New DNA Biosensors As Techniques for Rapid Determination of Water Borne Pathogens

Lee Yook Heng [1, 2, *], Dedi Futra [1], and Tan Ling Ling [2]

[1] Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia;
[2] Southeast Asia Disaster Prevention Research Initiative (SEADPRI-UKM), LESTARI. Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia;

Corresponding author: leeyookheng@yahoo.co.uk *(L.Y.H)*

Abstract. New DNA biosensors for the rapid determination of water borne pathogens are presented in this paper. Pathogenic organisms are routinely used as indicator organisms to evaluate potential water pollution and enteric pathogenic contamination of waters. Highly sensitive electrochemical DNA biosensors based on silica nanospheres and gold nano-composites for specific detection of *E. coli* and *V. cholera*e DNA in water samples (i.e. river water, sea water, tap water and bottled drinking water) were developed. Under optimum conditions, the DNA biosensors could specifically determine both *E. coli* and *V. cholera*e DNA in the range of 1.0×10^{-17}–1.0×10^{-7} M target DNA, with a limit of detection (LOD) as low as 1.25×10^{-18} -1.3×10^{-17} M and long-term stability of up to 55-60 days. The DNA biosensors demonstrated good reproducibility (relative standard deviation (RSD) <5.0%, n=5) and regenerability (RSD below 6.0%, n = 5). The DNA biosensors showed satisfactory recoveries for both *E. coli* and *V. cholera*e DNA in river water samples between 94.0-104.0 %. The DNA quantity evaluated by the DNA biosensors was found to be proportional to the amount of bacterial colonies in the water samples determined by a cell culture method.

Keywords: Water borne pathogens**,** DNA biosensor; *Escherichai coli; Vibrio cholerae,* biological water contamination

1. INTRODUCTION

Water-borne bacterial pathogens are a main causes of various health issues especially in terms of the safety of drinking water worldwide. The major sources of the contamination are from composting farm manure, industry and domestic waste, flooding, surface run-off, etc (Cabral, 2010; Croun et al., 2010). For example, *Escherichia coli* (*E. coli*) are negative gram bacteria and naturally found in the intestinal tracts of humans and warm-blooded animals. Many strains of *E coli* are known to be human pathogens. One of them, strain *E coli* O157:H7 bacteria could produce a toxin that damages the intestine membranes, cause anemia, stomach cramps, bloody diarrhea, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Cabral, 2010). On the other hand, cholera, a waterborne disease, which is an acute intestinal infection marked by exhaustive diarrhea is caused by the pathogenic bacteria *Vibrio cholerae* (*V. cholerae*). Cholera infection can be endemic, epidemic or pandemic especially after a natural disaster such as a flood or in areas with poor sanitation. *V. cholerae* is a gram-negative bacterium producing enterotoxin responsible for the watery diarrhea. It is non-virulent and only serogroups O1 and O139 strains contribute to the widespread epidemic cholera (Louis et al., 2003; Gubala, 2006).

Traditional methods for the determination of pathogenic organisms in water are mainly based on microbiological techniques, which involves the growth of the organism on selective agar media and then isolates and identifies the bacteria colonies according to their morphological, biochemical and immunological characteristics (Lu and Breidt, 2015). However, this conventional test is a time-consuming, laborious process and requires sterile conditions. Another conventional method commonly used for specific determination of water borne pathogenic organisms is molecular method based on polymerase chain reaction (Garrido et al., 2013). However, the main weakness of this method is that it is laboratory based, time-consuming and uses of toxic chemicals. Recently, an electrochemical DNA biosensor strategy has appeared as a promising alternative method for evaluation of *E. coli* DNA due to the simple and rapid procedure, small dimension, and high selectivity and specificity from the determination of DNA hybridization by the biosensor.

Sensitive electrochemical DNA biosensors based on silica nanospheres and gold nano-composites modified carbon screen-printed electrodes (SPE) for the determination of pathogenic *Escherichia coli* and *Vibrio cholerae* in water has been developed in this work. The colloidal gold nanoparticles (AuNPs) were firstly deposited onto the electrode surface to enhance the electron transfer. The aminated silica nanospheres were used to immobilize the pathogens' recognition DNA probes. The complementary DNA of pathogenic organisms from the water sample were allowed to bind with the immobilized probe DNA via hybridization reaction and the binding event was indicated by different pulsed voltametry (DPV) using sodium antraquaoinone-2-sulfonic acid monohydrate (AQMS) as a redox hybridization label.

2. RESULTS AND DISCUSSIONS

When the electrochemical DNA biosensor was exposed to the various target DNA concentrations of the organisms, the current response was increased with the increasing target DNA concentrations, due to the increasing DNA hybridization reaction and AQMS intercalation on the double stranded DNA. For the determination of *E coli*, a linear response was obtained where the limit of detection of the DNA biosensor was 1.0×10^{-16} to 1.0×10^{-9} M and 1.3×10^{-17} M (130 aM) respectively. Selective study of the *E. coli* DNA biosensor for the detection of complementary DNA, 1-base mismatched and non-complementary DNA at 5 µM and 0.5 µM for *E coli* DNA showed that 67.9 -70.8 % of the current response was observed during hybridization with the 1-base mismatched DNA compared with complementary DNA whilst the response of the DNA hybridization with non-complementary DNA were below 5.0 %. Therefore, the DNA biosensor for *E coli* was specific to the target DNA of *E coli.* Analysis of water *E coli* samples from river, seawater, tap water and bottled drinking water using the DNA biosensor indicated contamination of the pathogens in both river and seawater, but not in tap water or bottled water. The results agreed well with determination of *E coli* via cell culture method.

The dynamic linear concentration range of the *V. cholerae* DNA biosensor was investigated in the presence and absence of the reporter probe with various target DNA concentrations ranging from 10 to 1.0×10^{-13} µM (9.21×10^{-2}-9.21×10^{-16} µg/µL). The DNA biosensor response increased proportionally with the increasing target DNA concentration due to the increasing DNA hybridization reaction and AQMS intercalation on the DNA electrode. The DNA biosensor specificity towards *V cholerae* DNA was evaluated using 3-base mismatched DNA, non-complementary DNA and complementary DNA at 4.0 µM and 0.4 µM. About 46.0-52.1% of the current response was obtained for 3-base mismatched DNA relative to the response from complementary DNA. This confirmed the specificity of the DNA biosensor for the determination of *V. cholerae* DNA when a reporter probe was used. To evaluate the feasibility of the DNA biosensor in determining *V. cholera* DNA concentration in real water samples, various bacterial strains and water samples were used. The DNA concentrations for the respective *V. cholerae* strains such as *J3324*, *J2126*, *KM5802*, *J3330*, *CDHI5294* and *UVC1324* were obtained while the DNA for the control was obtained from the bacteria species *C. freundii, E. aerogenes* and *K. pneumonia*. The results showed that the DNA biosensor was highly specific towards the detection of *V. cholerae UVC1324* and *V. cholerae J3330*, whilst the response towards the control organisms such as *E. earogenes, C. feundii* and *K. pneumoniea* bacteria was lowest. *V. cholerae* DNA was found in river water samples in the range of 1.20×10^{-10} to 4.71×10^{-13} µg/µL and this was confirmed by using a cell culture method where *V. cholera* bacteria was in the range of 12-36 colonies.

3. CONCLUSIONS

The results presented here demonstrated that DNA biosensors can be potential tools for the rapid analysis of water samples to determine contamination by pathogenic organisms. The use of electrochemical DNA biosensors can allow water analysis for pathogenic organisms on site, within minutes, without the need of a large amount of chemical reagent and highly skilled manpower. The use of such an analytical device should assist in a rapid water quality monitoring program, especially for pathogenic organisms where the analysis is still confined to laboratory methods that required long hour of incubation time and well trained operators.

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