based on the published sequence of the β-glucuronidase gene of *Escherichia coli* were evaluated for the detection of *Escherichia coli* O157:H7. From the results obtained, the amplified product size produce from *Escherichia coli* O157:H7 by each primer pairs were the same as the size estimated during the process of designing the primers. Product size for primer pairs of UidA1, UidA2 and UidA3 were 352, 271 and 353 bp, respectively. However, primer pair of UidA2 was considered as more specific due to the high intensity and clarity of the amplified fragment compared to those of the UidA1 and UidA3 primer pairs. With the exception of the *Shigella sonnei* strain, which produced an amplicon of almost similar in size with that of the *E. coli* O157:H7 (Fig. 1, lane 8; maybe due to sequence homology between *E. coli* O157:H7 and *Shigella* spp.), none of the other bacterial species tested produced the expected amplicon. Extensive sequence homology in the 16 rRNAs of *E. coli*, *E. fergusonii* and *Shigella* spp. has been reported by Brenner (1984). Brenner also reported that DNA relatedness between *E. coli* and *Shigella* spp. were 70-100%. Hsu and Tsen (2001) reported that they found sequence homology between five *Shigella* spp. that they used in their experiment with *E. coli* with the percentage of homology of about 98.35% and higher.

The availability of a rapid, reliable and cost effective test systems to detect the presence or absence of contaminating pathogens has becomes increasingly more important for the food industry. In the normal traditional and standardized analysis of food, the detection for the presence of bacteria relies on the enrichment and isolation of presumptive colonies on solid media, followed by biochemical and/or serological identification. The introduction of the polymerase chain reaction (PCR) in microbial diagnostics has been established in research laboratories as a valuable alternative to traditional detection methods. In this study, primers were constructed from the *Uid*A gene (β-glucuronidase sequence). *Uid*A gene has some conserved sequences that are unique to *E. coli* O157:H7 serotype only (Feng and Lample, 1994 and Cebula *et al.*, 1995). The specificity of the primer pairs for the detection of *E. coli* O157:H7 were verified by using them in combination with published primer pairs for the H7 gene of *E. coli* O157:H7. The specificity of the primer pair of UidA2 was further evaluated by PCR assay of other closely related species of bacteria as shown in Table 1. The primer pair of UidA2 was able to discriminate between the *E. coli* O157:H7 with other *E. coli* and bacteria of other species tested. When combined with the primer for the H7 gene, the *E. coli* O157:H7 were able to be differentiated from all the other bacterial species used.

Conclusion

This study shows that the primer pair of UidA2 was able to detect *E. coli* O157:H7. Though the number of other bacterial species screened against the primer was low, the more encouraging results obtained suggest that PCR-based detection using the primer pair designed in combination with the H7 gene primers may be of some use in studying the epidemiology of this pathogen . Further studies are needed to detect target sequences in more bacterial species that could allow for a more efficient and specificity of the primer pair designed in the detection of *E. coli* O157:H7.

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