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Development of an immunoFET biosensor for the detection of biotinylated PCR product

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Abstract

ImmunoFET (IMFET) biosensor is a simple platform for the detection of biotinylated products of polymerase chain reaction (PCR). Construction of the IMFET biosensor started with adsorption of 1.5 mg/mL of protein A (PA) onto the insulated gate surface of ISFET for 90 min. Next, the immobilized 1/500 dilution of anti-biotin antibody was adsorbed onto the PA layer for 60 min. The IMFET biosensor was subsequently ready for detection of the biotinylated amplicon. The IMFET biosensor showed highly specific binding to the biotinylated PCR product of the *phaE* gene of *Haloquadratum walsbyi* DSM 16854. The *phaE* gene is a biomarker of polyhydroxyalkanoate (PHA) producers that contain PHA synthase class III. The lowest amount of DNA template of *H. walsbyi* DSM 16854 that the IMFET biosensor could detect was 125 fg. The IMFET biosensor has a lower

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amount of detection compared with a DNA lateral flow biosensor from our previous study. The degree of linearity of the biosensor signal was influenced by the concentration of the biotinylated amplicon. The IMFET biosensor also has a short response time (approximately 30 times) to detect the *phaE* amplicon compared to an agarose gel electrophoresis. The IMFET biosensor is a promising tool for the detection of the biotinylated PCR product, and it can be integrated into a micro total analysis system (μTAS).

Keywords: Engineering, Biochemistry, Bioengineering, Biotechnology, Microbiology

1. Introduction

Polymerase chain reaction (PCR) is a molecular method for amplification of a specific DNA sequence [1]. Amplification of DNA target relies on the use of different temperatures for the three steps of the reaction, denaturation, annealing and extension [1]. PCR is an *in vitro* assay that has been applied in many fields of research [2, 3, 4]. It also becomes a routine method for DNA analysis including diagnostic of certain infection and genetic diseases in clinical laboratory [5, 6]. Besides, PCR has been applied in environmental research such as for screening of polyhydroxyalkanoate (PHA)-producing haloarchaea [7]. PHAs are utilized as bioplastics because they can be easily degraded by microorganisms [8]. Of the screening, PCR was performed for targeting a biomarker gene (*phaE*) of PHAs-producing haloarchaea [7]. Amplicon of the *phaE* gene can be observed on agarose gel electrophoresis (AGE), which was a time consuming assay and required a skilled person. A simple platform for the detection of PCR product was development such as DNA lateral flow biosensor. The combination of PCR and a DNA lateral flow biosensor reduces the cost and time for *phaE* screening [7]. Although the DNA lateral flow biosensor increases the speed required for *phaE* detection, the biosensor cannot be reused and requires a labeled PCR product [7]. Therefore, non-labelling or reusable platforms for the PCR product detection is an interesting issue for development. The field-effect (FET)-based biosensor is an example device that can be modified for detection of non-labelling biomolecules [9, 10]. FET have been applied as a transducer for construction of biosensor including EnFET [11, 12, 13], GenFET [14] and IMFET [15, 16]. The FET is useful to detect an electrical field changing when biomolecules have interacted with the insulated gate surface of FET [17, 18]. The ion-sensitive field-effect transistor (ISFET) is an electronic transducer that has been applied to a biosensor. This biosensor-based ISFET-transducer is available for various types of biomolecule detection, such as DNA, proteins and organic compounds [19, 20, 21, 22]. ISFET was first developed to measure ion concentration [17]. Biomolecules, such as an antibody, can be immobilized on the ISFET gate to detect a specific antigen [21]. Biomolecules could be immobilized on the sensing

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membrane of ISFET using cross-linker molecules such as poly-L-lysine (PLL) [9], (3-aminopropyl)triethoxysilane (APTES) [15], protein A (PA) [19], etc. PA is a cell surface protein of *Staphylococcus aureus*. It consists of a single polypeptide chain (42 kDa of MW) [23, 24]. PA has high affinity for Fc fragment of immunoglobulin G (IgG) from a large number of species such as human, mouse, monkey, rabbit, etc. [17, 18, 20]. PA could be immobilized on a solid support including acrylic or silica beads are often development for monoclonal and polyclonal IgG purification [21, 22, 25].

The aims of the present study were to develop a simple electronic platform for detection of biotinylated PCR products of the *phaE* gene. ISFET was a transducer for the biosensor development. Protein A was applied for a cross-linker of the transducer and biorecognition molecule (anti-biotin antibody).

2. Materials and methods

2.1. Bacterial genomic DNA

Genomic DNA of *Bacillus megaterium* DSM 319, *Cupriavidus necator* DSM 428, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027 and *Streptomyces hygroscopicus* BTCC 7028 were obtained according to Muangsuwan et al. [7]. Genomic DNA of *Haloquadratum walsbyi* DSM 16854 was obtained from the Lieibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.

2.2. Polymerase Chain Reaction

PCR was performed to amplify the *phaE* gene of PHA-producing haloarchaea according to Muangsuwan et al. [7]. Genomic DNA of *H. walsbyi* DSM 16854 was used as a positive control. *H. walsbyi* DSM 16854 is a halophilic archaeon that is able to produce PHA, and it also contains the *phaE* gene [7]. The forward primer (PhaE3A-F) was labeled with biotin at the 5′-end, while the reverse primer (PhaE3A-R) was not labeled. Thus, the *phaE* gene amplicon (224 bp) contained a biotin only at the 5′-end. The PCR product was analyzed using an agarose gel electrophoresis and visualized under a UV transilluminator according to Muangsuwan et al. [7].

2.3. Measurement of the gate voltage of ISFET

The gate voltage shift (ΔV_G) of ISFET was monitored using the digital multimeter (Trektronik, DMM4050, USA). PBS buffer pH 7.4 (Calbiochem, 524650, Germany) was a buffer for the measuring process. The ISFET pH sensor, Ag/AgCl reference electrode, and read-out circuit box were obtained from the ISFET pH Sensor Kit (Winsense, WIPSK, Thailand). They were interconnected

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via a current control box (Winsense, WIPSK-CB1, Thailand). The I_{ds} of ISFET was constantly supplied for 30 μA by the current control box. The interfering negatively charged molecules including dNTPs, DNA template and the excess amount of coating biomolecules such as PA and anti-biotin antibody had to be removed before performed the measurement process. The non-targeting molecules were removed from the biosensor by submerging in cleaning solution (PBS buffer pH 7.4) for 30 s.

2.4. Adsorption of protein A solution onto the insulated gate surface of the ISFET

Stock solutions of protein A (Sigma, P6031, Germany) was dissolved in phosphate buffer saline (PBS) pH 7.4 (Calbiochem, 524650, Germany) to final concentration of 2.0 mg/mL. In order to determine the optimal concentration of PA and incubation time for adsorption of PA to the insulated gate surface of ISFET, the tests at different concentrations and incubation periods were performed. Various concentrations of PA (0.25, 0.50, 1.00, 1.50 and 2.00 mg/mL) were optimized for the optimum concentration of PA. Ten microliters of PA solution was adsorbed to the insulated gate surface of ISFET (Winsense, WIPSK-S, Thailand) at room temperature for 60 min. Then, the selected concentration was used to optimize the incubation time at 30, 60, 90, and 120 min.

2.5. Adsorption of anti-biotin antibody on PA layer

Ten microliters of an anti-biotin antibody (Santa Cruz Biotechnology, sc-57636, USA) was adsorbed onto the PA layer at room temperature for 60 min. The optimum concentration of the anti-biotin antibody was determined by adsorption onto the PA layer by dilution of the anti-biotin antibody to 1/250, 1/500, 1/1000, 1/1500 and 1/2000-fold. The optimum incubation time for adsorption of the anti-biotin antibody onto the PA layer was determined by varying the incubation time for 30, 60, 90 and 120 min.

2.6. Construction of the ISFET-based biosensor for detection of a biotinylated amplicon

First, the insulated gate surface of the ISFET was cleaned with 0.5% w/v of sodium dodecyl sulfate (SDS) and 70%v/v ethanol. Second, 1.5 mg/mL of PA (Sigma, P6031, Germany) was immobilized onto the insulated gate surface of ISFET for 90 min. Excess PA was removed by phosphate buffered saline (PBS) pH 7.4. Next, a 1/500 dilution of the anti-biotin antibody (Santa Cruz Biotechnology, sc-57636, USA) was adsorbed onto the PA layer for 60 min. An excess amount of anti-biotin antibody was removed using PBS buffer pH 7.4. The biosensor was ready for testing with the biotinylated PCR product.

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2.7. Optimization of the incubation time for biotinylated-amplicon detection

The biotinylated amplicon of *phaE* was used as a positive control. The negative control was obtained by non-amplifying PCR product that used deionized water instead of DNA template. Ten microliters of PCR product was added to the biosensor at room temperature for 1, 2, 5 and 10 min.

2.8. Specificity testing of the biosensor for *phaE* amplicon detection

The *phaE* gene amplicon was obtained by PCR according to Muangsuwan et al. [7]. Genomic DNA that had been used for a positive control was obtained from *H. walsbyi* DSM 16854. Sterile deionized water was added to the PCR mixture, instead of genomic DNA, to be used as a no template control (NTC) reaction. The genomic DNA of bacteria that cannot produce PHA was designated as negative control group 1 (NC1). The NC1 group included *E. coli* ATCC 25922 and *S. hygroscopicus* BTCC 7028. Negative control group 2 (NC2) included bacteria that can produce PHA but did not contain the *phaE* gene. The NC2 group contained *B. megaterium* DSM 319, *C. necator* DSM 428 and *P. aeruginosa* ATCC 9027. Ten microliters of the PCR product was incubated in the biosensor at room temperature for 10 min. The biotinylated PCR product was bound to the anti-biotin antibody that had been immobilized on the top layer of the biosensor.

2.9. Sensitivity testing of the biosensor for *phaE* amplicon detection

The genomic DNA of *H. walsbyi* DSM 16854 was diluted in sterile deionized water. The genomic DNA was diluted to 1,000 fg/μL, 500 fg/μL, 250 fg/μL and 125 fg/μL. Each dilution of the genomic DNA was used as a DNA template for the PCR reaction, which has been described previously by Muangsuwan et al. [7]. Ten microliters of the biotinylated amplicon was incubated in the biosensor at room temperature for 10 min.

2.10. Data analysis

Data obtained from six independent experiments and twenty devices. Each data point was expressed as mean ± SD. The data were fitted with nonlinear regression analysis. Statistical differences were calculated using ANOVA followed by Tukey-Kramer tests using the statistical analysis software SPSS version 18 (SPSS, USA). P-value of <0.05 was defined as statistically significant difference.

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3. Results

3.1. Adsorption of protein A solution on insulated gate surface of ISFET

The ΔV_G of ISFET was obtained by comparing the observation value (V_{PA}) to the baseline value of the gate voltage (V_B) of the ISFET ($\Delta V_G = V_{PA}$-V_B). Various concentrations of PA were tested and the results showed that the ΔV_G was gradually increased after PA had been adsorbed onto the insulated gate surface of ISFET (Fig. 1 and Table 1). The ΔV_G ranged from +3.500 ± 0.506 to +13.438 ± 0.282 mV (Table 1). There was no significant different of ΔV_G were observed between 1.5 mg/mL and 2.0 mg/mL ($P < 0.05$). Then, 1.5 mg/mL of PA was the selected concentration for the construction of the ISFET biosensor. The ΔV_G of ISFET increased to +12.500 ± 0.237 mV after 10 μL of 15 μg PA had been immobilized on the insulated gate surface of the ISFET (Table 1). The optimization of incubation time for adsorption of 1.5 mg/mL PA was observed for 30, 60, 90, and 120 min. The results showed that the increasing of ΔV_G was time dependent. The ΔV_G was gradually increased from 30 to 90 min. However, there was no significant different change in ΔV_G between 90 min and 120 min ($P < 0.05$). Therefore, the optimum incubation time for adsorption of the PA solution onto the insulated gate of ISFET was 90 min, which yielded +20.917 ± 0.401 mV of ΔV_G (Fig. 2 and Table 1).

3.2. Adsorption of anti-biotin antibody on PA layer

The Fc region of anti-biotin antibody adhered to the top of the PA layer. The ΔV_G of ISFET was obtained by comparing the observed value (V_{AB}) to the baseline value of the gate voltage (V_{PA}) of the ISFET ($\Delta V_G = V_{AB}$-V_{PA}). The ΔV_G was

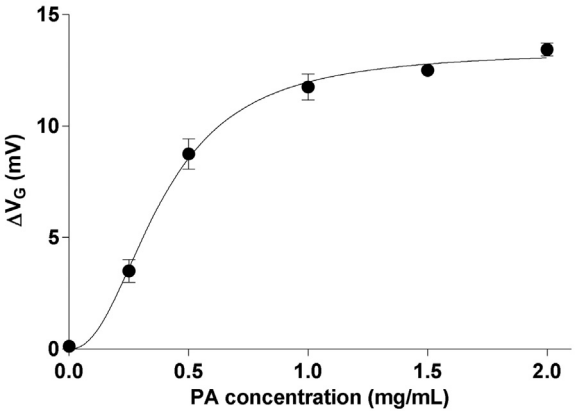

Fig. 1. Optimization of the concentration of protein A (PA) adsorption onto the insulated gate surface of ISFET. The analytical curve was fitted using nonlinear regression model ($R^2 = 0.9907$).

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Table 1. Optimization of PA concentration and incubation time for adsorption of protein A solution (1.5 mg/mL) onto the sensing membrane of ISFET.

Parameter	Value	ΔV_G (mV)
Concentration (mg/mL)	0.00	+0.125 ± 0.194
	0.25	+3.500 ± 0.506
	0.50	+8.750 ± 0.680
	1.00	+11.750 ± 0.586
	1.50	+12.500 ± 0.237
	2.00	+13.438 ± 0.282
Incubation time (min)	0	+0.125 ± 0.158
	30	+3.813 ± 1.382
	60	+11.708 ± 0.528
	90	+20.917 ± 0.401
	120	+21.479 ± 0.639

increased after anti-biotin antibody had been adsorbed onto the PA layer of the ISFET (Fig. 3 and Table 2). The ΔV_G value ranged from +1.604 ± 0.533 to +15.688 ± 0.511 mV (Table 2). The highest ΔV_G value was obtained from 1/250 dilution of anti-biotin antibody (+15.688 ± 0.511 mV) with significant higher than those of others studied dilution ($P < 0.05$). However, the 1/500 dilution of anti-biotin antibody was a selected dilution for the study. Because, it required less amount of the antibody but yielded enough signal for the IMFET biosensor development. The ΔV_G of ISFET was increased to +14.438 ± 0.360 mV after 10 μL of 1/500 dilution of the anti-biotin antibody had been immobilized onto the

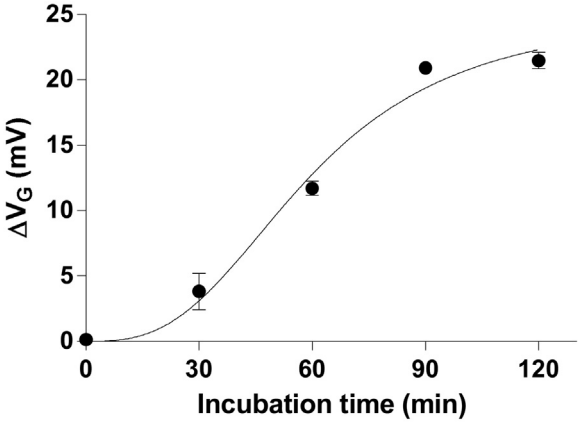

Fig. 2. Optimization of the incubation time for 1.5 mg/mL of protein A adsorption onto the insulated gate surface of ISFET. The analytical curve was fitted using nonlinear regression model ($R^2 = 0.9814$).

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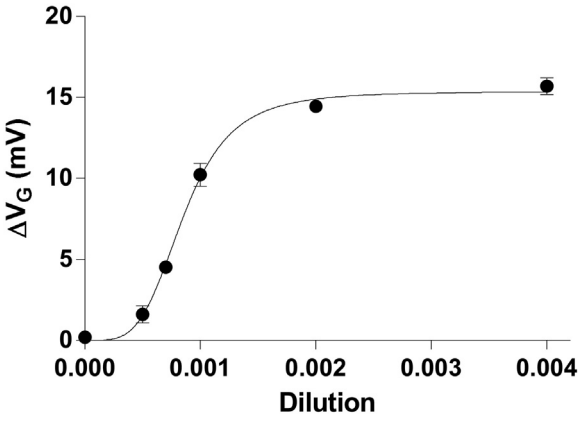

Fig. 3. Optimization of the concentration of the anti-biotin antibody adsorption onto the PA layer. The analytical curve was fitted using nonlinear regression model ($R^2 = 0.9931$).

PA layer (Table 2). In order to determine the suitable time for immobilize anti-biotin antibody (1/500 dilution) onto the PA layer, four different incubation times, 30, 60, 90 and 120 min were performed. The results showed that The ΔV_G values increased when incubated with longer time. Due to the development of biosensor, those in which give a good signal of the ΔV_G values are provided in a shorter period of time are more advantageous. The optimal incubation time, at which the increasing of ΔV_G values was not fall into the plateau curve, was 60 min which yielded +14.292 ± 0.292 mV of ΔVG (Fig. 4 and Table 2) whereas at 90 and 120 min the yield of ΔVG did not showed any significant different ($P > 0.05$).

Table 2. Optimization of the concentration of anti-biotin antibody and incubation time for adsorption 1/500 dilution of the antibody onto the PA layer.

Parameter	Value	ΔV_G (mV)
Dilution	1/2000	+1.604 ± 0.533
	1/1500	+4.521 ± 0.300
	1/1000	+10.229 ± 0.700
	1/500	+14.438 ± 0.360
	1/250	+15.688 ± 0.511
Incubation time (min)	0	+0.229 ± 0.348
	30	+3.479 ± 0.464
	60	+14.292 ± 0.292
	90	+15.583 ± 0.342
	120	+16.042 ± 0.626

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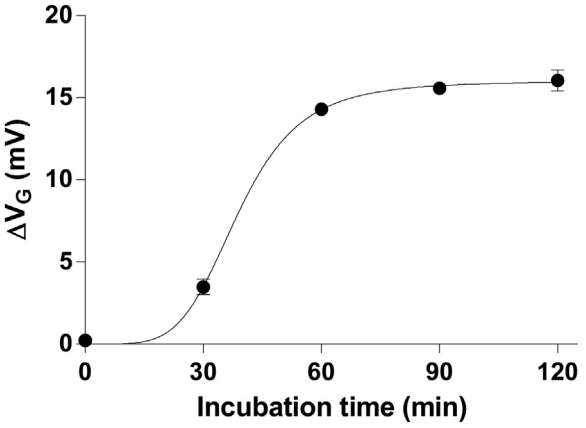

Fig. 4. Optimization of the incubation time for a 1/500 dilution of the anti-biotin antibody adsorbed onto the PA layer. The analytical curve was fitted using nonlinear regression model ($R^2 = 0.9962$).

3.3. Optimization of the incubation time for biotinylated-amplicon detection

The ΔV_G of the ISFET-based biosensor was observed after the PCR products had been incubated in the biosensor (Fig. 5 and Table 3). The ΔV_G of the biosensor was decreased when it was tested with the biotinylated PCR product (positive control). However, the opposite result (increasing of the ΔV_G) was observed when the negative control was tested in the biosensor (Fig. 5 and Table 3). Zero mV of the ΔV_G was the critical value that determined the detection results. The results indicated that one minute of the incubation time was sufficient to determine the detection results. The ΔV_G was significantly decreased to -5.771 ± 0.827 mV when the biotinylated PCR product was incubated for one minute as compared to negative control ($P < 0.05$, Table 3). However, agarose gel electrophoresis required 30 min for analysis of the PCR result.

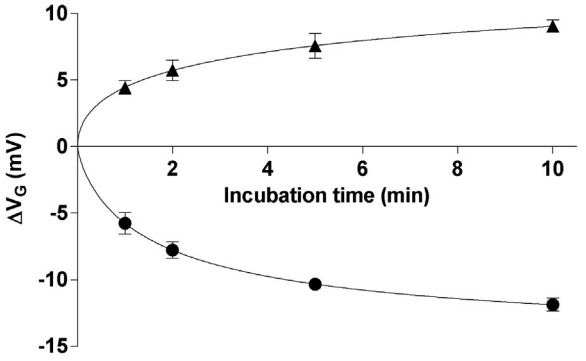

Fig. 5. Optimization of the incubation time to detect the biotinylated-amplicon by the ISFET-based biosensor. (●) Positive control showed the decreasing of gate potential and (▲) negative control showed the increasing of gate potential.

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Table 3. Optimization of the incubation time for biotinylated-amplicon detection.

Incubation time (min)	ΔV_G (mV)	
	Positive control	**Negative control**
1	−5.771 ± 0.827	+4.438 ± 0.461
2	−7.771 ± 0.609	+5.729 ± 0.698
5	−10.333 ± 0.351	+7.563 ± 0.847
10	−11.875 ± 0.487	+9.042 ± 0.419

3.4. Specificity and sensitivity testing of the biosensor for *phaE* amplicon detection

The biosensor displayed a high specificity to the PCR products that contained the *phaE* amplicon (Fig. 6 and Table 4). The ΔV_G of the positive control was decreased to −7.875 ± 0.354 mV. The ΔV_G of the no template control was increased to +8.125 ± 0.354 mV (Table 4). The lowest amount of the DNA template for PCR that the biosensor could detection was 125 fg, which yielded the ΔV_G of the biosensor to −11.250 ± 0.886 mV (Fig. 7 and Table 5).

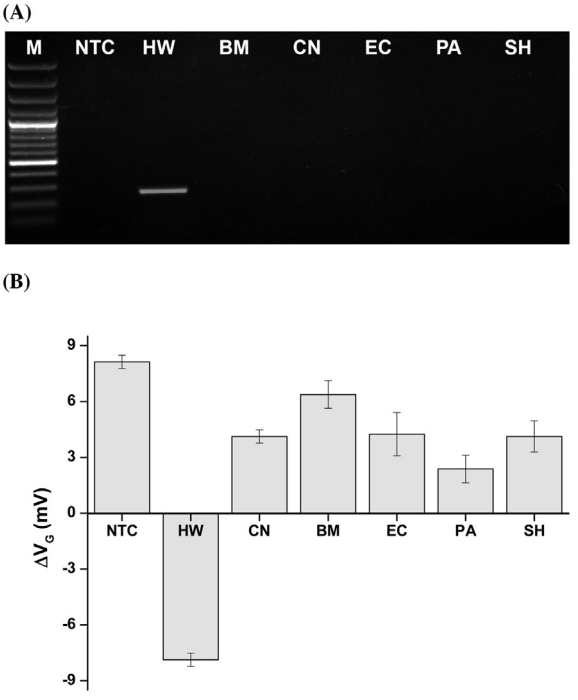

Fig. 6. Specificity testing of the ISFET-based biosensor to detect the *phaE* amplicon. (A) Agarose gel electrophoresis of the PCR product and (B) the ΔV_G of the biosensor. (M) DNA ladder, (NTC) No template control, (HW) *H. walsbyi* DSM 16854 (Positive control), (CN) *C. necator* DSM 428, (BM) *B. megaterium* DSM 319, (EC) *E. coli* ATCC 25922, (PA) *P. aeruginosa* ATCC 9027 and (SH) *S. hygroscopicus* BTCC 7028.

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Table 4. Specificity testing of the ISFET biosensor for detection the *phaE* amplicon.

Sample	ΔV_G (mV)
No template control (NTC)	+8.125 ± 0.354
H. walsbyi DSM 16854	−7.875 ± 0.354
C. necator DSM 428	+4.125 ± 0.354
B. megaterium DSM 319	+6.375 ± 0.744
E. coli ATCC 25922	+4.250 ± 1.165
P. aeruginosa ATCC 9027	+2.375 ± 0.744
S. hygroscopicus BTCC 7028	+4.125 ± 0.835

4. Discussion

Microbiological methods, such as culturing and microscopy, are essential tools for the screening of PHA-producing haloarchaea. These methods require expertise and

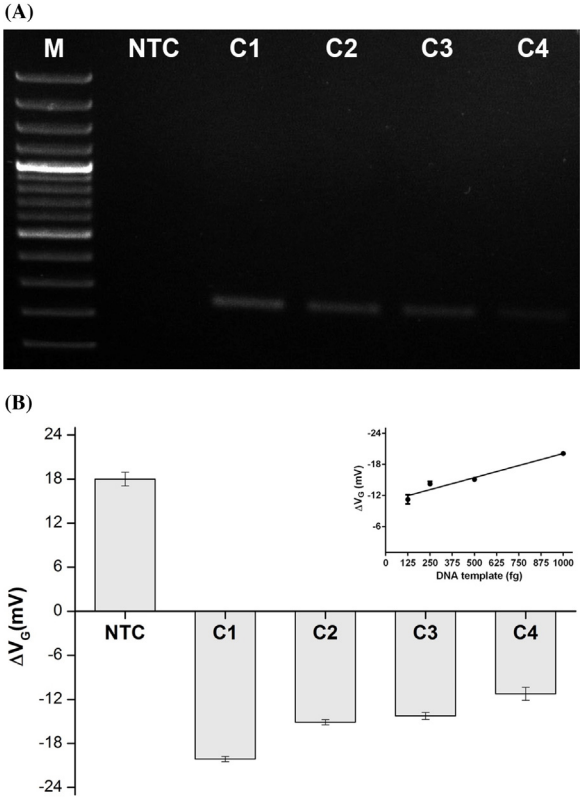

Fig. 7. Sensitivity testing of the ISFET-based biosensor to detect the *phaE* amplicon. (A) Agarose gel electrophoresis of the PCR product and (B) the ΔV_G of the biosensor. (M) DNA ladder, (NTC) No template control, (C1) 1000 fg, (C2) 500 fg, (C3) 250 fg and (C4) 125 fg. Inset is reduced views of the same graph showing data from all concentration of the biotinylated amplicon. The analytical curve was fitted using linear regression model ($R^2 = 0.9290$).

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Table 5. Sensitivity testing of the ISFET biosensor for detection the *phaE* amplicon.

Sample	DNA template for PCR (fg)	ΔV_G (mV)
NTC	0	+18.000 ± 0.926
C1	1000	−20.125 ± 0.354
C2	500	−15.125 ± 0.354
C3	250	−14.250 ± 0.463
C4	125	−11.250 ± 0.886

time to perform the experiments. Fast and accurate methods, such as the DNA lateral flow biosensor [7] and ISFET-based biosensor, have been developed for screening of the PHA-producing haloarchaea. The ISFET-based biosensor is a promising application for direct electrical detection, and this approach does not require a complicated procedure and has high detection sensitivity [26]. Resistance of the ISFET channel depends on the electric field that activates the insulated gate membrane or sensing membrane. The electric field is generated by an accumulation of charged molecules from a reaction solution [27]. The intrinsic carrier, such as an electron that is available in the ISFET channel, can be moved when the sensing membrane of the ISFET binds to a charged target molecule [26]. In the present study, the ISFET-based DNA-sensor is an immunoFET (IMFET) biosensor. This biosensor was developed for the detection of a biotinylated PCR product. The schematic of the IMFET biosensor development is shown in Fig. 8. The IMFET biosensor utilizes the anti-biotin antibody as a biorecognition molecule and use PA as a cross-linker. There are two essential steps for construction of the IMFET biosensor. The first step is PA immobilization and the second step is binding of anti-biotin antibody onto the PA layer (Fig. 8). The aims of using PA as a cross-linker because it adsorbs well on solid surface and has a binding specificity to the Fc fragment of IgG [24, 25, 28]. Mouse monoclonal IgG_{2b} of the anti-biotin antibody was a selected isotype for the experiment because it has strong affinity binding to PA [25]. Fab fragment of the antibody was turned up when Fc fragment was bound onto the PA layer (Fig. 8). Therefore, a positive result was obtained when the biotinylated PCR product was bound to the anti-biotin antibody (Fig. 8). Accumulation of the negatively charged DNA molecules (PCR product) affected the electrical field across the sensing membrane of IMFET, which resulted in a decrease in the ΔV_G. However, the ΔV_G of IMFET was increased when there was no PCR product bound to the sensing membrane. Only the biotinylated primer accumulated onto the sensing membrane of IMFET (Fig. 8). Ionic strength of the buffer is one important parameter that affects to the sensitivity of ISFET [16]. Consequently, the Debye radius (λ_D) should be considered before performed the experiments. It is necessary for ISFET measurements because the biological

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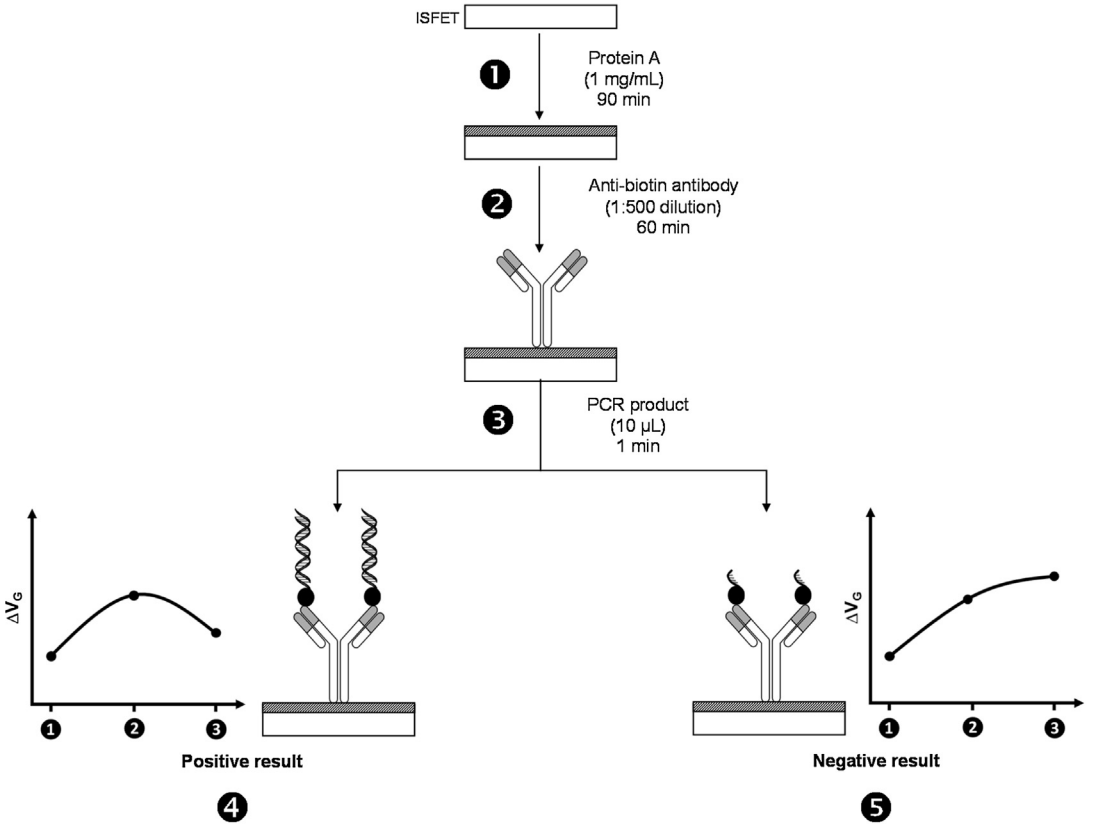

Fig. 8. Schematic of the IMFET biosensor construction. (1) PA was immobilized onto the sensing membrane of ISFET. (2) Anti-biotin antibody was adsorbed onto the PA layer. (3) Detection of biotinylated amplicon. (4) Biotinylated PCR product bound to the IMFET biosensor (Positive result) and (5) the biotinylated primer bound to IMFET biosensor (negative result).

sensing should take place within the λ_D [21]. Therefore, a low ionic strength buffer such as PBS had been applied for FET measurement. Moreover, the sensing membrane of the IMFET biosensor can be cleaned with a Piranha solution [29, 30]. The transducer of the biosensor can be reused several times by re-immobilization of PA and the anti-biotin antibody onto the cleaned sensing membrane. The reusing of the transducer led to reduce the cost of IMFET biosensor production.

The IMFET biosensor can detect the biotinylated *phaE* amplicon at a lower amount of the DNA template (125 fg) compared with the DNA lateral flow biosensor (250 fg [7]). It was found that the degree of linearity of the IMFET biosensor signal was influenced by the concentration of the biotinylated amplicon (Fig. 7B). The IMFET biosensor can also be utilized as a platform to detect biotinylated PCR products. The apparatus for this platform is smaller than an electrophoresis apparatus. The biosensor is simple to use and requires a short response time (1–10 min) to detect biotinylated PCR products. Therefore, the IMFET biosensor may be integrated into a lab-on-chip to detect biotinylated amplicons.

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Declarations

Author contribution statement

Wannaporn Muangsuwan: Performed the experiments; Analyzed and interpreted the data.

Chamras Promptmas, Wutthinan Jeamsaksiri, Prapimpun Wongchitrat: Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

Win Bunjongpru, Awirut Srisuwan, Charndet Hruanun, Amporn Poyai: Contributed reagents, materials, analysis tools or data.

Montri Yasawong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

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Additional information

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References

[1] K.B. Mullis, F.A. Faloona, Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction, Methods Enzymol. 155 (1987) 335–350.

[2] C.G. Haanshuus, S. Chandy, A. Manoharan, R. Vivek, D. Mathai, D. Xena, et al., A high malaria prevalence identified by PCR among patients with acute undifferentiated fever in India, PLoS One 11 (7) (2016) e0158816.

[3] T.W. Prins, I.M. Scholtens, A.W. Bak, J.P. van Dijk, M.M. Voorhuijzen, E.J. Laurensse, et al., A case study to determine the geographical origin of

 http://dx.doi.org/10.1016/j.heliyon.2016.e00188

unknown GM papaya in routine food sample analysis, followed by identification of papaya events 16-0-1 and 18-2-4, Food Chem. 213 (2016) 536–544.

[4] M. Yasawong, S. Areekit, A. Pakpitchareon, S. Santiwatanakul, K. Chansiri, Characterization of thermophilic halotolerant *Aeribacillus pallidus* TD1 from Tao Dam hot spring, Thailand, Int. J. Mol. Sci. 12 (8) (2011) 5294–5303.

[5] M.J. Espy, J.R. Uhl, L.M. Sloan, S.P. Buckwalter, M.F. Jones, E.A. Vetter, et al., Real-time PCR in clinical microbiology: applications for routine laboratory testing, Clin. Microbiol. Rev. 19 (1) (2006) 165–256.

[6] K.A. Harris, J.C. Hartley, Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service, J. Med. Microbiol. 52 (Pt 8) (2003) 685–691.

[7] W. Muangsuwan, P. Ruangsuj, P. Chaichanachaicharn, M. Yasawong, A novel nucleic lateral flow assay for screening of PHA-producing haloarchaea, J. Microbiol. Methods. 116 (2015) 8–14.

[8] B.H. Rehm, Polyester synthases: natural catalysts for plastics, Biochem. J. 376 (Pt 1) (2003) 15–33.

[9] J. Fritz, E.B. Cooper, S. Gaudet, P.K. Sorger, S.R. Manalis, Electronic detection of DNA by its intrinsic molecular charge, Proc. Natl. Acad. Sci. U. S. A. 99 (22) (2002) 14142–14146.

[10] K.Y. Park, Y.S. Sohn, C.K. Kim, H.S. Kim, Y.S. Bae, S.Y. Choi, Development of FET-type albumin sensor for diagnosing nephritis, Biosens. Bioelectron. 23 (12) (2008) 1904–1907.

[11] S.V. Dzyadevych, A.P. Soldatkin, A.V. El'skaya, C. Martelet, N. Jaffrezic-Renault, Enzyme biosensors based on ion-selective field-effect transistors, Anal. Chim. Acta. 568 (1–2) (2006) 248–258.

[12] X.L. Luo, J.J. Xu, W. Zhao, H.Y. Chen, A novel glucose ENFET based on the special reactivity of MnO_2 nanoparticles, Biosens. Bioelectron. 19 (10) (2004) 1295–1300.

[13] W. Torbicz, D.G. Pijanowska, M. Dawgul, Urea biosensors and their application in hemodialysis–perspective of EnFET application, Front. Med. Biol. Eng. 10 (2) (2000) 139–145.

[14] K.S. Song, G.J. Zhang, Y. Nakamura, K. Furukawa, T. Hiraki, J.H. Yang, et al., Label-free DNA sensors using ultrasensitive diamond field-effect transistors in solution, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 74 (4 Pt 1) (2006) 041919.

 http://dx.doi.org/10.1016/j.heliyon.2016.e00188

[15] E. Eteshola, M.T. Keener, M. Elias, J. Shapiro, L.J. Brillson, B. Bhushan, et al., Engineering functional protein interfaces for immunologically modified field effect transistor (ImmunoFET) by molecular genetic means, J. R. Soc. Interface 5 (18) (2008) 123–127.

[16] R.B. Schasfoort, R.P. Kooyman, P. Bergveld, J. Greve, A new approach to immunoFET operation, Biosens. Bioelectron. 5 (2) (1990) 103–124.

[17] P. Bergveld, Development of an ion-sensitive solid-state device for neurophysiological measurements, IEEE Trans. Biomed. Eng. 17 (1) (1970) 70–71.

[18] M.J. Schoning, A. Poghossian, Recent advances in biologically sensitive field-effect transistors (BioFETs), Analyst 127 (9) (2002) 1137–1151.

[19] N. Hom, C. Promptmas, K. Wat-Aksorn, Detection of DNA hybridization using protein A modified ion sensitive field effect transistor, Anal. Lett. 48 (7) (2014) 1128–1138.

[20] D.S. Kim, Y.T. Jeong, H.J. Park, J.K. Shin, P. Choi, J.H. Lee, et al., An FET-type charge sensor for highly sensitive detection of DNA sequence, Biosens. Bioelectron. 20 (1) (2004) 69–74.

[21] C.S. Lee, S.K. Kim, M. Kim, Ion-sensitive field-effect transistor for biological sensing, Sensors (Basel) 9 (9) (2009) 7111–7131.

[22] G. Scarpa, A.L. Idzko, A. Yadav, S. Thalhammer, Organic ISFET based on poly (3-hexylthiophene), Sensors (Basel) 10 (3) (2010) 2262–2273.

[23] M. Graille, E.A. Stura, A.L. Corper, B.J. Sutton, M.J. Taussig, J.B. Charbonnier, et al., Crystal structure of a *Staphylococcus aureus* protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity, Proc. Natl. Acad. Sci.U. S. A. 97 (10) (2000) 5399–5404.

[24] M. Uhlen, B. Guss, B. Nilsson, S. Gatenbeck, L. Philipson, M. Lindberg, Complete sequence of the staphylococcal gene encoding protein A: a gene evolved through multiple duplications, J. Biol. Chem. 259 (3) (1984) 1695–1702.

[25] D.A. Palmer, M.T. French, J.N. Miller, Use of protein A as an immunological reagent and its application using flow injection. A review, Analyst 119 (12) (1994) 2769–2776.

[26] A. Kim, C.S. Ah, C.W. Park, J.H. Yang, T. Kim, C.G. Ahn, et al., Direct label-free electrical immunodetection in human serum using a flow-through-

apparatus approach with integrated field-effect transistors, Biosens. Bioelectron. 25 (7) (2010) 1767–1773.

[27] M. Yuqing, G. Jianguo, C. Jianrong, Ion sensitive field effect transducer-based biosensors, Biotechnol. Adv. 21 (6) (2003) 527–534.

[28] T. Moks, L. Abrahmsen, B. Nilsson, U. Hellman, J. Sjoquist, M. Uhlen, Staphylococcal protein A consists of five IgG-binding domains, Eur. J. Biochem. 156 (3) (1986) 637–643.

[29] A. Hai, D. Ben-Haim, N. Korbakov, A. Cohen, J. Shappir, R. Oren, et al., Acetylcholinesterase-ISFET based system for the detection of acetylcholine and acetylcholinesterase inhibitors, Biosens. Bioelectron. 22 (5) (2006) 605–612.

[30] P. Saengdee, W. Chaisriratanakul, W. Bunjongpru, W. Sripumkhai, A. Srisuwan, W. Jeamsaksiri, et al., Surface modification of silicon dioxide, silicon nitride and titanium oxynitride for lactate dehydrogenase immobilization, Biosens. Bioelectron. 67 (2015) 134–138.

 http://dx.doi.org/10.1016/j.heliyon.2016.e00188