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Optimization and Application of a Custom Microarray for the Detection and Genotyping of *E. coli* O157:H7 in Fresh Meat Samples

Biao Suo · Yiping He · Peter Irwin · Andrew Gehring

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Abstract DNA microarrays are promising high-throughput tools for multiple pathogen detection. Currently, the performance and cost of this platform has limited its broad application in identifying microbial contaminants in foods. In this study, an optimized custom DNA microarray with flexibility in design and content for foodborne pathogen detection was developed through the systematic evaluation of spotting buffers, probe lengths, scanning conditions, and nucleic acid amplification and labeling strategies. Briefly, by robotic contact printing, a spotting solution of 50 % dimethylsulfoxide produced uniform and high-quality spots on UltraGAPS glass slides coated with aminopropyl silane. The use of 60 % photomultiplier tube gain in scanning ~70-mer oligonucleotide probes resulted in strong signals and low background. For sample preparation, multiplex PCR amplification coupled with fluorescent labeling of DNA using the Klenow fragment and random hexamers achieved higher specificity than whole genome random amplification. To minimize the cost of the assay, the quantities of probes, Klenow fragment, and Cy5 were substantially reduced in each assay without noticeably affecting the detection efficiency. Applying the optimized microarray assay to 26 fresh meat samples, three different isolates of *Escherichia coli* O157:H7 were found in four individual packages, demonstrating that the assay has a great potential for identifying and genotyping multiple pathogens in a real food system.

B. Suo
College of Food Science and Technology, Henan Agricultural University, Zhengzhou 450002, China

Y. He (✉) · P. Irwin · A. Gehring
Molecular Characterization of Foodborne Pathogens Research Unit, Eastern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, 600 East Mermaid Lane,
Wyndmoor, PA 19038, USA
e-mail: yiping.he@ars.usda.gov

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Introduction

Foodborne illness, caused by the ingestion of food containing pathogenic bacteria or bacterial toxins, is a serious public health problem with substantial economic cost. The Centers for Disease Control and Prevention estimated that 48 million people become ill each year from various foodborne infectious agents whereupon 128,000 are hospitalized and over 3,000 die (http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html). Of these 48 million cases, only 9.4 million were caused by known pathogens, and the majority (38.4 million) of them were from unknown infectious agents. Hence, there is a need to develop effective methods for rapid identification and characterization of pathogenic agents, which is a critical basis for disease prevention, outbreak investigation, and successful clinical treatment (Shi et al. 2010).

Most culture-based diagnostic methods are laborious, time consuming, and incapable of simultaneously detecting multiple pathogens. PCR assays are fast and sensitive but limited to the detection of a small number of DNA targets and insufficient to differentiate viable vs. dead cells. Immunochemical-based assays depend on the availability of reagents, such as target-specific antibodies, and are generally limited to one antigen per assay. The common drawbacks of these methods are low detection capacity of multiple organisms and poor genotypic characterization (Nugen and Baeumner 2008). By overcoming these limitations, microarray technology offers simultaneous analysis of a large number of targets from many microbial agents in parallel, which greatly increases the power of detection and molecular characterization of multiple pathogens that may be present in a given sample (Severgnini et al. 2011).

In recent years, while numerous microarrays have been described for the detection of a wide range of microorganisms (Wang et al. 2007, Chen et al. 2010, Suo et al. 2010a, b), the performance and cost remain limiting factors for routine microbial identification in food samples. Sensitivity and specificity are the major challenges encountered with the detection of low levels of pathogenic microorganisms in a large sample volume, which can typically contain high levels of background microflora as well as inhibitory materials which substantially interfere with molecular analyses including PCR and microarray assays.

The process of microarray analysis includes: (1) custom design of oligonucleotide probes and microarray fabrication; (2) sample preparation and enrichment including DNA extraction, amplification, and fluorescent labeling; (3) hybridization of the labeled DNA to immobilized probes based on the sequence-specific binding; and (4) image scanning and data analysis. In order to reduce cost and increase sensitivity, specificity, and robustness of the assay, protocols and procedures for microarray genotypic analysis need to be systematically examined and improved.

To evaluate and optimize microarray assay conditions, *Escherichia coli* O157:H7 was chosen as the testing organism for the design and fabrication of a custom microarray. *E. coli* O157:H7, a Shiga toxin-producing *E. coli*, is notable for causing foodborne illness, which can result in kidney failure associated with complications from hemolytic uremic syndrome (Gould et al. 2009). Public availability of genome sequences of several *E. coli* O157:H7 strains in the National Center for Biotechnology Information database allows appropriate design of microarray probes targeting both pathogen-specific sequences (*rfb*E and *fli*C) for detection and virulence genes (*hly*A, *eae*, *stx*1, and *stx*2) for pathogenicity classification and genotyping. The custom microarray design and assay optimization from this study should be applicable to comprehensive detection and genotyping of any group of microorganisms of interest.

This study focused on a systematic evaluation of microarray design and printing, probe selection, sample DNA amplification and labeling, and data acquisition procedures. The optimized microarray assay parameters and improvements in the detection of a foodborne pathogen are reported herein. Finally, application of the optimized assay for the identification and genotyping of *E. coli* O157:H7 in naturally contaminated meat samples was demonstrated.

Materials and Methods

Bacterial Strain and Growth Conditions

E. coli O157:H7 MD 380-94 was originally isolated from salami by USDA Food Safety Inspection Service, Athens, GA. This strain has been previously characterized as positive for the *hlyA*, *eae*, *stx1*, *stx2*, *rfbE*, and *fliC* genes (Uhlich et al. 2008). The bacterial culture was aerobically grown in brain–heart infusion broth (Becton Dickinson Co., Sparks, MD) overnight at 37 °C.

Selection of Target Genes and Microarray Probes

The target genes selected for microarray detection and characterization of *E. coli* O157:H7 are listed in Table 1. Specific nucleotide sequences from each target gene were used for designing one 70-mer microarray probe and a set of 20-mer reverse and forward PCR primers or microarray probes. The sequences and melting temperatures of the probes and primers, including a positive-control probe designed from the *hexon* gene of human adenovirus type 7, were described previously (Suo et al. 2010a, b). All of the primers and probes were commercially synthesized by Integrated DNA technologies (Coralville, IA), and no extra chemical modifications were amended to any of the probes.

Microarray Printing

A printing buffer consisting of 3× standard saline citrate (SSC), 0.05 % sodium dodecyl sulfate (SDS), and 0.001 % (3-[(3-cholamidopropyl) dimethylammonia]-1-propane sulfonate) (CHAPS) (Dawson et al. 2005) was compared to 50 % dimethylsulfoxide (DMSO) in microarray printing. A 20-μL printing solution comprised of the SSC-based buffer or 50 % DMSO and the probes (at a final concentration of 25 μM) were added into wells of a 384-well plate, and then printed onto UltraGAPS glass slides coated with aminopropyl groups (Corning Inc., Corning, NY). A robotic OmniGrid Accent® Microarrayer (GeneMachine, San Carlos, CA) equipped with ArrayIt Stealth SMP4 Pins (Telechem International, Inc., Sunnyvale, CA) was used for fabricating low-density microarrays. According to the product description, SMP4 pins typically uptake approximately 0.2-μL printing solution and generate microarray spots of 135-μm diameters. The distance between the centers of two adjacent spots was programmed 390 μm apart. Each chip had both a negative (50 % DMSO) and positive control probe. After printing, the chips were exposed to 600 mJ ultraviolet (UV) light for cross-linking the oligonucleotides to the surface of UltraGAPS slides, and then stored at room temperature in a vacuum desiccator. To assure the quality of microarray printing and avoid false-negative results, Syto 61 (Invitrogen, Inc. Carlsbad, CA) staining of a couple of randomly selected slides was performed (Battaglia et al. 2000). For the evaluation of printing buffers, 0.2 μL of Cy5 fluorescent dye (GE Healthcare, Piscataway, NJ) was directly added into the printing solution.

Table 1 Gene targets used for microarray detection of *Escherichia coli* O157:H7 MD 380-94

Gene target	Accession No.	Encoded protein	Reference
hlyA	X94129	Hemolysin toxin protein A	Schmidt et al. (1994)
eae	AF081182	Intimin adherence protein	Louie et al. (1993)
stx1	M16625	Shiga-like toxin1	O'Brien and Holmes (1987)
stx2	X07865	Shiga-like toxin2	O'Brien and Holmes (1987)
rfbE	S83460	O157 somatic antigen	Bilge et al. (1996)
fliC	AF228488	H7 flagellar antigen	Reid et al. (1999)

DNA Amplification

Target-Specific Multiplex PCR Bacterial genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA) and quantified with a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE) as described previously (Suo et al. 2010a, b). A six-plex PCR (for *hlyA*, *eae*, *stx1*, *stx2*, *rfbE*, and *fliC*) was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) using the QIAGEN multiplex PCR kit (Qiagen, Valencia, CA). Briefly, a 20-μL reaction mixture was prepared using 2 μL of DNA template (approximately 10 ng/μL), 10 μL of 2× QIAGEN multiplex PCR master mixture, 2 μL of Q-Solution, and 2 μL of a primer mixture containing six sets of primers (2 μM each) (Suo et al. 2010a, b). This multiplex PCR was performed under the following conditions: 95 °C for 15 min for initial activation of Taq DNA polymerase; 30 cycles of denaturing at 94 °C for 30 s, annealing at 58 °C for 90 s, and extension at 72 °C for 60 s; 72 °C for 10 min for a final extension. PCR product sizes were determined with a 2 % GenePure 3:1 agarose gel (ISC Bioexpress, Kaysville, UT) stained with ethidium bromide.

Whole Genome Amplification Genomic DNA was randomly amplified using a GenomePlex whole genome amplification (WGA) kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's recommendations. Briefly, a total of 9 μL genomic DNA (4 ng) was incubated with 1-μL 10× fragmentation buffer at 95 °C for 4 min to randomly fragment the DNA and was then cooled on ice. An Omniplex library was generated by linking flanking universal adapters onto each DNA molecule as follows: 2 μL of 1× library preparation buffer and 1 μL of 1× library stabilization solution were added to the fragmented DNA; the mixture was denatured at 95 °C for 2 min and rapidly chilled on ice. After adding 1 μL of library preparation enzyme, the reaction was carried out by sequentially incubating the mixture at 16 °C for 20 min, 14 °C for 20 min, 37 °C for 20 min, and 75 °C for 5 min. Subsequently, the Omniplex library was amplified using universal primers and Jumpstart Taq DNA polymerase (1.0–2.5 units) at the conditions of: 95 °C for 3 min for polymerase activation, followed by 14 cycles of denaturing at 94 °C for 15 s and annealing and extension at 65 °C for 5 min. The amplified DNA products were purified using the Qiagen PCR purification kit, and then subjected to Cy3 labeling.

Genomic DNA Fragment Labeling

A high concentration of Klenow fragment (50 U/μL; New England Biolabs, Ipswich, MA) was used for Cy5 labeling of DNA. Approximately 0.5 to 2 μg PCR products were mixed with 7.5 μg random hexamers or octamers (Invitrogen, Inc., Carlsbad, CA) and 25, 50, or 100 U Klenow in a 25-μL volume. The DNA-labeling reaction and subsequent removal of unincorporated dye and dNTPs were performed as described previously (Suo et al. 2010a, b). The efficiency of Cy5 incorporation and rate of DNA recovery were measured at 650 and 260 nm wavelengths, respectively, using a NanoDrop ND-100 spectrophotometer.

Microarray Hybridization and Scanning

Microarray slides were prehybridized and hybridized under the same conditions as previously described (Suo et al. 2010a, b). ScanArray 3000 laser scanner (Packard BioScience, Billerica, MA) was used to scan all the slides at excitation wavelengths of 667 nm for the Cy5 dyes. The ScanArray Express software was used for fluorescent image processing and data analysis.

Examination of Fresh Meat Samples

Twenty-six packages of fresh meat were obtained from local retailers and analyzed within 24 h. To promote the growth of potential *E. coli* O157:H7 contaminating strains in these meat samples, which contain various other background microorganisms, an *E. coli* O157:H7 selective enrichment medium (SEL) was prepared by adding antibiotics to buffered *Listeria* enrichment broth (Becton Dickinson) to achieve final concentrations of 0.01 g/L acriflavine (ICN Biomedical, Aurora, OH), 0.05 g/L cycloheximide, 0.05 g/L fosfomycin, and 0.002 g/L nalidixic acid (Sigma, St. Louis, MO) (Kim and Bhunia 2008). For each sample, 25 g of meat was massaged with 225 mL SEL medium in a Whirl-Pak filter stomacher bag (eNasco, Modesto, CA) for 2 min, and

then incubated at 37 °C for 16 h with shaking at 150 rpm for cell growth. Aliquots of each liquid sample were subjected to genomic DNA extraction and subsequent microarray analysis as described above.

Results and Discussion

Optimization of Microarray Printing Conditions

The printing buffer, consisting of 3× SSC, 0.05 % SDS and 0.001 % CHAPS was compared to 50 % DMSO for the quality and morphology of microarray spots printed on UltraGAPS slides. After dissolving oligonucleotide probes together with Cy5 in a printing solution, the mixture was deposited onto the surface of the aminopropyl silane-coated slides by a robotic contact printing technique (Barbulovic-Nad et al. 2006). The printed microarrays were directly subjected to image scanning without requiring the hybridization process. A scanned image of the printed spots is shown in Fig. 1. Consistently, all the spots printed from 50 % DMSO were uniform with a nearly circular shape and small diameter (~78 μm). However, the SSC-based solution generated larger spots with irregular diameters (~136 μm). Each of these spots consumed approximately two times more volume of the probe than a spot printed from 50 % DMSO based upon the number of microarrays produced from each uptake of the printing solution. The dispensed spot from the SSC-based solution spread over a greater surface area, which not only diminished the array densities but also was more prone to cross-contamination during the handling of array printing and hybridization. Reproducible results for spot size and shape were obtained in multiple trials of printing regardless of whether the probes were 20 or 70 mer. Therefore, on aminopropyl silane-coated slides, 50 % DMSO not only produced higher-quality microarray spots but also substantially saved on the cost of probes.

Type of spotting solution, substrate surface property of the slide, and parameter settings of the robotic arrayer are the primary factors contributing to the quality of microarray spots. Dawson et al. (Dawson et al. 2005) reported that the SSC-based printing solution generated the best quality spots, and DMSO resulted in poor reproducibility on aldehyde substrate coated glass slides printed with amino-terminated oligos. In this study, amine-modified glass slides (UltraGAPS), the most commonly used for DNA arrays, were chosen for the fabrication of low-density custom microarrays. In this way, there was no need for additional chemical modification during the synthesis of oligonucleotides. The electrostatic interaction between the DNA backbone and amino groups promotes covalent bonding of the nucleic acid to the microarray surface after the UV cross-linking treatment. On aminopropyl group coated slides, 50 % DMSO was found to produce better quality microarray spots with good morphology and uniformity presumably due to its viscosity and moderate evaporation rate. Using a 50 % DMSO solution as the spotting fluid, appears to be more effective than the SSC-based buffer because of the higher density of a spot and less consumption of the probe.

Fig. 1 Effect of spotting solutions on the quality of microarray spots. A scanned image of quadruplicate sets of the microarray spots containing 70-mer oligonucleotide probes mixed with the Cy5 dye. The quality of the microarray spots were compared between 50 % DMSO and the SSC-based solution used for dissolving and depositing the probes

Including the Cy5 dye in the printing solution allows direct monitoring of the quality of the spots after printing the microarray slides, which expedites the optimization of microarray printing conditions without the requirement of DNA labeling and hybridization steps. After establishing the optimal printing conditions, Cy5 was excluded from the printing solution during the microarray fabrication. For quality control, Syto61 was used to stain a randomly selected microarray from each batch of productions to assure the quality and correctness of each spot including both negative and positive controls.

Mechanical deposition of oligonucleotides on glass slides is a relatively inexpensive approach for the production of low-density custom microarrays. It can consistently produce good quality microarrays and requires low maintenance. This detection system provides high flexibility in probe design and updating, which facilitates the identification of a range of different organisms. Therefore, it is suitable in clinical settings, as well as for the food industry for the detection and genotyping of various pathogenic microorganisms.

Comparison of Bacterial DNA Amplification Methods

A major challenge in applying microarray detection of pathogens in food samples is acquiring a sufficient amount of sample DNA. It is impractical to directly extract 1–10 μg DNA from a non-abundant target microorganism in food samples without enrichment. In order to increase sample DNA to a detectable level and obtain a DNA amplification approach suitable for microarray analysis, target-specific multiplex PCR and random WGA methods were compared by using the genomic DNA of *E. coli* O157:H7 MD 380-94 as a template.

For target-specific multiplex PCR containing the primer sets for *hlyA*, *eae*, *stx1*, *stx2*, *rfbE*, and *fliC*, six DNA fragments with known sizes were concurrently amplified in a single reaction tube (Fig. 2a). In WGA, after the Omniplex library generation and random amplification steps, random DNA fragments with good quality and quantity were produced (Fig. 2b). With a starting concentration of 4.4 ng genomic DNA, a yield of 5–10 μg DNA ranging in size from 75 to 1,500 bp with a mean of ~400 bp was generated based upon spectrophotometric analysis at an absorbance of 260 nm as well as from DNA from signal intensity from agarose gels. Each array hybridization reaction requires only 1.6 μg of sample DNA.

In order to reduce the cost of the assay, quantities of Plantinum Taq DNA polymerase of 1.0, 1.5, 2.0, and 2.5 units/reaction were tested for the amplification efficiency. Apparently, there were no major differences in terms of the product yield and size between 1.5 and 2.5 units/reaction of Taq polymerase when used in WGA. However, 1.0 unit/reaction of Taq polymerase resulted in a noticeable reduction of DNA amplification (Fig. 2b).

Overall, both random and target-specific amplifications significantly increased the quantity of sample DNA to above a detectable level by amplifying the DNA over a thousand fold. However, cross-hybridization was observed between the randomly amplified DNA of *E. coli* O157:NM and the *fliC* probe, which might be due to the interference from background DNAs or relatively low specificity of the *fliC* probe. On the other hand, the selective amplification of targeted DNA in the multiplex PCR produced specific hybridization signals (data not shown). However, the low multiplex potential of PCR may limit the advantages of a microarray system which has the capability of analyzing hundreds to thousands of targets. Recently, Palka-Santini et al. (2009) developed a multiplex amplification of several dozen DNA targets in a single PCR tube, which might increase the compatibility of PCR with microarray detection to a certain extent. OmniPlex WGA is a useful approach for producing sufficient genomic DNA from low numbers of microorganisms without bias (Uda et al. 2007), but the assay selectivity remains to be improved on some less specific targets such as the *fliC* probe.

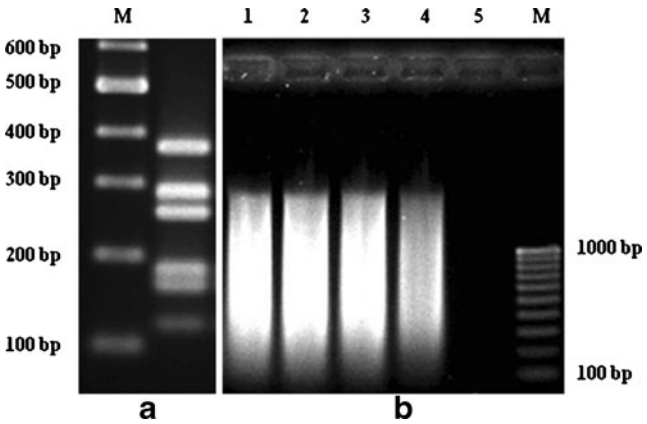

Fig. 2 Comparison of different nucleic acid amplification strategies. **a** Amplified DNA fragments by a multiplex PCR containing six sets of *E. coli* O157:H7 primers. **b** Randomly amplified DNA by GenomePlex WGA and the effect of different amounts of Taq DNA polymerase on WGA. *Lane M*, 100 bp DNA marker; amplification using varying concentrations of Taq DNA Polymerase–2.5 (*lane 1*), 2.0 (*lane 2*), 1.5 (*lane 3*), 1.0, (lane 4), and 0 U (*lane 5*)

Evaluation of DNA-labeling strategies

Random octamers and hexamers (7.5 μg) were compared in Klenow-based reactions to label the DNA fragments amplified from a multiplex PCR. The fluorescent labeled DNA was hybridized to the microarrays and results were compared (Fig. 3). The labeled DNAs using both random primers were positively hybridized to all the probes. However, the samples labeled using hexamers resulted in stronger hybridizing signals than those from octamers. Moreover, two concentrations of hexamers (7.5 and 15 μg) were tested in 50-μL labeling reactions. The primer in the lower concentration was able to produce the same signal intensity as that in the higher concentration (data not shown).

The DNA-labeling efficiency was further optimized regarding the quantities of Klenow and Cy5 used in each reaction. Three concentrations of Klenow and Cy5 were tested in different combinations, and the results are summarized in Table 2. Based on the absorbance measured at 260 and 650 nm, reduced concentrations of Klenow and Cy5 resulted in slightly lower yield of the total DNA and Cy5-labeled DNA, respectively. However, the labeling efficiencies denoted as the percentage of Cy5-DNA/total DNA were not significantly decreased. Thus, considering the labeling efficiency and cost of the assay, the combination of 25 U Klenow, one fourth vial Cy5, and 7.5 μg random hexamer in each labeling reaction was optimal.

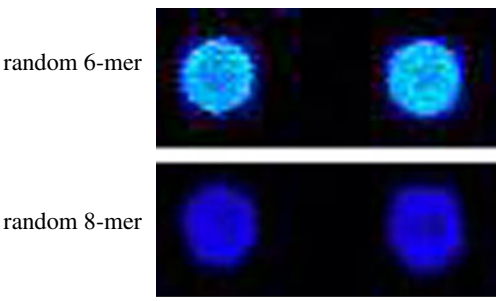

Fig. 3 Effects of random primers of different length on the DNA-labeling and microarray hybridization. Random hexamers and octamers were used in the Klenow-based DNA-labeling reactions. After hybridizing the labeled DNAs to the custom microarray, a representative image of the microarray spots is shown

Table 2 Effects of Klenow fragment and Cy5 on the DNA-labeling efficiency

	A	*B*	*C*	*D*	*E*
Klenow (U/reaction)	25	25	50	100	100
Cy5 (vial/reaction)	1/4	1	1/2	1	1/4
Total DNA (ng/μL)	131.1±9.4	167.0±6.6	160.4±8.2	211.1±10.8	221.9±9.7
Cy5-labeled DNA (ng/μL)	25.1±0.8	39.7±0.9	30.3±0.8	46.1±1.3	36.7±1.1
Labeling efficiency[a] (%)	19.1	23.8	18.9	21.8	16.5
Cost/reaction[b] ($)	10.2	23.6	20.5	41.0	27.6

[a] Labeling efficiency is the ratio of Cy5-labeled DNA/Total DNA

[b] Cost of each labeling reaction was calculated based on the prices of $232/1,000 U Klenow fragment and $213/pack of 12 vials Cy5

There are both direct and indirect methods for fluorescent labeling of DNA. In this study, the indirect DNA-labeling approach was chosen because of the low efficiency of directly incorporating Cy5-dCTP into DNA (Manduchi et al. 2002). Reduced quantities of hexamers, Klenow enzyme, and Cy5 in each labeling reaction allowed us to save up to four times the reagent cost without considerably affecting DNA-labeling efficiency.

Microarray Scanning of Different Lengths of Oligonucleotide Probes

Twelve short (~20 nt) and six long (~70 nt) oligonucleotides were spotted onto the same array and hybridized with the Cy5-labeled DNA from *E. coli* O157:H7 and scanned with a 60 % photomultiplier tube (PMT) gain whereupon the ~70 nt probes displayed strong fluorescent signals and low background (Fig. 4a). In contrast, at the same hybridization and scanning conditions, the fluorescent signals from the ~20 nt probes were barely visible (data not shown). When the PMT gain of the scanner was increased to 90 %, the signals of the short probes were enhanced but the background on the array was increased, as well, suggesting that increasing the PMT gain can improve detection sensitivity but also increases background noise (Fig. 4b). Thus, the better condition for diagnostic microarrays is to use 70-mer oligonucleotides as probes and 60 % PMT gain for scanning.

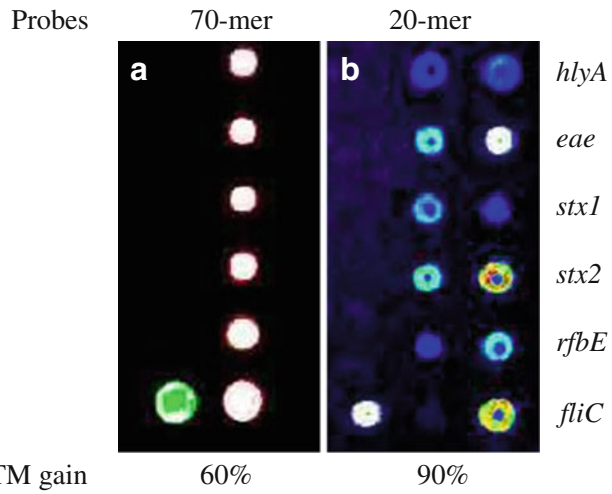

Fig. 4 Effect of scanning conditions on different lengths of oligonucleotide probes. **a** A microarray image of 70-mer oligonucleotide probes scanned at 60 % PMT gain. **b** A microarray image of 20-mer oligonucleotide probes scanned at 90 % PMT gain

To appropriately analyze microarray data, background noise should be subtracted from the probe-based signal. Saturation of signal intensities, typically not background levels, could underestimate the true results. In the past, chemical saturation related to the concentration and length of probes was addressed (Chudin et al. 2002). Optical saturation also may happen if the scanner PMT gain is set too high. Under that circumstance, the true probe signals could be underestimated due to the threshold of the maximum intensity value. Based on the results of comparing different settings of PMT gain in scanning 70- and 20-mer oligonucleotide probes (Fig. 4), 60 % PMT gain appears to be optimal for scanning microarrays printed with 70-mer probes.

Application of the Optimized Microarrays in Pathogen Detection

Twenty-six packages of fresh meat obtained from local supermarkets over various periods of time were tested for *E. coli* O157:H7 specific (*rfbE* and *fliC*) and virulence genes, including the *eae*, *hlyA*, and Shiga-like toxin genes (*stx1* and *stx2*) using the optimized microarray assay. Prior to microarray analysis, each individual meat sample was selectively enriched in SEL medium to promote the growth of viable *E. coli* cells and therefore to reduce the chance of detecting nonviable cells potentially existing in these samples. After overnight enrichment, four packages were found to be positive for *E. coli* O157:H7 by displaying at least 10-fold greater fluorescent signals from the *hlyA*, *rfbE*, and *fliC* probes than a negative control on the same array. The scanned images of the positive samples are shown in Fig. 5.

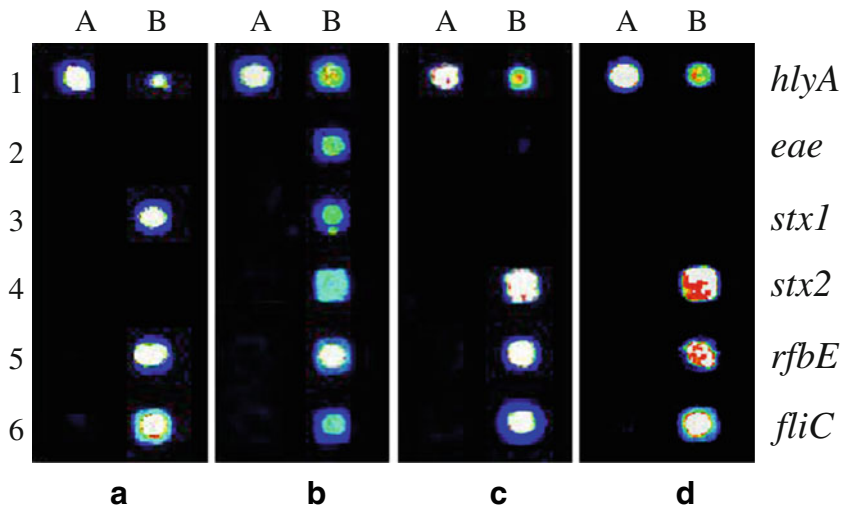

Fig. 5 Application of the optimized microarray to the detection of *E. coli* O157:H7 in fresh meat. Positive microarray results were obtained from 4 (samples **a**–**d**) out of 26 packages of ground beef after selective enrichment of *E. coli* O157:H7 followed with target DNA amplification. There are three different genotypes of *E. coli* O157:H7 based on the target gene profiles in the samples. Column *A1* is the positive control, *A2* is the negative control, and *B1–6* are the *E. coli* O157:H7 probes

The occurrence of *E. coli* O157:H7 in these meat samples was confirmed by conventional assays using CHROMagar O157 and sorbitol MacConkey agar with cefixime and tellurite. The O157 and H7 antigens were verified using the RIM *E. coli* O157:H7 latex agglutination kit. The specific immunological reactions of the isolated colonies with the O157 and H7 antisera suggest the presence of O157 and H7 antigens (data not shown) and were consistent with the positive microarray results for *rfb*E and *fli*C in these strains. The prevalence rate (15 %) of *E. coli* O157:H7 found in these retail meat is comparable to other reports, such as *E. coli* O157 ranged from 0.1 to 54.2 % in ground beef, 0.1 to 4.4 % in sausage, 1.1 to 36.0 % in retail cuts, and 0.01 to 43.4 % in whole carcasses (Hussein 2007). Compared with the culture-based methods together with the immunoassays, the microarray detection is reliable and effective for the identification of *E. coli* O157:H7 in food matrices (Kostic et al. 2011).

Interestingly, based on the hybridization patterns with the *eae*, *stx1*, and *stx2* probes in Fig. 5 and the validation results with the conventional assays, there could be three different genotypes of *E. coli* O157:H7 existing in four positive samples. Sample A was eae^{-}, $stx1^{+}$, and $stx2^{-}$; B was eae^{+}, $stx1^{+}$, and $stx2^{+}$; and both C and D were eae^{-}, $stx1^{-}$, and $stx2^{+}$, suggesting that these contaminants might be transmitted from different sources. The important information on the occurrence of virulence genes (*eae*, *stx1*, and *stx2*) revealed from this assay can assist in the understanding of the pathogenicity and track the transmission sources of these strains. Finally, since these results were obtained from direct analysis of food samples, not individual isolates, there might be a low probability of false positives or co-contamination of multiple strains and/or serotypes of *E. coli* in the same package. To avoid any erroneous conclusions, the food samples that test positive should then be subjected to a thorough investigation, e.g., strain isolation, verification, and specific tests to confirm the contaminants as well as their serotypes and virulence information.

Table 3 Optimized conditions for fabrication and process of pathogen detection microarray

Key element	Optimized condition
Spotting buffer	50 % DMSO
Oligonucleotide probe	~70 mers and 25 μM
DNA amplification	Target-specific multiplex PCR
DNA labeling	Klenow-based labeling
Random primer for DNA labeling	7.5 μg hexamers
Klenow	25 U/reaction
Cy5	0.25 vial

Conclusions

Conditions for microarray printing and analysis have been optimized in this study (Table 3). With these improvements, the custom designed and fabricated microarray is a cost effective and reliable platform for microbial pathogen detection and characterization. The applications of the microarray assay to specific identification and genotyping of different *E. coli* O157:H7 strains were also demonstrated in naturally contaminated meat samples. The design of the microarray probes can be periodically improved based upon updated microbial genome sequences in GenBank, which allows this flexible assay to be expanded to the detection and genotyping of additional microorganisms of food safety concern.

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Conflict of Interest Biao Suo declare no conflict of interest. Yiping He declare no conflict of interest. Peter Irwin declare no conflict of interest. Andrew Gehring declare no conflict of interest. This article does not contain any studies with human or animal subjects.

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