Development of rapid qualitative detection DNA microarray platform for *Salmonella typhimurium* for food safety

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Development of rapid qualitative detection DNA microarray platform for *Salmonella typhimurium* for food safety

C Samphaongern[1], C Pipatpanukul[2] and T Srikhirin[1,*]

[1]Materials Science and Engineering Program, School of Materials Science and Innovation, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[2]Materials Engineering Program, Faculty of Engineering, Burapha University, Chonburi 20131, Thailand

*Corresponding author: toemsak.sri@mahidol.ac.th

Abstract. Screening of possible bacteria contamination in food products is one of the measures implemented to avoid potential health hazards. In food industry, standard cell culture technique is widely used to monitor bacterial contamination. However, the main drawback of this technique is its inherited time consumed during the culturing step, which requires about 72 hours. In this research, an alternative DNA microarray technique was developed for a qualitative screening of a possible *Salmonella typhimurium* contamination in chicken, with the aim of reducing the detection time down to 3 hrs. The identification of *S. typhimurium* in food samples was carried out by hybridization of the possible contaminant with specific probe immobilized on a biochip. Novel DNA probes were designed as 16-50 base pairs of nucleotides to recognize with specific parts in *S. typhimurium* genomic. At least two DNA probes were identified as the candidate probes which had the potential to promote the best hybridization. DNA microarray was fabricated by mixing candidate DNA probes with photoactive polymer network, (poly(DMAA-mABP-SSNa)), and was printed onto a plastic substrate by non-contact microspotter. DNA probe was covalently immobilized onto the surface by 254 nm of UV lamp with 1.25 J/cm2. PCR product functionalized with biotin was hybridized with DNA probe and labelled with streptavidin-cy5. Specific binding yielded fluorescence signal. The intensity image signal was read-out by a fluorescent microarray reader. Two genes specific to *S. typhimurium* (*fimC* and *invA*), were investigated by using specially designed DNA primers and DNA probes. From the assay optimization, it was found that 1 mg/ml of polymer hydrogel concentration, 10 µM of DNA probe concentration, 10 µg/ml of labelling concentration, and 2 nL of array volume yielded the highest signal intensity. The results were calibrated into CFU/ml (cell forming unit). The system was applied successfully for the detection of *S. typhiurium* without any contamination. The biochip validation with spiked sample (DNA standard) is currently underway.

1. Introduction

Food pathogen refers to microorganisms that cause disease in humans and animals by food contamination. The major food pathogen that can lead to foodborne illness is bacteria. Many types of bacteria are concerned with fresh chicken but the one that plays an important role is *Salmonella typhimurium*. Recently, two important genes that are used for identification of *S. typhimurium* such *fimC* and *invA* were reported [1,2]. However, many methods have been recently developed for microbial identification based on cell culture technique in the standard technique, the growth of target cells on a specific media, still commonly used because of its cost effectiveness. Contrarily, the

identification process can take up to 24-72 hours, which can be a major drawback, especially, in the food industry.

DNA microarray technology is a novel technique that can be fabricated to contain thousands of the genetic code in a single chip. The technique relies on designing smaller fragments of DNA probes to represent the known genes of target cells. These DNA probes are then spotted on fixed positions on a substrate. DNA microarray technique is an alternative technique for the multiplex detection where the multiplex of DNA probes are being immobilized to surface of substrate. Photoactive polymer network applied to DNA microarray fabrication was proposed by previous work. the method begins with mixing designed DNA probes and synthesized photoactive polymer network, (poly(DMAA-mABP-SSNa)), which are then printed onto a plastic substrate (PMMA sheet) by non-contact microspotter. In a previous study, DNA probe was covalently immobilized onto surface by UV irradiation at 254 nm wavelength with 1.25 J/cm2 energy. PCR product functionalized with biotin were then hybridized with the DNA probe at the annealing temperature and labelled with streptavidin-cy5 [3]. This specific binding yields fluorescence signal and the intensity image signal can be read-out by a fluorescent microarray reader.

In this study, primers and probes as specific to *fimC* and *invA* were designed to amplify DNA product and promote specific hybridization, respectively as shown in table1. However, a step of food pathogen DNA microarray fabrication was optimized to obtain the best intensity signal. Important parameters need to be optimized, including polymer concentration, array volume, labelling concentration, and DNA probe concentration. In this study, DNA array sensor was fabricated for the detection of *S. typhimurium* using probes based in *invA* and *fimC* genes and was tested with DNA standard sample for initiating the platform for real sample testing.

Table1. Target genes, designed primer and designed probe

Target genes	Designed primers (5'-3')	Number of designed probes
fimC	F: CTCTGGCAATCAGTAATAGCGA R: CTGATATTGACCAGCGTGAG	2
invA	F: TTAGGACTGATTGGCGATCTC R: CTCAACTTCAGCAGATACCA	3

2. Materials and Methods

2.1. Optimization of polymer concentration

The photoactive polymer network was synthesized followed by previous study [3]. Concentration of polymer solution was diluted as 0.1, 0.5, 1, 2, 5 mg/ml with distilled water and mixed with 100 µg/ml of labelling solution (streptavidin-Cy5 (GE Healthcare)). Spot volume was 0.4 nL (lowest volume of droplet) which was printed on PMMA substrate (Rungart company, Thailand) by microspotter machine (sciFLEXARRAYER, SCIENION) and incubated in a 254nm-UV chamber (UV Stratalinker 1800, STRATAGENE) of 1.25J. The substrate was washed with PBS buffer (Sigma Aldrich) and observed with a microarray reader (Agilent High-Resolution Microarray Scanner).

2.2. Optimization of microarray spot volume

1mg/ml of polymer solution was mixed with 100 µg/ml of labelling The spot volume was varied as follows 0.4, 1.2, 2.0, 2.8, 3.6 nL and was printed on PMMA substrate and incubated in a 254nm-UV chamber of 1.25J. The substrate was washed with PBS buffer and observed with a microarray reader.

2.3. Optimization of the labelling

1mg/ml of polymer solution mixed with labelling solution that varied in concentration of staining as follows 0.001, 0.01, 0.1, 1, 10 and 100 µg/ml respectively. Spot volume was 2 nL of solution which was printed on PMMA substrate and incubated in a 254nm-UV chamber of 1.25J. The substrate was washed with PBS buffer and observed with a microarray reader.

2.4. Optimization of DNA probe density

1mg/ml of polymer solution mixed with DNA probe-biotin that varied in concentration of probe as follows 0.01, 0.1, 1, 10, and 20 µM. Spot volume was 2 nL which was printed on PMMA substrate

and incubated in 254nm-UV chamber of 1.25J, followed by staining with 10 µg/ml of labelling for 10 minute. The substrate was washed with PBS buffer and observed with a microarray reader.

2.5. Optimization of DNA standard sample detection

Standard culture of *S. typhimurium* was cultured as 24 hours. Bacterial suspensions were prepared and standardized (according to Standard McFarland solution) to $1.5x10^8$, $1.5x10^7$, $1.5x10^6$, $1.5x10^5$, $1.5x10^4$, $1.5x10^3$, and $1.5x10^2$ CFU/ml (cell density) respectively, and bacterial DNAs were extracted from each solution. 1 mg/ml of polymer solution was mixed with 10 µM a designed probe and immobilized onto the PMMA surface by UV irradiation at 254 nm with 1.25 J/cm2. DNA standard PCR product functionalized with biotin was hybridized with the designed probe at annealing temperature and labelled with 10 µg/ml streptavidin-cy5 for 10 minutes. The PMMA substrate was washed with PBS and the results were observed with microarray reader. First platform was designed for *fimC* designed probe testing; the platform was divided to 3 fields including positive field (blue), designed probe field (yellow) and negative field (red), blue field for biotin tagged probe, 1-yellow field for *fimC* designed probe1, 2-yellow field for *fimC* designed probe2, 3-red field for *L. monocytogenes* probe, 4-red field for E. coli probe and 5-red field for bare polymer solution without probe [figure1, a]. Second platform was designed for *invA* designed probe testing; platform was divided to 3 field including positive field (blue), designed probe field (yellow) and negative field (red), blue field for biotin tagged probe, 1-yellow field for *invA* designed probe1, 2-yellow field for *invA* designed probe2, 3-yellow field for *invA* designed probe3, 4-red field for *L. monocytogenes* probe, 5-red field for *E. coli* probe and 6-red field for bare polymer solution without probe [figure1, b].

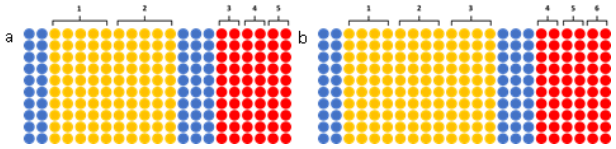

Figure 1. a) *fimC* testing platform b) *invA* testing platform

3. Results and discussion

3.1. DNA microarray fabrication

From results, it found that concentration of polymer solution is 1mg/ml, due to light intensity signal increased from 0.1 mg/ml until it reached a plateau around 1 mg/ml. As the signal did not vary much beyond this point, 1mg/ml of polymer solution was fixed for other experiments [figure2, a]. For the spot volume optimization, there were no significant differences in term of intensity among the spot volumes 1.2, 2.0, and 2.8 nL. From these, 2.0 nl was selected as it had the most uniform morphology and the lowest standard deviation in terms of intensity [figure2, b]. Intensity of labelling conc. trended to increase significantly from 1 µg/ml to 10 µg/ml and attained 85% intensity around 10 µg/ml (97.75% intensity with 100 µg/ml) [figure3, a]. For the limit of reagent, DNA probe density (probe conc.) could be read up to the highest conc. at 20 µM, and also exhibited a stable range around 10 µM [figure3, b]. Following optimization of these parameters, the first platform was fabricated and tested with the standard DNA sample.

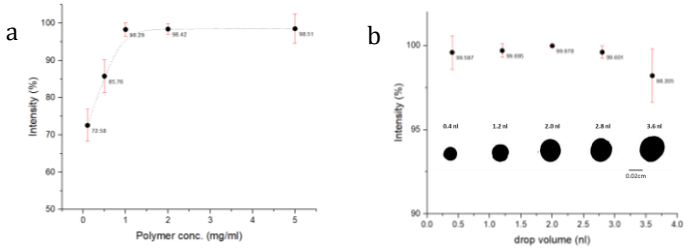

Figure 2. a) effect of polymer solution conc. to signal
b) effect of drop volume to signal

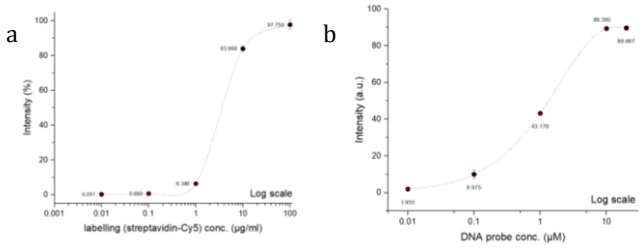

Figure 3. a) signal limitation of labelling
b) effect of DNA probe density to signal

3.2. DNA standard sample testing

For first platform, both designed probes detected DNA product from $1.5x10^8$ CFU/ml until the last concentration of product $1.5x10^2$ CFU/ml, *fimC* probe2 show higher intensity than *fimC* probe1. These results were obtained without contamination with other probes [figure4, a]. In the second platform, only invA probe3 detected DNA product and showed limitation or cut of value at $1.5x10^4$ CFU/ml, without contamination with other probes as well [figure4, b].

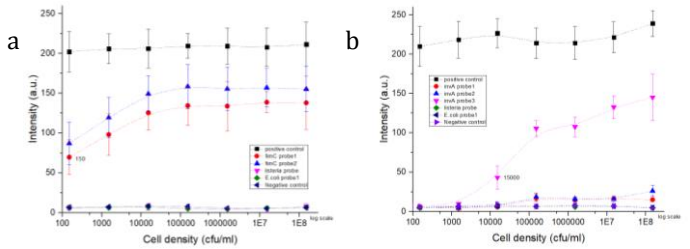

Figure 4. a) detection of DNA sample with *fimC* designed probes b) detection of DNA sample with *invA* designed probes

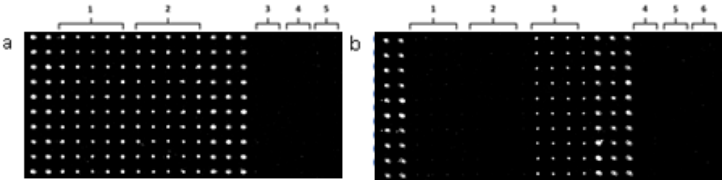

Figure 5. a) *fimC* array at $1.5x10^7$ CFU/ml b) *invA* array at $1.5x10^6$ CFU/ml

4. Conclusions

In this study, important parameters were optimized and comprised of 1 mg/ml of polymer solution, 2 nL of array volume, 10 µg/ml of labeling concentration, and 10 µM of DNA probe concentration for the detection of *S. typhimurium* using DNA micro array platform. With the standard DNA tests, *fimC* gene and *invA* gene could be detected with designed probes and presented a cut off value at $1.5x10^2$ CFU/ml and $1.5x10^4$ CFU/ml, respectively, without any contamination with others probes. The propose platform can qualitatively detect the PCR product of *S. typhimurium*.

5. Reference

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