

Detecting and genotyping *Escherichia coli* O157:H7 using multiplexed PCR and nucleic acid microarrays

Douglas R. Call, Fred J. Brockman, Darrell P. Chandler
International Journal of Food Microbiology (2001) 67:71–80

Abstract

Rapid detection and characterization of food borne pathogens such as *Escherichia coli* O157:H7 is crucial for epidemiological investigations and food safety surveillance. As an alternative to conventional technologies, we examined the sensitivity and specificity of nucleic acid microarrays for detecting and genotyping *E. coli* O157:H7. The array was composed of oligonucleotide probes (25–30 mer) complementary to four virulence loci (intimin, Shiga-like toxins I and II, and hemolysin A). Target DNA was amplified from whole cells or from purified DNA via single or multiplexed polymerase chain reaction (PCR), and PCR products were hybridized to the array without further modification or purification. The array was 32-fold more sensitive than gel electrophoresis and capable of detecting amplification products from <1 cell equivalent of genomic DNA (1 fg). Immunomagnetic capture, PCR and a microarray were subsequently used to detect 55 CFU ml⁻¹ *E. coli* O157:H7 from chicken rinsate without the aid of pre-enrichment. Four isolates of *E. coli* O157:H7 and one isolate of O91:H2, for which genotypic data were available, were unambiguously genotyped with this array. Glass-based microarrays are relatively simple to construct and provide a rapid and sensitive means to detect multiplexed PCR products; the system is amenable to automation.

Below are examples of PCR product hybridizations and genotyping experiments:

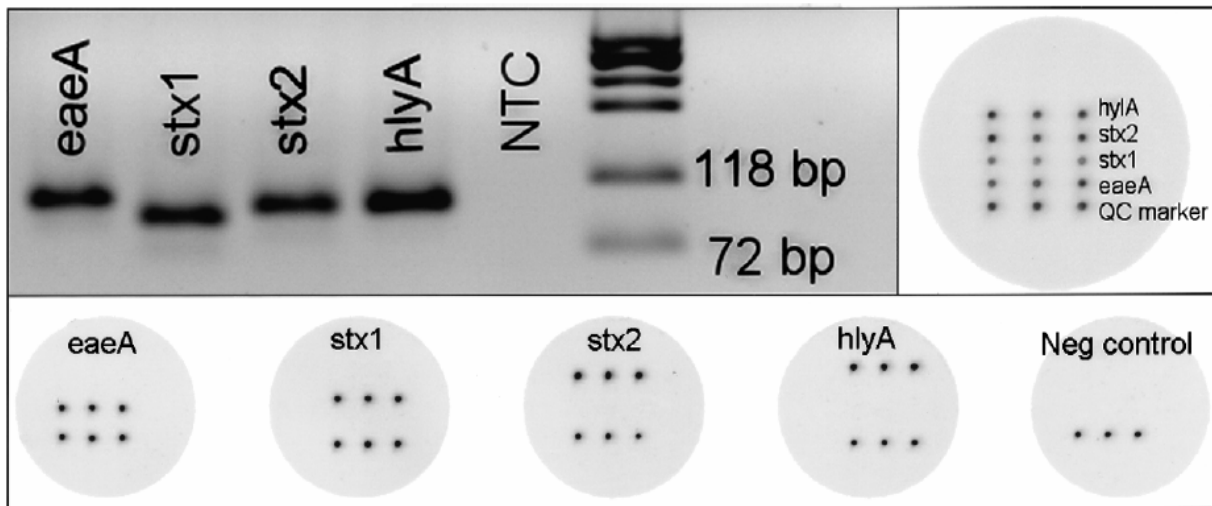


Fig. 4. Hybridization specificity for *E. coli* O157:H7 virulence loci. Upper left panel illustrates electrophoretic separation of *eaeA*, *stx1*, *stx2*, and *hlyA* PCR products. Upper right panel illustrates hybridization of a multiplex PCR reaction to an array with probes complementary for each PCR target and for QC. The lower panel illustrates probe specificity for individual primer sets when PCR products were hybridized to arrays identical to the upper right panel. All microarray incubations were at 23°C.

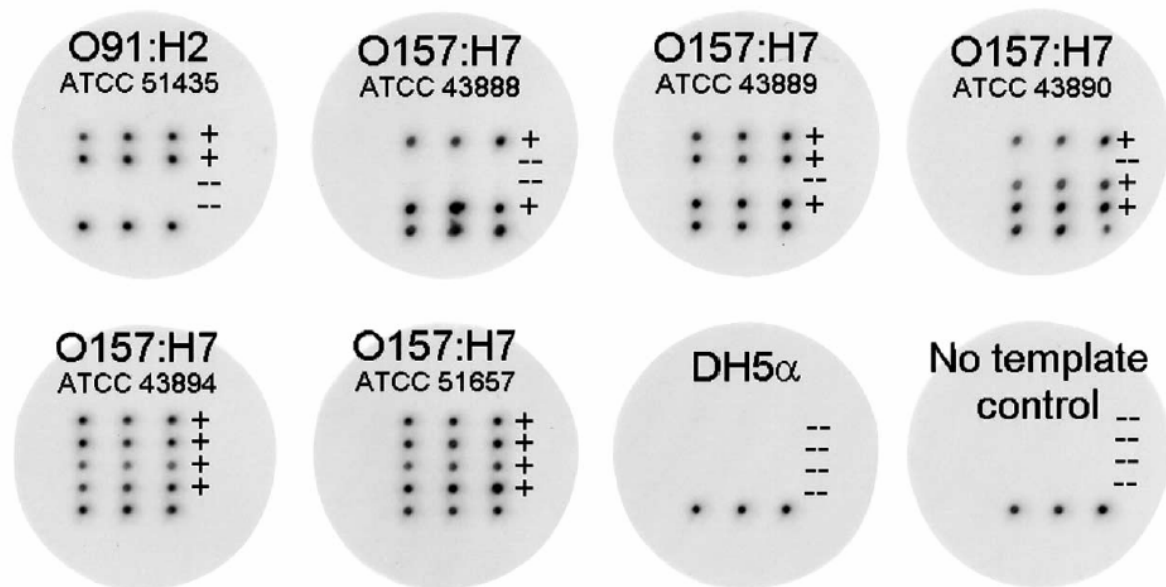


Fig. 5. Genotyping results for seven strains of *E. coli* using multiplex PCR for *eaeA*, *stx1*, *stx2*, and *hlyA* loci (see Fig. 4 for spot legend). Genotypes for ATCC 51435, 43888, 43889, 43890, and 43894 match respective genotypes for *stx1* and *stx2*. All microarray incubations were at 23°C.