

# USING RAPD-PCR TECHNIQUE TO DEVELOP THE SPECIFIC PCR PRIMERS FOR CONSTRUCTING THE RAPID DETECTION SYSTEM OF ESCHERICHIA

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## ABSTRACT

ESCHERICHIA COLI HAS BEEN USED AS A MICROBIOLOGICAL INDICATOR FOR FECAL CONTAMINATION IN WATER OR FOODS FOR A LONG TIME. THUS, QUALITATIVE AND QUANTITATIVE DETECTION OF E. COLI CE -LLS IN WATER AND FOODS IS IMPORTANT. CURRENTLY, THE CONVENTIONAL AND MODIFIED METHODS BAS -ED ON SELECTIVE CULTIVATION FOR THE IDENTIFICATION OR ENUMERATION OF E.COLI ARE TIME-CONS -UMING AND THE RATES OF FALSE- POSITIVE ARE RELATIVELY HIGH.WHILE,METHODS OF DNA PROBE OR POLYMERASE CHAIN REACTION ( PCR ) FOR DETECTION OF E. COLI REPORTED WERE SHOWN POOR SPECIF -ICITY. RECENTLY, RAPD ( RANDOMLY AMPLIFIED POLYMORPHIC DNA ) PROFILE GENERATED BY ARBITRA -RILY PRIMED PCR HAS BEEN USED FOR RANDOM SCREENING DNA PROBES. THE PURPOSES OF THIS STUDY ARE TO USE SUCH RAPD-PCR TECHNIQUE FOR DEVELOPING DNA PROBES SPECIFIC TO E.COLI.WHEN OPQ5 OPQ4 AND U69 WERE SEPARATELY USED AS PCR PRIMERS, 240 BP AND 1675 BP RAPD FRAGMENTS,WHICH WERE ABSENT IN RAPD PROFILES OF NON-E. COLI STRAINS, WERE GENERATED AND NAMED AS Q54E01AND U69E3. WHEN PRIMER OPQ3, U76 AND U82 WERE USED, 456 BP, 404 BP AND 506 BP RAPD FRAGMENTS, WHICH WERE PRESENT ONLY IN PROFILE OF EHEC, WERE CHOSEN AND NAMED AS Q3H01,U76H15 AND U82H -01 SEQUENTIALLY.THESE RAPD FRAGMENTS WERE THEN SUBJECTED TO SPECIFICITY EVALUATION THROUG -H BLOT HYBRIDIZATION.THE RESULTS SHOW THE DETECTION RATE OF E.COLI STRAINS WAS 58.8 % AND 100 % WHEN Q54E01 AND U69E3 FRAGMENTS USED AS PROBES,RESPECTIVELY.BUT BOTH ALSO SHOW THE C -ROSS REACTION FOR SOME STRAINS OF SHIGELLA.THE RESULTS ARISED FROM U76H15 AND U82H01 FRAG -MENTS WERE SIMILAR TO THAT OF U69E3.IN OTHER HAND,Q3H01 FRAGMENT WAS PROVED TO BE SPECIFI -C FOR SEROTYPE O157:H7 AND O157:NM BY DOT BLOT HYBRIDIZATION.THE PRIMER SET AF1/AR1 DESIG -NED FROM SEQUENCES OF Q3H01 FRAGMENT WAS SYNTHESIZED AND USED IN PCR DETECTION OF EHEC.TH -E RESULTS ALSO SHOWED THE SPECIFICITY FOR THESE TWO SEROTYPES.AFTER 8-HOUR PRE-CULTURE ST -EP WITH LB BROTH, THE DETECTION SENSITIVITY COULD REACH TO 100 TARGET CELLS INOCULATED IN PER GRAM OF BEEF.

Keywords : ESCHERICHIA COLI, O157:H7 SEROTYPE, RAPD, DOT HYBRIDIZATION, POLYMERASE CHAIN REACTION

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