Early diagnosis of blast fungus, *Magnaporthe oryzae*, in rice plant by using an ultra-sensitive electrically magnetic-controllable electrochemical biosensor

Weijuan Yang [a], Hongyan Zhang [a], Mengxue Li [a], Zonghua Wang [b], Jie Zhou [c], Shihua Wang [c], Guodong Lu [b,**], FengFu Fu [a,b,*]

[a] Key Lab of Analysis and Detection for Food Safety of Ministry of Education, Fujian Provincial Key Lab of Analysis and Detection for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350108, China
[b] Department of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[c] School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

HIGHLIGHTS

- An electrochemical biosensor for the sensitive detection of *M. oryzae* in rice plant was developed.
- The *M. oryzae* in rice plant can be detected before any symptomatic lesions were observed.
- The electrochemical biosensor is simple, rapid, cost-effective and ultra-sensitive.
- The proposed biosensor can be used to help farmers timely manage the rice blast disease.

GRAPHICAL ABSTRACT

An ultra sensitive and specific magnetic-controllable electrochemical biosensor for the early diagnosis of blast fungus, *Magnaporthe oryzae*, in rice plant was developed in this study.
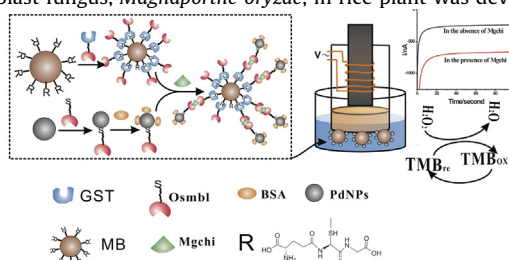
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ABSTRACT

As one of the most destructive and widespread disease of rice, *Magnaporthe oryzae* (also called *Magnaporthe grisea*) has a significant negative impact on rice production. Therefore, it is still in high demand to develop extremely sensitive and accurate methods for the early diagnosis of *Magnaporthe oryzae* (*M. oryzae*). In this study, we developed a novel magnetic-controllable electrochemical biosensor for the ultra sensitive and specific detection of *M. oryzae* in rice plant by using *M. oryzae*'s chitinases (Mgchi) as biochemical marker and a rice (*Oryza sativa*) cDNA encoding mannose-binding jacalin-related lectin (Osmbl) as recognition probe. The proposed biosensor combined with the merits of chronoamperometry, electrically magnetic-controllable gold electrode and magnetic beads (MBs)-based palladium nano-particles (PdNPs) catalysis amplification, has an ultra-high sensitivity and specificity for the detection of trace *M. oryzae* in rice plant. It could be used to detect *M. oryzae* in rice plant in the initial infection stage (before any symptomatic lesions were observed) to help farmers timely manage the disease. In comparison with previous methods, the proposed method has notable advantages such as higher sensitivity, excellent specificity, short analysis time, robust resistibility to complex matrix and low cost etc. The success in this study provides a reliable approach for the early diagnosis and fast screening of *M. oryzae* in rice plant.

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* Corresponding author at: Key Lab of Analysis and Detection for Food Safety of Ministry of Education, Fujian Provincial Key Lab of Analysis and Detection for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350108, China. Tel.: +86 591 22866135; fax: +86 591 22866135.
** Corresponding author.
E-mail addresses: lgd@fafu.edu.cn (G. Lu), fengfu@fzu.edu.cn (F. Fu).

1. Introduction

The increasing population poses enormous pressure on the current limited food-producing resources. As a main food and primary income source in many developing countries, rice production will have to increase markedly to keep pace with demand from population growth. However, the increase of rice production will have to face the decrease of cultivated land and water source [1]. It was reported by FOA (Food and Agriculture Organization of UN) that diseases, insects and weeds are responsible for about 25% failures of rice crops. As one of the most destructive and widespread diseases of rice, rice blast caused by the ascomycete fungus of *Magnaporthe oryzae* (*M. oryzae*, also called *Magnaporthe grisea*) has a significant negative impact on rice production [2,3]. It was estimated that the amount of rice destroyed by rice blast disease every year is enough to feed 60 million people [4]. In general, the blast is asymptomatic lesions in the initial infection stage (within 3 days), and it will massively propagate on the 4th day when rice was infected by *M. oryzae*. Therefore, the most effective method to save the crops from blast is timely detection of *M. oryzae* in the initial infection stage (within 3 days when rice was infected by *M. oryzae*).

Traditionally, the main technique used to identify plant pathogens is culture-based morphological observation [5,6]. However, this method usually costs a long time, about 1 week to culture and identify the pathogen, and its accuracy and reliability largely depend on the skill and taxonomical knowledge managed by analysts [7]. Therefore, it cannot be used to diagnose blast in the initial infection stage to help farmers timely control the disease. In order to overcome the limitations of morphological observation, some nucleic acid-based methods and immunoassays have been developed for the diagnoses of plant pathogens [6,8]. Especially, polymerase chain reaction (PCR) based methods are more specific, sensitive and accurate for blast diagnosis [9–12]. For example, Qi and Yang developed a method for the quantification of *M. oryzae* growth in rice plant based on DNA-based real-time PCR and RNA-based northern blot/phosphor-imaging analysis [13]. Su'udi et al. developed a sensitive method for the specific detection of *M. oryzae* by detecting as low as 1 pg *MHP1* DNA from *M. oryzae* [14], this strategy was able to analyze the amount of *M. oryzae* in the infected leaf tissues quantitatively. However, PCR-based methods have obvious disadvantages including complicated and long nucleic acid extraction procedure and relatively high assay cost etc. [15]. Especially, PCR-based methods target only a single pathogen, which make comprehensive screening of complex samples relatively unprofitable. Another sensitive and specific method used for the detection of plant pathogenic fungi is enzyme-linked immunosorbent assay (ELISA) [8,16–18]. However, the application of this method is limited by the utilization of specific antibody, since, it is very difficult to obtain species-specific antibodies of fungi.

Not long ago, stimulated by the experimental results of Dahiya et al. and Jiang et al. [19,20], we expressed and screened the *M. oryzae*'s chitinase (Mgchi) and a rice (*Oryza sativa* L., *japonica*) cDNA encoding mannose-binding jacalin-related lectin (Osmbl) with Mgchi-related gene [21]. We further demonstrated that Mgchi could be used as biochemical marker for the detection of *M. oryzae* in rice plant, and there was a specific interaction between Osmbl and Mgchi. By using the Mgchi as the biochemical marker and the Osmbl as recognition probe (receptor), we developed a novel method for the visual and specific detection of rice blast fungus, *M. oryzae*, based on the PdNPs-catalyzed TMB (3,3′,5,5′-tetramethylbenzidine sulfate)/H_2O_2 system. However, the previous method has a relatively low sensitivity, which made, it cannot be used to identify *M. oryzae* in rice plant in the initial infection stage (within 3 days when rice was infected by *M. oryzae*). Electrochemical detectors hold potential as a next-generation molecular detection strategy for

the diagnostic purpose due to their high sensitivity, rapid response, low cost and excellent compatibility with miniaturization technologies [22,23]. By using the electrochemical detector, we herein developed an ultra-sensitive electrochemical biosensor for the early diagnosis of *M. oryzae* in rice plant based on an electrically magnetic-controllable electrode together with magnetic beads (MBs)-based catalysis amplification. The proposed biosensor has notable advantages including ultra-high sensitivity and specificity, simple operating process, short analysis time and low cost etc., and can be used to identify rices' *M. oryzae* in the initial infection stage to help farmers timely manage the disease.

2. Experimental

2.1. Materials and instruments

Osmbl and Mgchi were prepared with the previous method [21]. Glutathione-modified MBs (1 μm, 10 mg mL^{-1}) were purchased from Promega Company (China). The solid PdNPs were purchased from DK Nano Technology Co., Ltd. (China). TMB (3,3′,5,5′-tetramethylbenzidine sulfate)/H_2O_2 solution was purchased from Neogen Corporation (USA). The buffer solutions used in the experiment are as follows: PB buffer is the mixture of 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 solution (pH 7.4), PB-T buffer solution is the mixture of 10 mM KH_2PO_4, 10 mM Na_2HPO_4 and 0.01% (w/v) Tween 20 solution (pH 7.4), the buffer solution used to extract proteins from rices' leaf is the mixture of 137 mM NaCl, 2.7 mM KCl, 10 mM KH_2PO_4, 1 mM EDTA and 0.3 mM DTT (pH 7.0). All chemicals are of analytical grade. All solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}).

M. oryzae (Wild-type strain Guy-11)-infected samples of rices' leaf (from day 1 to day 7) were obtained from Fujian Agricultural and Forestry University.

The electrically magnetic-controllable gold working electrode used in this study is the same as that previously reported [22,23]. All electrochemical measurements were performed with a CHI-660C electrochemical workstation (CH Instrument, USA). The transmission electron microscopy (TEM) image was taken with a H-9000NAR instrument (Hitachi, Japan). Zeta potential (ζ) was performed on a zeta potential analyzer (Brookhaven Instruments Corporation, USA). A gel electrophoresis (DYY-6C, Beijing LiuYi Instrument Company, China) together with a gel imaging system (Bio-Best 200E, SIM International Group Co., Ltd.) was used for the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) experiment.

2.2. Preparation of Osmbl–MBs bio-conjugates

The Osmbl–MBs bio-conjugates was prepared by immobilizing Osmbl on the surface of glutathione-modified MBs. Firstly, 5 μL of glutathione-modified MBs (10 mg mL^{-1}) was washed three times with PB buffer under the magnetic field and re-suspended in 50 μL of PB buffer. Then, about 7.5 μg (15 μL, 500 μg mL^{-1}) of Osmbl was added into MBs solution and the total was incubated for 2 h under 37 °C and gentle stirring. By this procedure, Osmbl was immobilized on the surface of glutathione-modified MBs via the strong affinity between glutathione and glutathione-*S*-transferase, since, the Osmbl was expressed and purified as a glutathione-*S*-transferase fusion protein. Subsequently, the Osmbl-modified MBs were washed for three times with PB-T buffer (pH 7.4) and re-suspended in 50 μL of PB buffer. The final solution of Osmbl-modified MBs was stored at 4 °C for next use.

2.3. Preparation of Osmbl–PdNPs bio-conjugates

PdNPs were coated with Osmbl via the interaction between cysteine or NH_3-lysine residues of Osmbl and PdNPs. Firstly, about