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CARBOXYLATED MULTI-WALLED CARBON NANOTUBES MODIFIED SCREEN-PRINTED ELECTRODES FOR EARLY DETECTION OF RICE DISEASES USING IMMUNOSENSOR APPROACH

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ABSTRACT

Normal practice in detecting rice disease relies on symptoms' confirmation by pathologists. Serological and molecular analysis, on the other hand, require the infected samples to be sent to the laboratories, are time-consuming, and only can be operated by technical personnel. In recent years, the electrochemical biosensor technique has been gaining attention in rapid test analysis owing to the system's practicality, portability, and simplicity of construction. We described an antibody-based electrochemical biosensor development for the early detection of three major rice diseases in Malaysia, namely bacterial leaf blight (BLB), bacterial leaf streak (BLS) and blast. Polyclonal antibodies against causal agents of the diseases, i.e., *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) and *Pyricularia oryzae* were produced and purified in-house. Modification of screen-printed carbon electrodes (SPCEs) was achieved via electrodeposition of functionalized multi-walled carbon nanotubes (MWCNTs) and polypyrrole (Ppy) network on the surface, followed by antibody immobilization. Using an enzyme labelled primary antibody via chronoamperometry measurement, the functionalized SPCEs showed good R^2 values in the standard curves' development. The immunosensor technique exhibited a good correlation of more than 80% with the polymerase chain reaction (PCR) method for all artificially infected samples. A stability study showed that the functionalized SPCEs are stable for up to 44 weeks at 4ºC storage for a bacterial concentration of 10^4 CFU mL^{-1}.

Keywords: early detection, electrochemical biosensor, immunosensor, multi-walled carbon nanotubes, rice diseases

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of Asians and the crops are cultivated in most Asia regions. Unfortunately, its production is constrained by the recurrent attacks of several diseases, leading to yield and economic losses. In Malaysia, rice diseases may cause a susceptible variety's crop yield loss as high as 70% [1]. Normal practise in rice disease detection relies on symptom confirmation by plant pathologists. However, the diseases are yet to be determined scientifically for precise identification of plant pathogens. In the laboratory, techniques for rice disease detection include the conventional agar plate method, serological assay, enzyme-linked immunosorbent assay (ELISA), a nucleic acid-based technique such as multiplex polymerase chain reaction (PCR), real-time PCR and loop-mediated isothermal amplification (LAMP) [2]. Albeit

those aforementioned techniques are widely accepted, with the PCR method regarded as a gold standard and sensitive, the major drawbacks are that they require trained personnel, costly and time-consuming. Latest trends employing advanced image processing techniques and machine-learning technology also have been widely reported for rice disease detection [2-5]. These non-destructive methods, however, have limitations in terms of data volume, noises from the sensor and calibration errors; besides being unable to differentiate between very low infection and healthy plants. With regard to this, a detection method that fulfils the requirement of being rapid, sensitive, reliable, ready to be performed on-site and user-friendly is hence being sought.

The biosensor method is gaining tremendous interest among researchers due to its practicality (ease of use and user-friendliness), portability and simplicity of construction. It offers advantages over conventional methods, including rapid and sensitive detection for practical on-site application. The biosensor principle lies in binding of the bioreceptors on a transducer surface with their specific analytes; these interactions are then converted to a measurable signal. The electrochemical biosensor is favoured over other classes of biosensor (e.g., optical sensor) as the signal generated is not susceptible to light interference (i.e., colour changes), portability on a custom-made handheld device, low cost (i.e., local fabricated plastic-based electrodes) and user-friendly [6]. Its vast applications include medicine/clinical [7], food safety [8], environment [9] and agricultural plant disease management [10]. Antibody-based biosensors, also known as immunosensor, employ antibodies as their biosensing element. Immunosensor has great potential for detecting agricultural plant pathogen as the detection mechanism is viable in various matrix such as water, leaves, and seeds with on-site application feature [11].

We report here the development of an electrochemical immunosensor for the detection of three major rice diseases in Malaysia, namely bacterial leaf blight (BLB), bacterial leaf streak (BLS) and blast. The causal agents for the diseases are listed in Table 1; *Xanthomonas* spp. is bacteria causing both rice BLB and BLS, while *P. oryzae* is an ascomycete fungus responsible for the fatal rice blast disease.

Table 1. Major rice diseases highlighted in the study with their causal pathogens.

Rice Disease	Causal agents
Bacterial leaf blight (BLB)	*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)
Bacterial leaf streak (BLS)	*Xanthomonas oryzae* pv. *oryzicola* (*Xoc*)
Blast	*Pyricularia oryzae*

In this study, polyclonal antibodies against the rice disease agents were produced in-house and utilized as bioreceptors in immunosensor development. The working electrode area of screen-printed carbon electrodes (SPCEs) was first modified with conducting electroactive polymer and nanomaterial network for antibody immobilization. Visual and electrochemical characterizations of the SPCEs' surfaces were performed to assess the modification procedure. Standard curves of the pathogens were established with optimized parameters and the immunosensor system developed was then applied to real samples with artificially infected pathogens.

MATERIALS AND METHOD

The summary of research activities conducted in the rice disease immunosensor development is depicted in Figure 1 as follows.

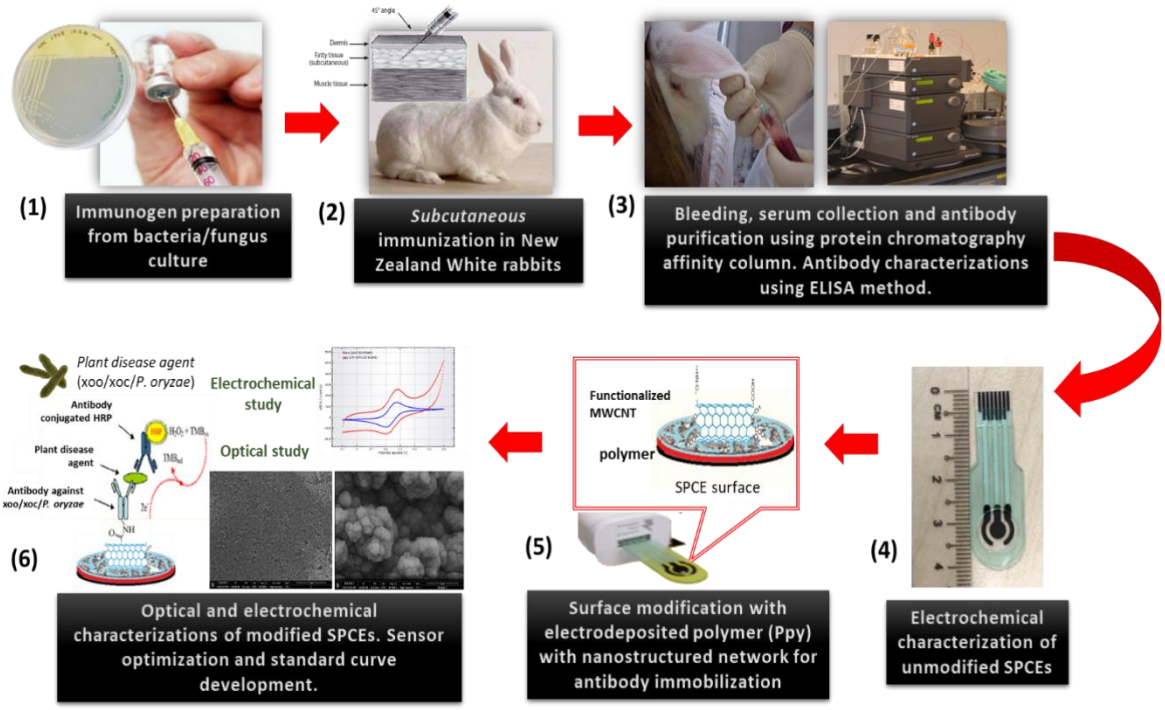

Figure 1. Workflow for research activities involved in the development of rice disease immunosensor.

Polyclonal antibodies against rice diseases agents

Polyclonal antibodies against *Xoo*, *Xoc* and *P. oryzae* were developed in-house at Biotechnology & Nanotechnology Research Centre, MARDI. All animal procedures involving rabbits were approved by the MARDI Animal Ethics Committee (approval number 20171103/R/MAEC27). The immunization protocol was performed based on guidelines by Leenars & Hendriksen (2005) [12]. In total, ten immunization shots were performed, and blood was collected until the seventh bleed.

Chemicals and biological reagents

Xoo and *P. oryzae* cultures of identified strains were obtained from MARDI Seberang Perai. *Xoc* cultures were procured from the National Collection of Plant Pathogenic Bacteria (NCPPB), UK. Standard chemicals used in the study were purchased from Sigma-Aldrich, USA, unless stated otherwise. *N*-(3-Dimethylaminopropyl) - *N*'-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were from Sigma-Aldrich, Japan. Functionalized multi-walled carbon nanotubes (MWCNT-COOH) were from Dropsens (Spain), while plastic-based SPCEs were from Biogenes Technologies Sdn. Bhd. (Malaysia). Blotto, non-fat dry milk was from Santa Cruz

Biotechnology and EZ-Link™ Plus Activated Peroxidase Kit for enzyme-antibody conjugation was from Thermo-Scientific, USA. All solutions were prepared using deionized (DI) water from Sartorius Arium® pro VF Ultrapure Water System with a resistivity of 18.2 MΩcm or conductivity of 0.05 μS/cm.

Modification of SPCE surface and characterizations

The electrodeposition of Ppy and functionalized-MWCNT on SPCEs was achieved by one-step electropolymerization of 0.075 M pyrrole and 0.1 mg mL^{-1} MWCNT-COOH via chronoamperometry technique in PBS (900 s, fixed potential at 1.0 V). The carboxylic acid group of MWCNT-COOH was activated in the EDC-NHS mixture (1:1) for 15 mins before polyclonal antibodies against the plant pathogens were immobilized on the electrode surface [13]. For optical characterization, field emission scanning electron microscope (FESEM) and energy dispersive X-ray (EDX) analysis were carried out at INFRA Analysis Laboratory, University Malaya, Kuala Lumpur. FESEM images were taken on a Quanta FEG 450, while EDX was performed by EDX-OXFORD.

Electrochemical measurements were performed via cyclic voltammetry (CV) and chronoamperometry (CA) on an Autolab PGSTAT 20 potentiostat (Eco Chemie, Netherlands) with NOVA 1.10 software. A potential scanning study of *Xoc*, *Xoo*, and *P. oryzae* detection at a range potential from -0.6 V to +0.6 V were performed to find the set potential that will be applied in the chronoamperometry method. The experimental set-up for set potential determination is adapted from Bunawan et al. (2016) [14].

Real sample application and validation study

The sample application was performed on artificially inoculated rice plants of selected varieties carried out in a controlled netted house environment at MARDI Serdang and MARDI Seberang Perai for BLB and BLS disease, respectively. As a controlled study, sterile distilled water was used for the inoculation instead of bacterial cultures. The experiments were carried out in triplicates and the plants were arranged in random orientations. The symptoms development was observed for 14 days-after-inoculation (DAI), and rice leaves were collected on 1, 3, 5, 7 and 15 DAI. For blast disease, 15 different rice varieties were planted in the blast hotspot at the MARDI Seberang Perai rice plot; the sample was taken on week 6 after sowing.

Samples were analyzed using the functionalized strips with simple sample extraction (Figure 2). Briefly, rice leaves were cut to 7 cm and washed with 80% ethanol. The leaves were then rinsed and immersed in deionized water before being cut into small pieces and re-immersed in 0.1 M carbonate-bicarbonate buffer, pH 9.6. A 10 μL of the sample aliquot was then dropped on the modified SPCEs, and electrochemical measurements were taken. For the validation study, ELISA and polymerase chain reaction (PCR) (Bio-Rad thermocycler) methods were used to compare the analyzed results.

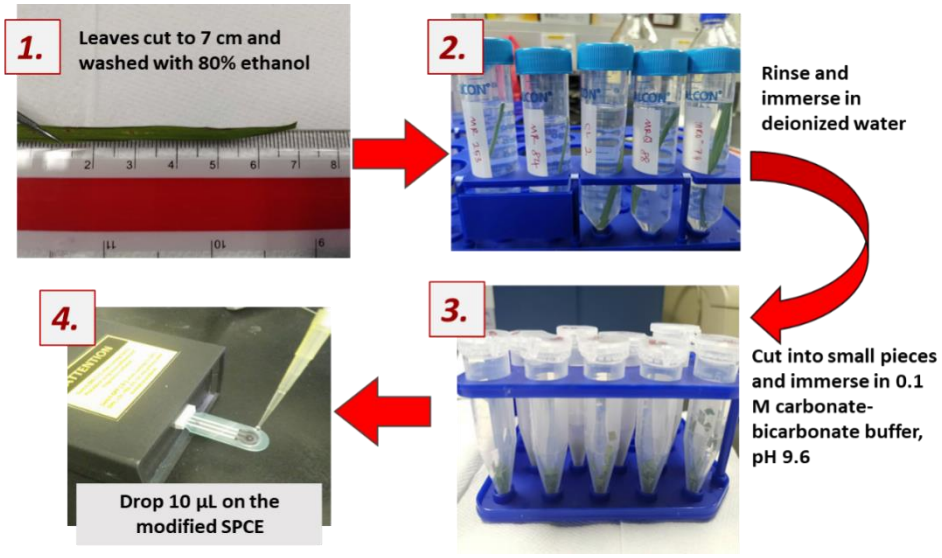

Figure 2. Rice leaf sample preparation for electrochemical immunosensor measurements on the modified SPCEs.

Storage stability study

This study used a BLS sensor employing antibodies against *Xoc* on the SPCEs as a model. The functionalized SPCEs' stability study was carried out at two different storage conditions of 4ºC and room temperature (RT) for 48 weeks. Two sets of bacterial concentrations were studied for the storage, i.e., a low bacterial concentration of 10^4 CFU mL^{-1} and a high bacterial concentration of 10^8 CFU mL^{-1}. Sensor strips were analyzed weekly for the first three months and every fortnightly for the subsequent months.

RESULTS AND DISCUSSION

Rice BLB disease is regarded as the oldest bacterial rice disease in Asia; its attack is reported to reduce rice production by up to 81%. The discovery of seed-borne rice BLS, on the other hand, is more recent and was observed in the hybrid rice cultivars in Asia [15]. BLS disease may decrease yield by as much as 30%, and its occurrence in Africa has been associated with climate change issues [16]. At the early stage, BLS infection shows symptoms of water-soaked lesions that develop into translucent, yellow interveinal streaks of various lengths along the rice leaf blades (Fig. 3b). The problem arises later at advanced stages, both diseases are difficult to distinguish based on symptoms' identification (Fig.3b and 3d). BLS and BLB can occur simultaneously on rice plants and since the pathogens are closely related, it is difficult to differentiate them morphologically and genetically; hence, the need for rice disease sensors for accurate detection of the pathogens. SEM images captured for the *Xoc* and *Xoo* (Fig. 3c and 3e) exhibited rod-shaped bacterium and polar flagellated as described by Chompa et al. (2022) [17] with the approximate size of 0.55-0.75 x 1.35-2.17 µm.

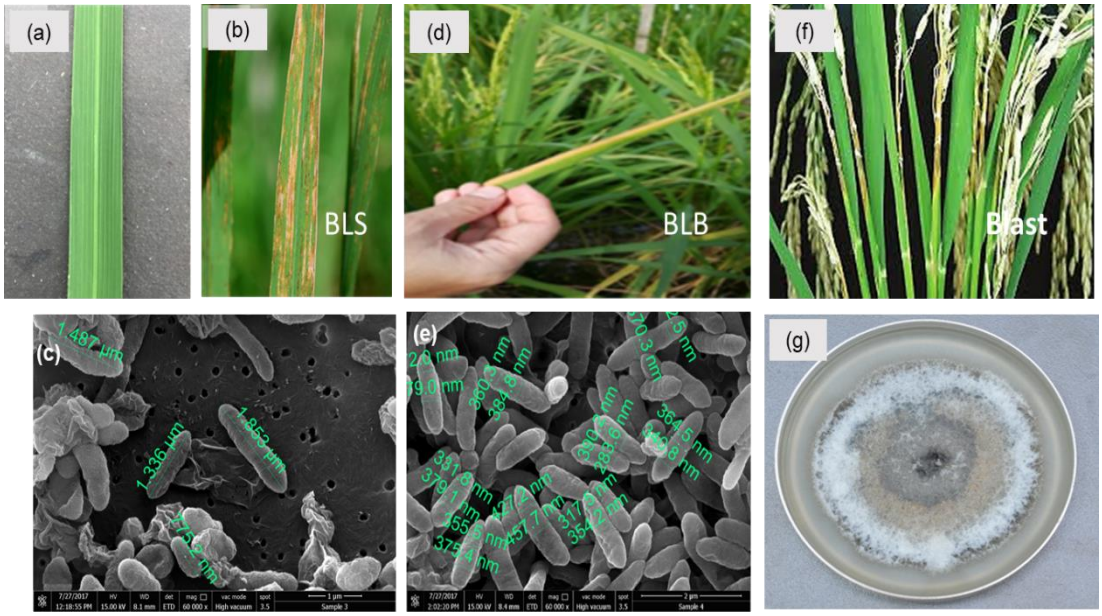

Figure 3. Picture of (a) a healthy rice leaf; infected rice leaf with (b) bacterial leaf streak (BLS) with SEM image of its causal bacteria, (c) *Xoc* (magnification 60,000x, scale bar 1 µm); infected rice leaf with (d) bacterial leaf blight (BLB) with the SEM image of its causal bacteria, (e) *Xoo* (magnification 60,000x, scale bar 2 µm); and infected leaves and panicles with (f) blast disease with the causal fungus culture, (g) *P.oryzae*.

For rice blast disease, there are two types of blast commonly found in Malaysia, namely, foliar/leaf blast and panicle blast (Fig. 3f). *P. oryzae* produces many sexual spores (conidia) dispersed by wind and water to a susceptible host, causing new infections. Upon infection, blast symptoms can be either lesions or spots. Unfortunately, these symptoms vary depending on the environmental conditions and the host plants' resistance level. Due to the uncertainties in accurately determining the pathogens and rice disease solely based on the symptoms, immunosensor thus lends itself well as a precise detection tool in the identification of on-site plant pathogens, especially at early stages.

Polyclonal antibody characterization

Selective strains of the most pathogenic and major pathovar/patotypes were used in the antibody development. Purified antibodies were first characterized via enzyme-linked immunosorbent assay (ELISA). As reported before, the antibody against rice bacterial disease exhibited ten to forty-times higher activity compared to the pre-immune antibody (control antibody) at an antibody concentration of 0.01 mg mL^{-1} [18], and a high titer at 1:100000 dilution ratio was successfully achieved [19]. As for antibodies against blast disease, the antibody from 4th bleed showed the highest activity than the other bleed batch. Its activity was found 8-9 times higher than the pre-immune antibody at an antibody concentration of 0.01 mg mL^{-1} (data not shown). In general, antibodies from the third bleed onward will be used in subsequent studies.

A specificity study of the antibodies also was performed by ELISA. Anti-*Xoo* and anti-*Xoc* antibodies showed the highest reaction with their respective strains, followed by

other *Xanthomonas* spp. (16.7%). No cross-reactions were observed with other plants bacterial such as *Pseudomonas* spp. and *Pantoea* spp. As for anti-*P. oryzae* antibody, while the antibody is specific towards *P. oryzae* itself, it also showed 60% of cross-reaction with *Rhizoctonia solani*, *Helminthosporium oryzae* and *Soracladium oryzae*. However, no cross-contamination between *Xoo* and *Xoc* was observed.

Modified SPCEs for rice disease detection

As the surface of SPCEs contains chemically inert carbon conductive ink, the surface needs to be functionalized first to increase its sensitivity and enhance the current generated from biological interactions. Surface modification was achieved by electrodeposition of multi-walled carbon nanotubes (MWCNTs) and polypyrrole (Ppy), allowing precise and controllable polymer coating formation over the electrode surface for antibody immobilization.

Surface modification of the SPCEs was performed by electrodepositing functionalized MWCNTs and Ppy, followed by antibody immobilization via EDC-NHS as cross-linking agent (Figure 4). The mild condition of Ppy does not affect the nature or activity of most biological elements immobilized on the polymer matrix in biosensor application [20]. The incorporation of MWCNTs helps to induce carbon enhancement for signal amplification due to the conductive properties of Ppy and the high surface area of the carboxylated functionalized-MWCNT. The functional groups introduced within these films help preserve the hydration layer of antibodies in the polymer chains.

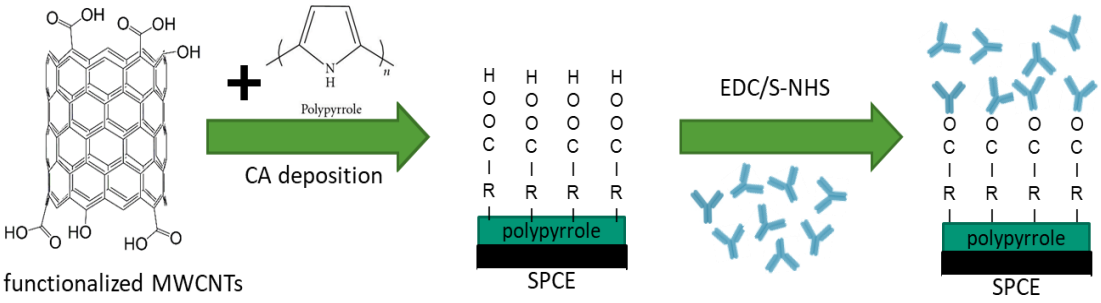

Figure 4. Schematic diagram of electrodeposition of functionalized-MWCNTs and polypyrrole (Ppy) achieved via chronoamperometry (CA) technique on SPCE, followed by antibody immobilization.

Comparison between modified and unmodified SPCEs was observed electrochemically and visually. Cyclic voltammetry (CV) response of an unmodified and modified SPCE with functionalized MWCNTs/Ppy was measured in a redox solution of 1 mM ferrocenecarboxylic acid (FCA) in PBS 0.01 M, pH 7.4 (Figure 5A). Peak current was observed to increase significantly from 14.6 µA to 50 µA after modification, indicating the successful formation of the electrodeposited nanomaterial-polymer composite on the SPCEs. SEM images (Figure 5B) showed a typical cauliflower structure associated with Ppy [21,22], which can be seen after surface modification. EDX analysis (data not shown) confirmed an increase of 26.3% in nitrogen and a decrease of 34.8% in carbon upon the MWCNT/Ppy network formation. The increase in nitrogen is derived from the base unit of pyrrole, consisting of a five-membered heteroaromatic ring

containing nitrogen. The large binding site of carboxylated MWCNT allows more antibody attachment on the modified electrode surface through EDC-NHS crosslink, thus increasing the sensitivity of the immunosensor.

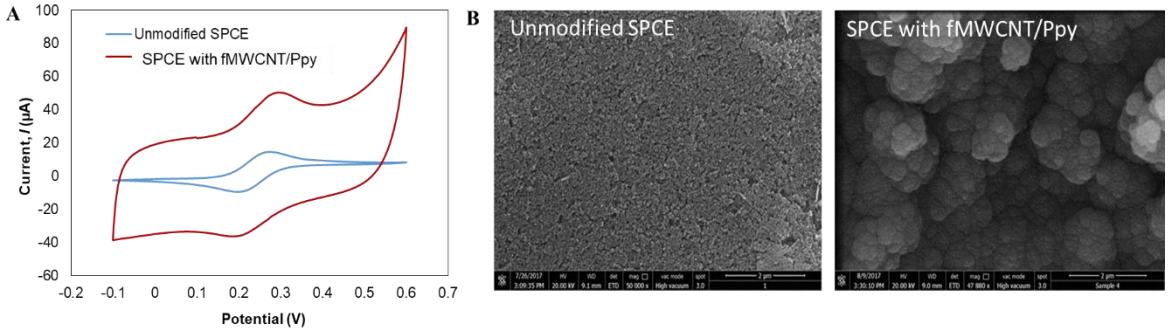

Figure 5. (A) Cyclic voltammetry (CV) response for unmodified (blue line) and modified SPCE with electrodeposited funtionalized MWCNTs/Ppy (red line) in 1mM ferrocenecarboxylic acid (FCA) in PBS 0.01 M, pH 7.4; (B) SEM images of SPCE surface for unmodified SPCE and modified SPCE surfaces (Magnification of 50,000x, scale bar 2 μm).

Besides SPCEs, the application of MWCNTs with zinc nanocomposites on glassy carbon electrodes has also been reported for detecting chili virus disease using the DNA-based biosensor method [23]. This study's electrochemical immunosensor system developed for rice disease detection was adapted from ELISA format based on sandwich immunoassay. Horseradish peroxidase (HRP) was employed as the enzyme label for the antibody, and TMB/H_2O_2 as the substrate/mediator system (Figure 6). The current generated from the enzyme-labelled antibody with its substrate (TMB) was recorded and measured accordingly via chronoamperometry technique at a fixed set potential.

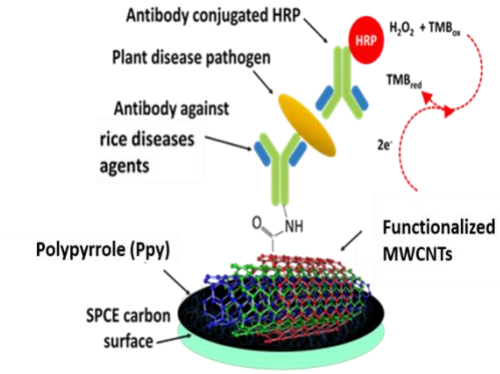

Figure 6. Schematic diagram of electrochemical immunosensor detection employing enzyme-linked immunosorbent assay (ELISA) format used for rice disease detection on a modified SPCE.

Sensor optimization

In further assay analysis, the dilution for *Xoo* and *Xoc* cell cultures as well as the *P. oryzae* conidias, will be carried out in 0.1 M carbonate-bicarbonate buffer pH 9.6 as this choice of buffer was found to have a lower background current as opposed to 0.01 M

PBS, pH 7.4 [24]. Besides that, 0.1% ethanolamine was chosen as a blocking buffer as ethanolamine was found to reduce the non-specific binding in the sensor development by 70% compared to 0.5% BSA [13].

Calibration curve development

The optimal potential for the current signal generated from the antibody-labelled HRP enzyme with TMB substrate was studied via the chronoamperometry method. The signal current to current background ratio (S/B ratio) was measured within the window potential of -0.6 V to +0.6 V for all BLB, BLS, and blast sensors. The current signal can differentiate between the negative control study (0 CFU mL^{-1} bacteria/spore, i.e., PBS solution) and bacteria/spore of several concentrations (Figure 7). The PBS appears as a flat baseline, whilst the strips with various pathogen concentrations gave out current signal measurements at certain potentials. Although BLB (Fig. 7A) and BLS (Fig. 7B) strips exhibited potential readings at two different points (-0.2 V and -0.1 V for the BLB sensor; -0.1 V and 0 V for the BLS sensor), eventually, the maximum or highest S/B difference with the control's baseline was selected. The optimized set potentials with the highest current generated indicate the maximum active area of the electrons involved in the reaction. For further sensor development study, the best-set potential selected is -0.2 V for BLB; and -01 V is for both BLS and blast sensor.

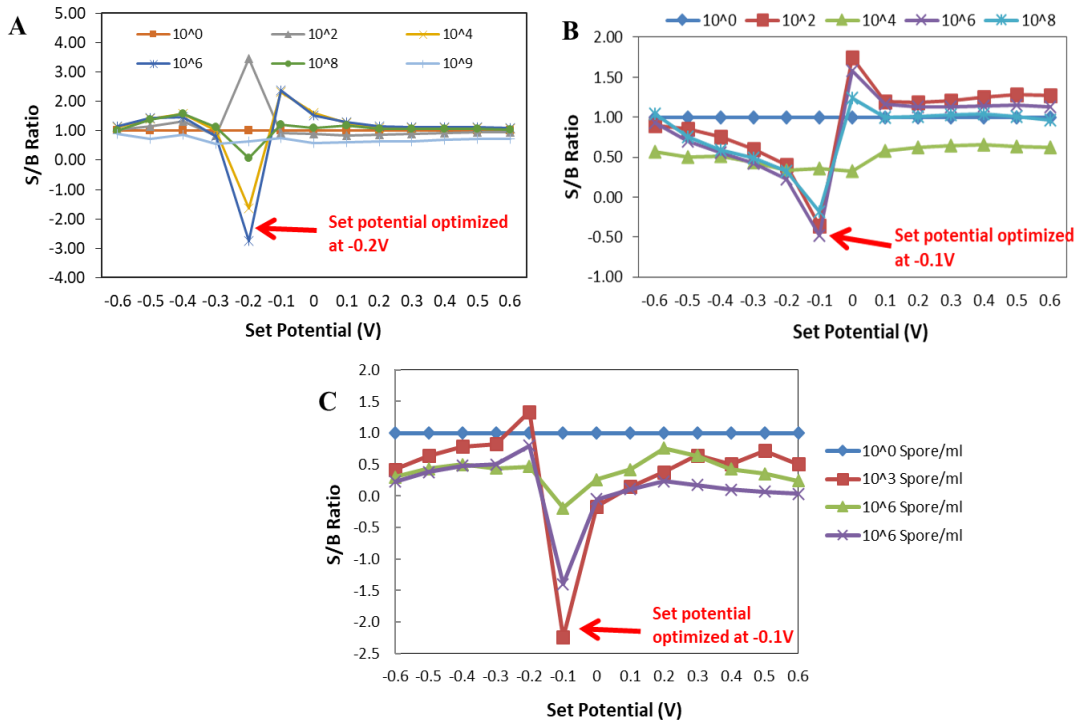

Figure 7. Set potential determination for detection of (A) rice bacterial leaf blight (BLB); (B) rice bacterial leaf streak (BLS); and (C) rice blast by scanning the potential from -0.6 to +0.6 V

Before developing standard curves for each rice disease detection, the optimization of antibody concentration was also conducted to determine the minimum concentration of immobilized antibody on a sensor surface that gave the highest current response. From Figure 8, the antibody was optimized at 0.03 mg mL^{-1} for BLB detection and 0.07 mg mL^{-1} of antibody for both BLS and blast disease detections. These concentrations will be utilized for further sensor development.

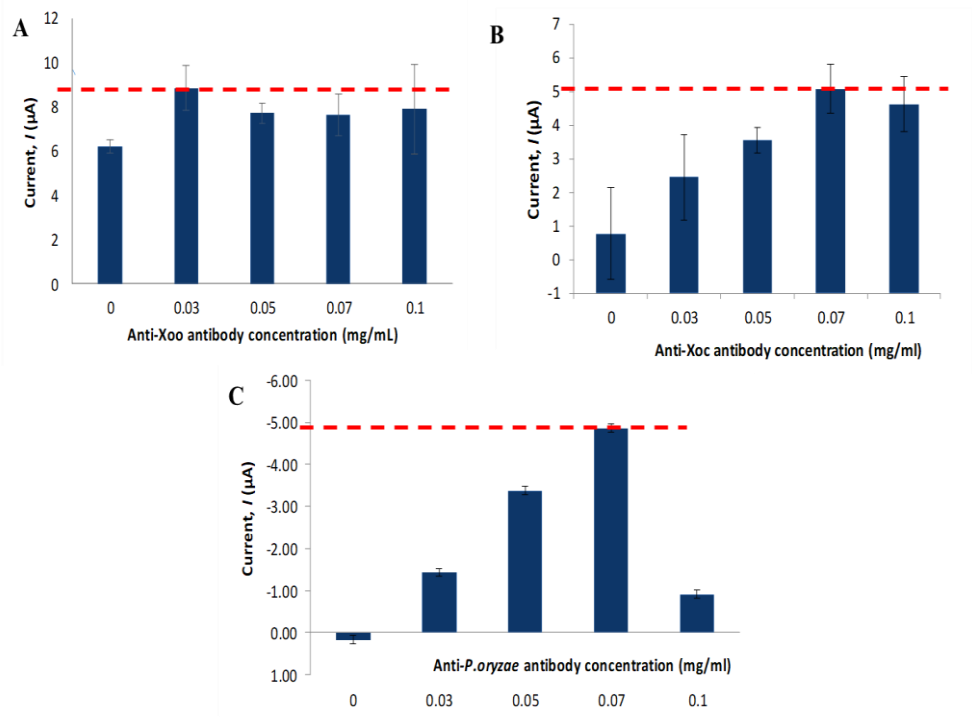

Figure 8. Optimization of antibody concentration on SPCEs for the detection of (A) rice bacterial leaf blight (BLB), (B) rice bacterial leaf streak (BLS); and (C) rice blast.

Standard curves for the respective rice diseases were then developed. Three (3) linear standard curves were successfully obtained for the detection of rice diseases agents for BLB, BLS and blast within the range of 10^2 to 10^8 for *Xoo* and *Xoc* cells (Fig. 9A and 9B) and 10^1 to10^5 conidia spores per mL for *P. oryzae* detection (Fig. 9C). From the standard curves, the lowest limit of detections (LOD) for the modified strips was 10^2 cell mL^{-1} for both *Xoo* and *Xoc*. The immunosensor detection limit for bacteria, albeit at 10^2 CFU mL^{-1}, is still superior when compared with other immuno-based assays, such as the ELISA method, that have detection limits of 10^4-10^5 CFU mL^{-1} [25]. Although Awaludin and co-workers reported a lower detection limit for *Xoo* detection (22 CFU mL^{-1}) with fluorescence-based immunoassay [25], this optical method is light-sensitive and requires a fluorescence-spectrophotometer for signal readout. The latter limited its application to on-field application. For the rice blast sensor, the detection limit for *P. oryzae* conidia achieved in this study is 10^1 spore mL^{-1} and is on par with other immuno-based assay methods that reported LOD of 21.60 conidia mL^{-1} [26].

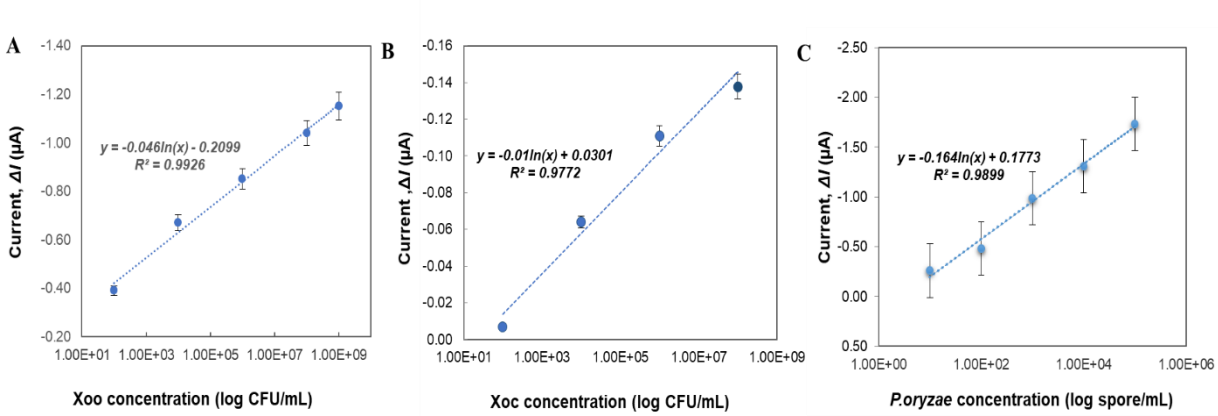

Figure 9. Standard curves development for the detection of (A) rice bacterial leaf blight (BLB), (B) rice bacterial leaf streak (BLS); and (C) rice blast diseases.

These detection limits of 10^2 CFU mL^{-1} for *Xoo* and *Xoc*; and 10^1 spores mL^{-1} for conidia are acceptable and indicate the feasibility of the developed immunosensor as an early warning system tool as symptoms of infected leaves only appear when pathogens' concentration reached up to 10^8 CFU mL^{-1}.

Real sample application

The application of the developed sensor in real samples, i.e., rice leaves, is crucial to assess the matrix interference background and to study the viability of the sensor in detecting rice disease. Although several researchers have reported the development of immunosensor in detecting rice tungro virus [22] and indirect ELISA for rice blast disease detection [26], their work has not been extended to real matrix samples and disease detection. Rice leaves extraction was performed by cutting the leaves into small pieces without the need to grind them. This method has proven to pose less background noise as opposed to the grinding method, as reported before [27]. Real sample applications for the detection of BLB and BLS diseases were carried out based on artificially infected rice plants with their respective bacteria cultures. The PCR method was used to compare the results and validate them. For 41 selected artificially infected rice leaves samples with *Xoo*, the immunosensor for BLB detection showed a 92.7% correlation with the PCR method (data not shown). For BLS disease, on the other hand, the percentage correlation recorded for 90 samples showed an 83.3% correlation with PCR. However, the correlation was higher when compared with the ELISA method (94.4%) as both immunosensor and ELISA are based on antibody format detection [28].

Meanwhile, for rice blast disease, the detection performed on 15 selected varieties showed a correlation of immunosensor and PCR at 80% (Table 2). These good correlations indicate the viability of the sensor developed based on the functionalized SPCEs for rice disease detection. Previously, in 2014, Yang and co-workers [29] reported an early and rapid detection of rice blast disease caused by *Magnaporthe oryzae* by employing its chitinases as biochemical marker and a cDNA encoding lectin as a recognition probe. This highly sensitive electrochemical biosensor is able to detect *M. oryzae* in rice plants within three days of infection. However, to the best of our knowledge, no antibody-based biosensor has been reported so far for detecting rice blast

disease. The sensitivity and selectivity of the blast immunosensor reported here can be improved by incorporating nanomaterials in the sensor development [30].

Table 2. Correlation between rice blast immunosensor and polymerase chain reaction (PCR) towards 15 selected rice varieties at week 6.

Rice Variety	Blast Sensor	PCR
263	+*ve*	+*ve*
220	+*ve*	+*ve*
211	+*ve*	+*ve*
307	+*ve*	+*ve*
303	+*ve*	-*ve*
74	+*ve*	+*ve*
88	+*ve*	+*ve*
76	+*ve*	+*ve*
CL2	+*ve*	+*ve*
84	-*ve*	-*ve*
253	+*ve*	-*ve*
269	+*ve*	+*ve*
232	-*ve*	+*ve*
297	+*ve*	+*ve*
219	+*ve*	+*ve*

To demonstrate the viability of the developed immunosensor, the developed sensor strips were subsequently applied to a portable reader for real-time on-site study. BLB strips were tested on an Android-based portable biosensor device for the detection of BLB in hotspot areas at Selangor Northwest. The sensor showed excellent potential as a monitoring tool as the system can successfully detect rice BLB disease within two weeks after transplant (DAT) before the appearance of infection symptoms [27].

Storage stability study

The functionalized SPCEs were then subjected to a storage stability study. This study is crucial in assessing the sensor's performance using the SPCEs with the readily immobilized antibody at a long storage period. As shown in Figure 10, SPCEs stored at 4ºC gave the most stable reading up to 44 weeks for low bacterial detection of 10^4 CFU mL^{-1}. The performance of SPCEs stored at RT was as good as 4ºC storage until week 40 for low bacterial detection. However, at high bacterial concentrations, the performance of SPCEs stored in both conditions fluctuated and started to decline after 22 weeks.

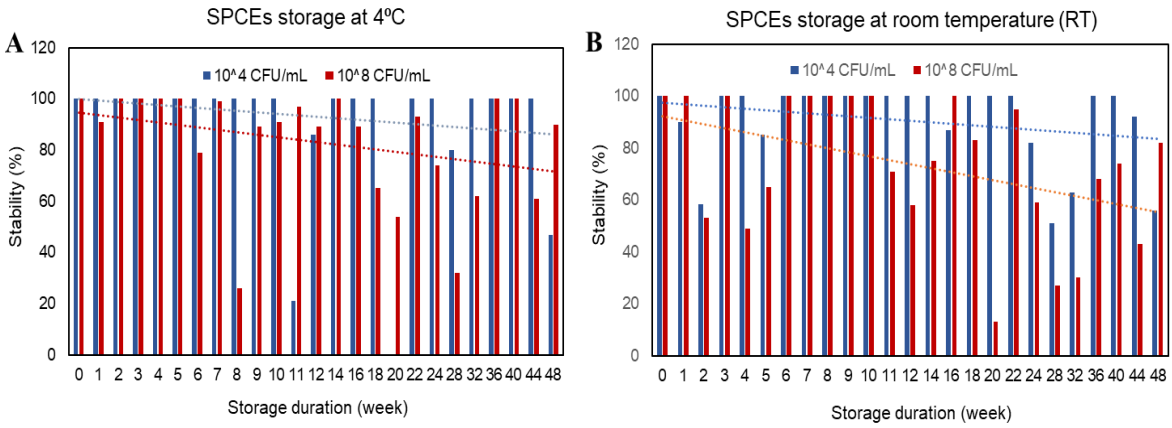

Figure 10. Storage stability of functionalized strips stored at (A) 4ºC and (B) room temperature (RT).

For future on-field application, the surface of the readily prepared functionalized strips can be protected using a single-use cartridge, as depicted in Figure 11. This casing provides protection against scratch or foreign matters on the modified SPCEs, particularly during storage and application on-site.

Figure 11. Picture of single-use cartridge encasing the SPCEs. Picture courtesy of Biogenes Technologies Sdn. Bhd.

Advantages of the developed immunosensor

In general, biosensors exhibited several advantages over other conventional methods for rice disease detection in terms of assay duration, detection limit, qualitative/quantitative detection, and portability (Table 3). The assay duration for the developed immunosensor is much shorter (15 minutes) than PCR and ELISA, which may take 4-5 hours. The detection limit for the developed immunosensor is also lower compared to those of ELISA. Immunosensor can serve as both qualitative and quantitative detection tools. Most importantly, when equipped with a portable handheld reader, the analysis can be performed on-site, thus giving rapid in-situ detection as opposed to the PCR machine and ELISA spectrophotometer that is limited to the laboratory.

Table 3. Comparison between the developed immunosensor and other conventional methods for rice diseases detection.

	Immunosensor	**PCR**	**ELISA**
Assay Duration	15 min	4 hours	5 hours
Detection Limit (CFU/mL or ng)	10^2 CFU mL^{-1}	55 fg	10^4 CFU mL^{-1}
Qualitative	Yes	Yes	Yes
Quantitative	Yes	No	Yes
Portable/on-site Instrumentation	Yes	No	No

CONCLUSION

We have demonstrated the effectiveness of the functionalized SPCEs with MWCNTs and Ppy as an ideal platform for antibody immobilization in developing immunosensors for the detection of rice bacterial leaf blight (BLB), bacterial leaf streak (BLS) and blast disease. By using a dilution buffer of 0.1 M carbonate-bicarbonate buffer, pH 9.6, and 0.1% ethanolamine, a lower background current was achieved, giving more significant current changes. Integration of nanomaterials (i.e., MWCNT) and conducting electroactive polymer networks (Ppy) have greatly improved the biosensor system's sensitivity and selectivity. The limit of detections (LODs) achieved using the developed sensors is 10^2 CFU mL^{-1} for *Xoo* and *Xoc* detection and 10^1 spores mL^{-1} for *P. oryzae*. For an antibody-based method, the LODs are considered low and acceptable, as the symptoms of infected leaves only appear when the bacterial concentration exceeds 10^8 CFU mL^{-1}. A good correlation study with the PCR technique on the inoculated sample indicates the sensitivity and selectivity of the SPCEs. Eventually, the modified SPCEs can be coupled with a smart Android-based portable reader for on-site detection. This provides great alternatives to the lab-based conventional methods that are lengthy and require tedious sample preparation.

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