

Use of photopolymerization for the rapid and cost-effective identification of Shiga toxin-producing *Escherichia coli* on DNA microarrays

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ABSTRACT

Shiga toxin-producing *Escherichia coli* O157:H7 is a leading cause of foodborne illness worldwide. To evaluate better methods to rapidly detect and genotype *E. coli* O157 virulent strains, the present study explored the use of photopolymerization, a colorimetric and photoinduced signal amplification detection method, for pathogen identification on DNA microarrays. A DNA oligonucleotide microarray was designed to target O157 genetic markers encoding Shiga toxin production, adherence and O-antigen factors. Analysis of the microarray data demonstrated polymer formation for only probes targeting virulence genes present in the tested *E. coli* O157 reference strains RM1625, RM1600, RM6011, and RM4876. Positive hybridization signals had average signal-to-noise ratio values above 10, while signal-to-noise ratio values below 1.5 were determined for the same virulence probes in the non-pathogenic *E. coli* strain RM5034. Thus, the use of DNA microarrays in combination with photopolymerization allowed the rapid and cost-effective identification of *E. coli* O157, compared to fluorescence methods that are more expensive and require several days for strain detection.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is an enteric pathogen known to cause human gastrointestinal illnesses (3). In the recent years, most risk management efforts have focused on identifying the presence and distribution of *E. coli* O157:H7 as an important part of food safety programs (3). Thus, the O157 serotype is considered to be the most commonly reported serotype linked to outbreaks in North America.

The rise in foodborne-related outbreaks of O157 STEC has heightened the importance of developing improved methods to rapidly detect and characterize virulent strains (1). Molecular-based technologies, such as DNA microarrays, offer a viable alternative to screen multiple markers simultaneously (6). One challenge of using the DNA microarray platform for pathogen detection has been developing cost effective and sensitive procedures that would indicate the positive signals on the array. Limitations of current assays can result in an inconsistent labeling of target DNA and utilize expensive and non-portable scanners for data acquisition and analysis (4). Improved procedures for pathogen surveillance are thus needed with sufficient sensitivity, cost-effectiveness, and suitability for routine testing (2, 4).

To implement the use of better detection methods for categorizing STEC strains, our studies have evaluated a novel and innovative procedure that is based on light-initiated signal amplification through polymerization (ampliPHOX™, InDevR, Boulder CO). This method proved to be simpler, rapid and cost-effective for DNA microarray-based pathogen identification by using reagents and instrumentation that is low cost and suitable for routine pathogen surveillance.

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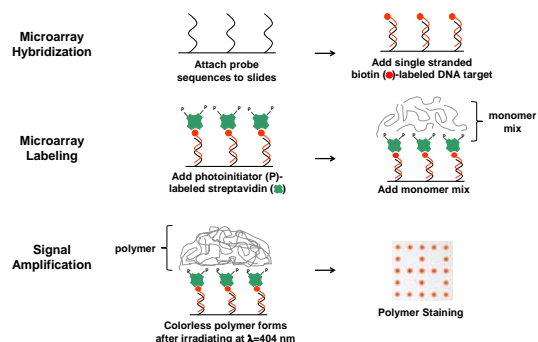


FIGURE 1. Steps in the use of photopolymerization with DNA microarrays. Photopolymerization, a light-initiated signal amplification through polymerization, is a colorimetric detection method for low-density microarray (ampliPHOX™ system, InDevR, Inc., Boulder, CO). A streptavidin-conjugated photoinitiator labels the microarrays that have been previously hybridized with a biotin-labeled target. After irradiating at a 404 nm wavelength, a colorless polymer forms where the probe and target sequences hybridized specifically on the microarray. Polymer formation is easily visualized after a short staining step.

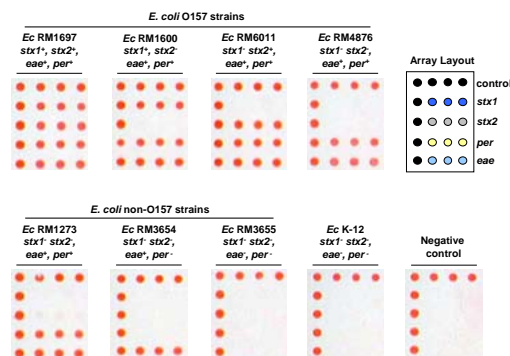


FIGURE 2. Specific identification of pathogenic *E. coli*. To validate the specificity of using photopolymerization with a low-density microarray, *E. coli* reference strains with different genotypes were examined. Multiplex PCR amplification of *eae*, *per*, *stx1*, and *stx2* loci was performed by using bacterial crude lysates as template. Microarray hybridization, labeling and signal amplification was performed according to the ampliPHOX detection protocol (InDevR, Inc.). Polymer formation was observed for only those probes targeting virulence genes known to be present in the *E. coli* O157 reference strains

Gene	30-mer Probe [5'-3']	Phosphorylated		Biotinylated
		Forward Primer [5'-3']	Reverse Primer [5'-3']	
<i>stx1A</i>	GTTCTTATCTAATGACTCTGTAAGATGTC	CTCTGCAAGAGCGATGTTA	CTCACTTCCCGAGTCAA	
<i>stx2A</i>	GTTAATGAGGTTGAGTGAATAACAATGAC	AATCAGTCGTCACTACTGGTT	CGCGTATCTGATACACAG	
<i>per</i>	TAGGCTACAATTATAGATGACAATATCT	GGTGAAGTGGATGGTGTTC	TCAGCAATTTACGTTTTGTC	
<i>eae</i>	GTTAATCTGCAGATGGTAATAACTTTGAC	CCGCTCTTGGTATGCGTGT	CGCTACCCCGACCTAAA	

TABLE 1. List of DNA oligonucleotides used in the DNA microarray. To construct this DNA microarray, 30-mer oligonucleotide probes, targeting intimin adherence protein (*eae*), perosamine synthetase (*per*), Shiga toxin 1 (*stx1*), and Shiga toxin 2 (*stx2*), were designed with a 5'-amino-C6 modification for covalent binding to the glass slide surface with an aldehyde surface coating. A multiplex PCR amplification of biotinylated fragments was conducted to generate fragments ranging in sizes between 150-350 base pairs. For a rapid and sensitive microarray hybridization, a lambda exonuclease digestion was performed on the PCR-amplified fragments to yield the biotinylated single-stranded DNA targets.

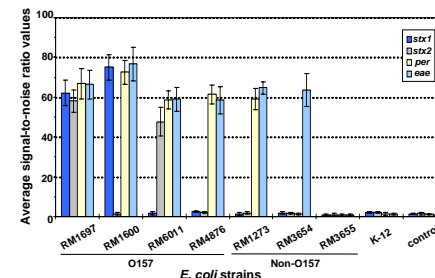


FIGURE 3. Quantification of signal-to-noise ratios. Quantification analysis of strain RM1697 showed that positive signals detected for all probes had signal-to-noise (SNR) ratio values, ranging from 58.2 ± 5.6 to 66.8 ± 7.5 , similar results were observed after analysis of positive signals for the other O157 and non-O157 *E. coli* reference strains. Negative signals where no specific hybridization was observed had signal-to-noise ratio values, ranging from 2.48 ± 0.49 to 69 ± 0.39 for all tested strains. The averages \pm standard deviations of three independent experiments with triplicate measurements for each probe are shown.

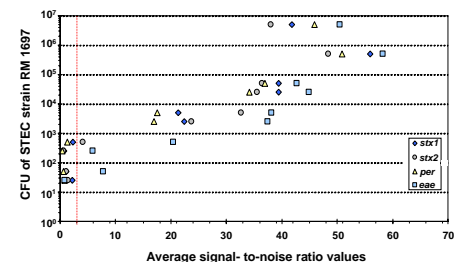


FIGURE 4. Detection sensitivity. To determine the sensitivity threshold, various cell concentrations of the reference STEC strain RM1697 were tested on the microarray, and the signal-to-noise ratio values were quantified. By establishing an SNR value of 3 as our detection limit for positive signals, the sensitivity threshold for detecting STEC strain RM1697 in the sample was between 100-1000 CFU. The averages of two independent experiments with triplicate measurements for each probe are shown.