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A sandwich ELISA method based on immunomagnetic beads for the rapid enrichment and detection of *Tilletia foetida* teliospores in wheat and flour samples

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ABSTRACT

Common bunt is a major disease of wheat worldwide that reduces crop yields and grain quality. Rapid and sensitive quantitative detection methods are required to diagnose and monitor this disease in wheat management programs and to ensure seed quality. In this study, an immunomagnetic beads-based enzyme-linked immunosorbent assay (IMBs-ELISA) was developed for the detection of *Tilletia foetida* teliospores in wheat and flour. An anti-*T. foetida* teliospores polyclonal antibody bound to immunomagnetic beads was used as the capture probe, and a polyclonal antibody labeled with horseradish peroxidase was used as the detector probe. The capture and detection conditions for the target spores were optimized to achieve the best determination results. Under optimal conditions, the proposed method took less than 2 h to complete. Its limit of detection was 300 teliospores per gram. The critical reaction in this IMBs-ELISA occurred on magnetic beads. This not only simplified the traditional ELISA process, but also shortened the detection time. This study has expanded the application of the IMBs-ELISA method to fungal spore detection. This method has potential applications in agriculture and seed management.

1. Introduction

Common bunt caused by two closely related fungi, *Tilletia caries* and *Tilletia laevis*, is one of the most devastating diseases of wheat crops worldwide. These pathogens are widely distributed, have strong potential to cause epidemics, and result in serious crop damage[1-2]. In China, the main pathogen causing common bunt is *T. laevis*, also known as *Tilletia foetida*[3]. As a seedborne pathogen, it germinates in the soil, infects the newly emerged coleoptiles, and then colonizes the plant asymptomatically. At seed maturity, the caryopsis contains a huge mass of black spores called a sorum or false caryopsis, which breaks down during harvest, infecting healthy kernels and releasing teliospores in the soil[4-6]. The *T. foetida*-infected grains produce an unpleasant odor like rotten fish, which significantly reduces the grain quality and seed vigor, and results in large economic losses[7-10]. In this scenario, pathogen detection is of fundamental importance.

The classical diagnostic protocol involves the tentative identification of teliospores based on morphology followed by germination of the teliospores and confirmation of their identity using molecular protocols[11]. Molecular biology techniques such as

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polymerase chain reaction (PCR) and immunodiagnostic methods have also been used to identify these fungi, with the advantages of low detection limits and high-throughput processing[12-15]. In our laboratory, a trimethylamine assay based on headspace solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry was developed to detect biomarkers in *T. foetida*-infected wheat, and it proved to be an effective detection method when there was a low infection rate[16]. Although the existing methodologies are useful, microscopic identification and teliospore germination are time consuming, and depend on experienced personnel. The molecular biological and trimethylamine determination methods are sensitive but require professional laboratories and technicians.

Magnetic beads can bind to an antibody to form immunomagnetic beads (IMBs), which are able to capture and enrich target antigens after dispersion in solution[17-20]. There are several features of IMBs that make them suitable for high-throughput detection: only simple equipment is required, the experimental procedure is easy, the binding is highly specific, the reaction is fast, and few samples are required. The analysis of samples using IMBs can be easily achieved without pre-enrichment, purification, or separation. Therefore, a range of assays based on IMBs have been used in various research fields, such as food safety, environmental monitoring, and clinical diagnosis[21-24].

In this study, a method based on IMBs for rapid enrichment and extraction of teliospores from *T. foetida*-infected samples, followed by sandwich enzyme-linked immunosorbent assay (ELISA) for sensitive detection, was developed. To establish a rapid, efficient, and simple detection method, we used a polyclonal antibody to *T. foetida* teliospores as the specific recognition element coupled with magnetic beads as the capture probe. Then, the whole target, i.e., *T. foetida* teliospores bound to IMBs, was recognized by a horseradish peroxidase (HRP)-labeled polyclonal antibody. The IMBs-ELISA method was transferred from a 96-well plate to magnetic beads, which simplified the traditional ELISA process and shortened the detection time. The results show that the lower limit of detection (LOD) was 300 teliospores per gram, and the whole analysis was completed within 2 h.

2. Materials and methods

2.1 Chemicals, reagents, and instruments

Low-electrolyte bovine serum albumin (BSA), phosphate-buffered saline (PBS), the HRP conjugation kit (periodate method), and HRP-conjugated mouse anti-rabbit immunoglobulin G (IgG) were obtained from Sangon Biotechnology Technology Company (Shanghai, China). Chemiluminescence substrate solutions (luminol) A and B were obtained from Huzhou InnoReagents Company (Huzhou, China). Streptavidin-coated magnetic beads were purchased from Nanoeast Biotech (Nanjing, China). Biotin 3-sulfo-*N*-hydroxysuccinimide ester sodium salt and dimethylsulfoxide (DMSO) were purchased from Sigma Chemicals (St Louis, MO, USA). All other reagents were of analytical grade and were purchased from Aladdin Bio-Chem Technology Company (Shanghai, China). All solutions were prepared using ultrapure water (18.2 MΩ·cm) purified by a Merck Milli-Q system (Millipore, Molsheim, France).

The magnetic separation rack was purchased from Biomagbeads Company (Wuxi, China). The orbital shaker was purchased from Ohaus Company (Shanghai, China). The Varioskan Lux enzyme-labeling measuring instrument used for chemiluminescence detection was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The polystyrene 96-well microtiter plates were obtained from Sangon Biotechnology Technology Company (Shanghai, China). The pH value of all buffer solutions was measured with pH meter (Mettler, Shanghai, China).

The washing buffer was 0.01 mol/L PBS (pH 7.6) with 0.05% Tween-20 (PBST). The stock solution was 0.01 mol/L PBS with 5% BSA and 0.05% (*V/V*) Tween-20 (5% BSA-PBST). The solution used to dilute the antigen and antibody was 0.01 mol/L PBS with 0.5% BSA and 0.05% (*V/V*) Tween-20 (0.5% BSA-PBST).

Aspergillus niger, *A. flavus*, and *Paecilomyces varioti* Bainier were provided by the Microbiological Testing Center of Nanjing Institute of Product Quality Inspection (Nanjing, China). The bacterial strains were cultured on Sabouraud agar medium at 28 ℃ for 3 weeks. The spores were collected and washed with PBS several times, centrifuged at 1 000 × *g* for 5 min, then resuspended in PBS. The spores were counted using a blood cell counting plate.

2.2 Preparation of immunogen and polyclonal antibody

The intact teliospores were extracted from the *T. foetida*-infected grains of wheat and used as an immunogen source. To extract the spores, the diseased wheat caryopses were cut open using a surgical blade. The seeds were incised, and teliospore masses were loosened and transferred to a centrifuge tube.

The anti-*T. foetida* teliospore polyclonal antibody was generated by immunizing 13-week-old male New Zealand white rabbits (approved by Animal Experimental Ethics Committee of Southeast University. No: 20250310001). The rabbits were immunized by administering three subcutaneous injections in the posterior neck at 7-day intervals with 1 mL intact teliospore suspension (1 mg teliospores emulsified with an equal volume of Freund's complete adjuvant). The blood was collected from the carotid artery 14 days after the final immunization. Plasma was incubated at 37 ℃ for 1 h before centrifugation at 500 × *g* for 30 min. The serum was collected, packaged, and then stored at –80 ℃ until use.

2.3 Validation of the polyclonal antibody

A modified ELISA was used to validate the prepared polyclonal antibody. The microtiter plates were coated with spores in 0.01 mol/L Na_2CO_3 buffer (200 μL/well). The plates were incubated for 1 h at room temperature and then at 4 ℃ overnight before washing three times with PBST. The wells were filled with 5% BSA-PBST and incubated for 2 h at 37 ℃ to prevent adventitious binding, and then washed three times with PBST. The prepared polyclonal antibody was diluted to 1∶100 concentration with 0.5% BSA-PBST, and then 100 μL diluted antibody was added to each well. The plates were incubated for 2 h at 37 ℃, and then washed three times with 0.5% BSA-PBST. HRP-conjugated mouse anti-rabbit IgG diluted in 0.5% BSA-PBST (1∶5 000) was added to the plates, and then they were incubated for 1 h at 37 ℃ before washing three times with PBST. Next, 100 μL chemiluminescence substrate solution (A:B = 1:1, *V/V*) was added to the microplates, which were then shaken in an orbital shaker at 500 r/min for 1 min in the dark. Each 96-well plate was then immediately placed in the enzyme-labeling measuring instrument for chemiluminescence detection.

2.4 Preparation of IMBs

2.4.1 Biotin-labeled polyclonal antibody

The polyclonal antibody was quantified and its concentration was adjusted to 1 mg/mL. Biotin 3-sulfo-*N*-hydroxysuccinimide ester sodium salt was thawed from frozen to room temperature within 30 min, and then used to prepare a 2 mg/mL biotin solution with DMSO. The biotin-labeled polyclonal antibody (bio-antibody) was prepared by adding 10 μL biotin solution to 300 μL polyclonal antibody solution and incubating the mixture at room temperature for 30 min in the dark. Then, to remove unbound biotin, the bio-antibody was dialyzed against PBS solution (at least 50 volumes) in the dark. The bio-antibody was stirred at room temperature during the day, and the PBS solution was changed every 4 h. At night, the bio-antibody was stirred at 4 °C, and the PBS was changed again. Finally, the bio-antibody was collected into a centrifuge tube and stored at –20 °C until use.

2.4.2 Synthesis of HRP-conjugated polyclonal antibody (HRP-pAb)

HRP was used for the detection reaction because of its small size and strong stability despite chemical modifications. The HRP-pAb was prepared using an HRP conjugation kit (periodate method) according to the kit's instructions. Details of the procedure are provided in the Supplementary material.

2.4.3 Preparation of IMBs

The bio-polyclonal antibody and streptavidin-coated magnetic beads were used to prepare the IMBs. The streptavidin-coated magnetic beads were washed three times with PBS. Then, the bio-antibody diluted in 0.5% BSA-PBST was added to the magnetic beads, which were then incubated for 30 min at room temperature. The supernatant was discarded and the IMBs were washed with PBST to remove the unbound antibodies. The IMBs were resuspended in 5% BSA-PBST for 30 min at 37 °C to block the sites on the magnetic beads that had not bound to antibodies. Finally, the IMBs were washed, resuspended in PBS buffer containing 0.02% NaN_3, and then stored at 4 °C until use in further experiments.

2.4.4 Establishment of the IMBs-ELISA method

The blocked IMBs were added to centrifuge tubes, washed twice with 200 μL PBST, and placed in a magnetic separation rack before carefully removing the supernatant. A solution of *T. foetida* teliospores diluted with PBST was added to the IMBs, and then the capture reaction was allowed to proceed at 37 °C for 30 min on an orbital shaker at 500 r/min shaking speed. After magnetic separation and washing three times with PBST, 100 μL HRP-pAb diluted with 0.5% BSA-PBST was added to each centrifuge tube and the mixture was incubated at 37 °C for 30 min. Then, the solution was transferred to a 96-well microtiter plate. After magnetic separation, removal of the supernatant, and washing with PBS three times, 100 μL chemiluminescence substrate solution (A:B = 1:1, *V/V*) was added to each well of the microplate. The microplate was then shaken on an orbital shaker at 500 r/min for 1 min in the dark, and then chemiluminescence was detected using an enzyme-labeling measuring instrument. A schematic diagram of the sandwich ELISA method based on IMBs for enrichment and detection of *T. foetida* teliospores is shown in Fig. 1.

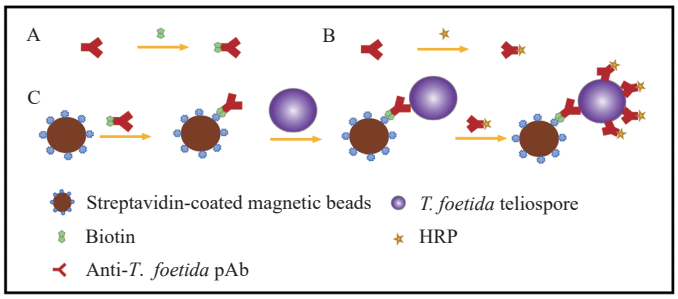

Fig. 1 (A) Biotin-labeled polyclonal antibody. (B) Synthesis of HRP-pAb. (C) Preparation of IMBs and establishment of the IMBs-ELISA method.

2.5 Optimization of IMBs-ELISA

The majority of magnetic separation techniques used to concentrate bacterial cells rely on antibodies[18]. Therefore, several key conditions were optimized to maximize the capture and determination efficiency of the method. The particle size of the magnetic beads, the incubation time for streptomycin-coated magnetic beads to bind to biotin-labeled antibodies, the incubation time and temperature for IMBs to bind to *T. foetida* teliospores, and the incubation time for IMBs-teliospores to bind to HRP-pAb were optimized based on the response value of chemiluminescence of the IMBs-ELISA. The magnetic beads, *T. foetida* teliospores, and IMBs-bound *T. foetida* teliospores were observed under a transmission electron microscope (TEM) (Malvern Instruments Ltd., Worcestershire, UK).

2.6 Analysis of T. foetida teliospores in real samples

The *T. foetida*-infected wheat and flour samples were obtained from Xi Zhang Grain & Oil Purchase and Sale Company (Zhangjiagang, China). The normal wheat and flour samples were provided by Nanjing Institute of Product Quality Inspection (Nanjing, China).

The crushed wheat samples and flour samples were weighed (0.1 g) and added to 50-mL polypropylene centrifuge tubes containing 1 mL PBST solution, then mixed and set aside. The established IMBs-ELISA method was used to detect pathogen spores in these samples, using the procedure described in Section 2.4.4.

2.7 Statistical analysis

All analyses were conducted in triplicate and the data were represented as mean ± standard deviation (SD). The statistical analyses were conducted using the SPSS 17.0 (SPSS, Chicago, IL, USA) software. Differences between groups were considered significant at $P < 0.05$ Significant differences among and between groups were determined by performing an analysis of variance (ANOVA) with Tukey's test for multiple comparisons. The figures were plotted with WPS (Kingsoft Corporation, Guangdong, China) software.

3. Results

3.1 Specificity of the polyclonal antibody

Spores from *A. niger*, *A. flavus*, and *P. varioti* Bainier, three different fungal species that commonly infect plants, were used to evaluate the specificity of the prepared polyclonal antibody. The spores from these three species were used as negative test groups, and PBST buffer alone was used as the blank group. The micrographs of spores of the 4 different fungi are shown in Fig. 2.

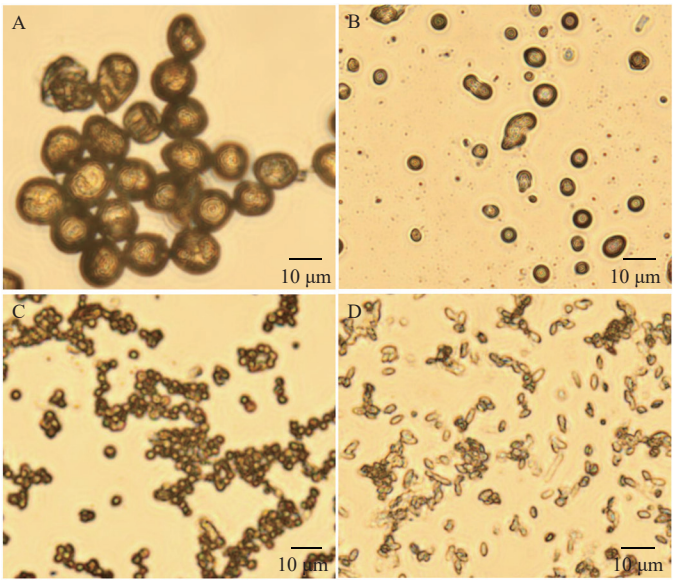

Fig. 2 Micrographs of spores of four different fungi. (A) *T. foetida* teliospores. (B) *A. flavus* spores. (C) *A. niger* spores. (D) *P. variotii* Bainier spores.

There was no significant difference in the chemiluminescence signal between the blank group (PBST) and the three negative test groups (Fig. 3). Compared with the blank group and the negative test groups, the positive test group showed a significantly stronger chemiluminescence signal. These results show that the prepared polyclonal antibody identified the spores of the target species with high specificity.

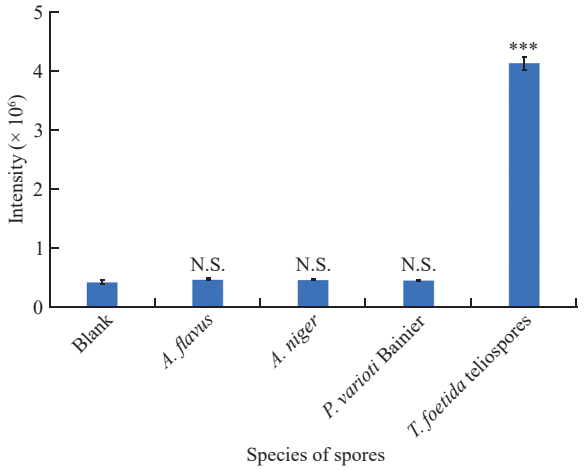

Fig. 3 Analysis of specificity of polyclonal antibody. ***$P < 0.001$. N.S. not significant compared with blank group.

3.2 Characterization of magnetic beads, teliospores, and IMBs bound to the spores

Streptavidin-coated magnetic beads, teliospores, and IMBs bound to the spores were analyzed by TEM at an acceleration voltage of 120 kV after depositing 5 μL of each diluted solution on a formvar-carbon-coated copper grid and evaporating to dryness. The images in Figs. 4A and B show the streptavidin-coated magnetic beads with a particle size of 1 μm at different magnifications. Fig. 4C shows the TEM images of *T. foetida* teliospores, and show that the spore diameter was approximately 14 μm. The image clearly shows that IMBs bound to the teliospores (Fig. 4D). This confirmed the selectivity of the assay—it demonstrated the efficient binding of the biotin-labeled polyclonal antibody to the streptavidin-coated magnetic beads

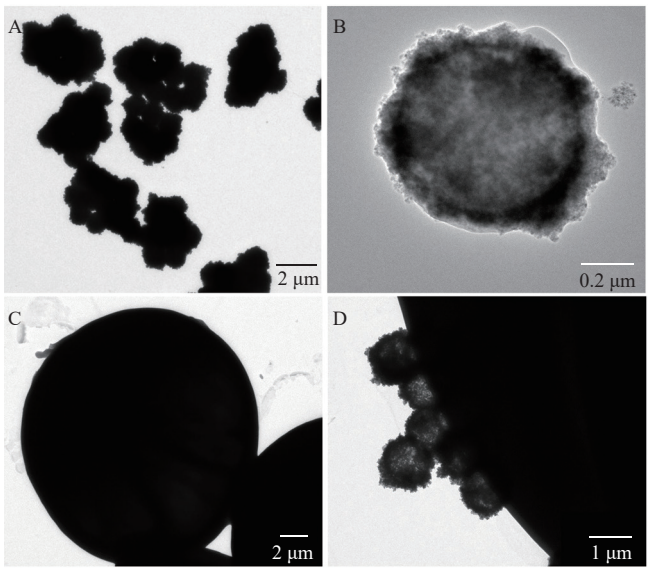

Fig. 4 TEM of magnetic beads and teliospores. (A, B) Streptavidin magnetic beads (particle size, 1 μm). (C) *T. foetida* teliospores. (D) IMBs-bound teliospores.

3.3 Optimization of detection conditions

To optimize the performance of the method, different detection conditions were explored. First, the diameter of the streptavidin-coated magnetic beads was optimized. As shown in Fig. 5A, the diameter of these beads significantly affected the results of the determination of *T. foetida* teliospores at both concentrations (1 000 and 2 000 teliospores per mL), with the highest fluorescence intensity obtained using particles with a diameter of 1 μm. When the particle diameter was smaller than 1 μm, the detection efficiency was decreased, possibly because more magnetic beads were needed to capture the spores (spore diameter, approximately 15 μm). When the particle diameter was larger than 1 μm, the detection efficiency also decreased, possibly because of steric hindrance. Based on the experimental results, streptavidin-coated magnetic beads with a particle size of 1 μm were selected as the carrier for capturing *T. foetida* teliospores.

Next, the incubation time to allow streptavidin-coated magnetic beads to bind to biotin-labeled antibodies was also a critical factor to be optimized. Several 100-μL aliquots of biotin-labeled antibodies were added separately to the same batch of streptavidin-coated magnetic beads, and then the mixtures were incubated with shaking at 500 r/min at 37 °C for different times. Fig. 5B shows the relationship between

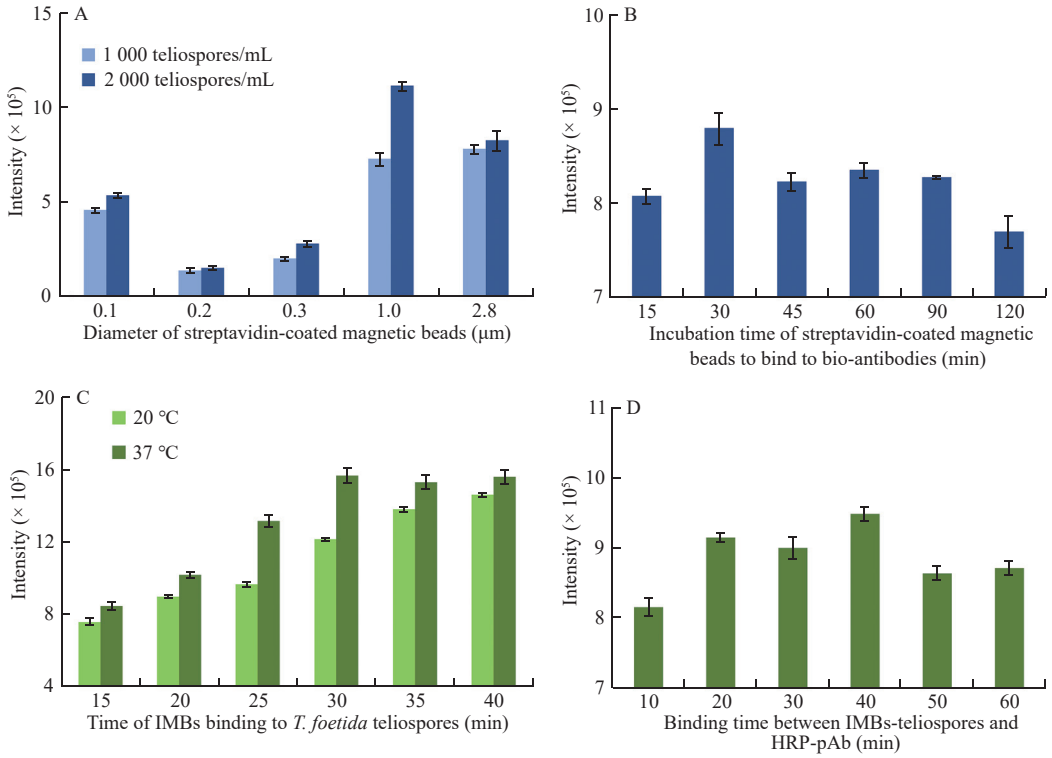

Fig. 5 Optimization of detection conditions. (A) Optimization of diameter of streptavidin-coated magnetic beads. (B) Optimization of incubation time to allow streptavidin-coated magnetic beads to bind to biotin-labeled antibodies. (C) Optimization of time and temperature for IMBs to bind to *T. foetida* teliospores. (D) Optimization of binding time between IMBs-teliospores and HRP-pAb.

test results and the incubation time between streptavidin-coated magnetic beads and biotin-labeled antibodies. On the basis of these results (Fig. 5B), 30 min was chosen as the optimal time for binding.

The incubation time and temperature during the binding of IMBs to *T. foetida* teliospores were also optimized. As shown in Fig. 5C, the chemiluminescence intensity was stronger after incubation at 37 °C than after incubation 20 °C. At the same time, when the incubation time was increased from 15 min to 30 min, more *T. foetida* teliospores were adsorbed and the chemiluminescence intensity was increased. When the incubation time at 37 °C was increased from 30 min to 40 min, the chemiluminescence intensity no longer increased significantly, indicating that the adsorption of spores by IMBs reached the limit by 30 min. Therefore, the optimal time and temperature for binding of IMBs to *T. foetida* teliospores were 30 min and 37 °C, respectively.

Finally, the incubation time for binding between IMBs-teliospores and HRP-pAb was optimized. As shown in Fig. 5D, as the incubation time extended, the chemiluminescence intensity first increased and then decreased. Therefore, although the maximum response was observed at 40 min, we selected 20 min as the optimal incubation time, considering the trade-off between time cost and response intensity.

3.4 Method validation

The calibration curve was obtained by plotting the chemiluminescence values against the logarithm of the concentration of teliospores. Solutions of teliospores with known concentrations were added to prepared blank wheat and flour samples, and then the mixtures were shaken for 30 min at room temperature. Then, the precision and trueness were determined for the IMBs-ELISA method.

3.4.1 Linearity, LOD, and limit of quantification (LOQ)

The teliospore solution was diluted to concentrations of 5−5 × 10^6 teliospores per gram and used for IMBs-ELISA detection. When the logarithmic concentration of *T. foetida* teliospores (*X*-axis) was plotted against the intensity value (*Y*-axis), an S-shaped curve was obtained (Fig. 6). When the concentration of *T. foetida* teliospores was in the range of 1 000−10^6 teliospores per gram, the logarithm of the concentration of *T. foetida* teliospores showed a linear relationship with the chemiluminescence intensity value—the equation for this relationship was $Y = 2\ 076\ 622.28X - 6\ 082\ 265.09$ ($R^2 = 0.990\ 2$). The calculated LOD and LOQ were 300 (signal-to-noise ratio, $R_{S/N} = 3$) and 1 000 ($R_{S/N} = 10$) teliospores per gram, respectively.

3.4.2 Precision and trueness

The precision and trueness of the detection method were also assessed. The recovery test was performed using blank samples mixed with *T. foetida* teliospores at concentrations of 1 000, 2 000, and 10 000 teliospores per gram, with a non-spiked sample as the blank control. As shown in Table 1, the recovery ranged from 88.15% to 113.39% and from 85.46% to 118.07% for wheat and flour samples, respectively. The intra-day and inter-day variations calculated ranged from 4.73% to 9.83% and from 11.4% to 15.6%, respectively, for wheat samples. The calculated intra-day and inter-day variations ranged from 5.23% to 7.95% and from 10.8% to 16.2%, respectively, for flour samples. These results show that the method had good precision and trueness.

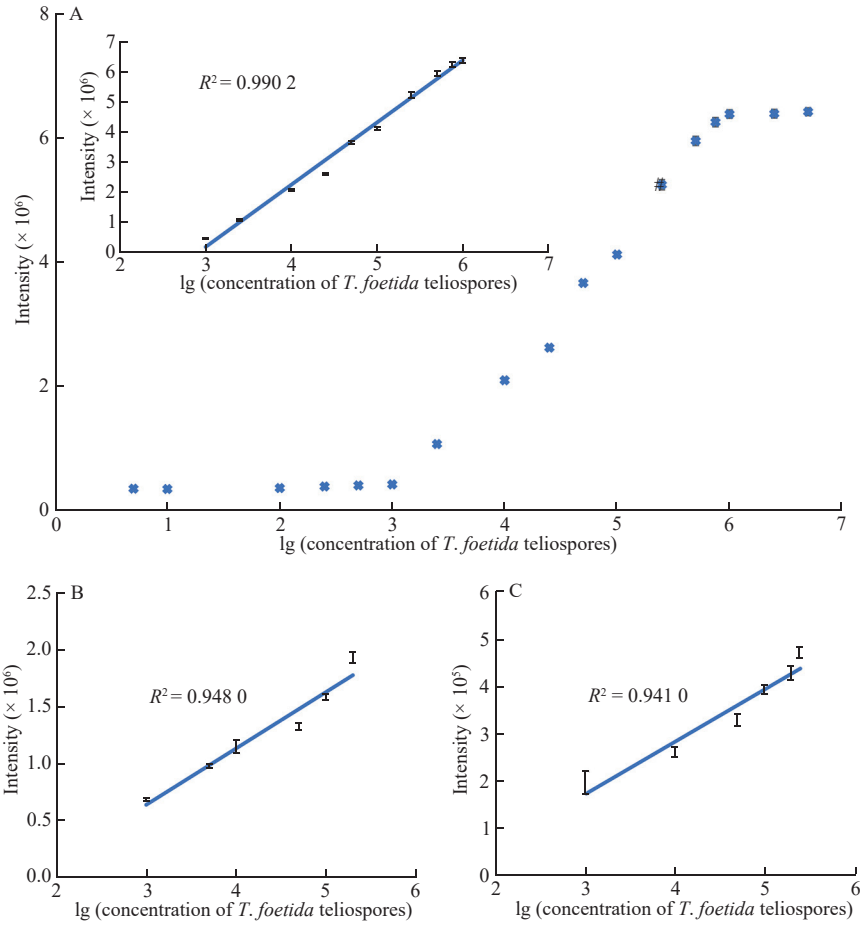

Fig. 6 Standard curve of IMBs-ELISA for the detection of *T. foetida* teliospores. (A) Standard curve in the standard solution. (B) Standard curve in wheat sample solution. (C) Standard curve in flour sample solution.

Table 1
Precision and trueness of the IMBs-ELISA method.

Sample	Spiked levels (teliospores per gram)	Recovery (%, mean ± SD)	Intra-day variations (%, n = 6, RSD)	Inter-day variations (%, n = 3, RSD)
Wheat	1 000	88.15 ± 15.45	9.83	15.6
	2 000	112.56 ± 7.43	5.44	13.2
	10 000	113.39 ± 9.23	4.73	11.4
Flour	1 000	85.46 ± 11.24	6.27	16.2
	2 000	118.07 ± 4.36	7.95	13.2
	10 000	108.61 ± 9.59	5.23	10.8

3.5 Analysis of wheat and flour samples

To demonstrate the applicability of the newly developed IMBs-ELISA method, 10 different batches of wheat and flour samples were assayed separately. According to the test results shown in Table 2, *T. foetida* teliospores were detected in 3 out of 10 batches of wheat and 2 out of 10 batches of flour. The results provided information about the *T. foetida* teliospores content in wheat and flour samples from Jiangsu Province, China, and demonstrated the applicability of this IMBs-ELISA method for detecting spores of an important pathogen.

3.6 Comparison with other methods

The goal of this work was to develop an accurate and reliable IMBs-ELISA method using a prepared polyclonal antibody for the detection of *T. foetida* teliospores in wheat and flour samples. This new method was simpler and faster than other quantitative analysis methods for *T. foetida* teliospores. The sample pre-treatment in the new method was much simpler than those required for other immunological and PCR techniques[12-14]. The use of IMBs improved the sensitivity of detection and shortened the time required for spore enrichment and isolation from a few days to a few hours. Another group established a specific immunoassay for Karnal bunt detection, and the process took approximately 24 h[25]. A dyed latex bead agglutination test was developed for immunodiagnostic detection of Karnal bunt teliospores. Its detection limit was 750 teliospores, making it suitable only for single seed analysis[26]. Table 3 summarizes the parameters of the different methods for detecting spores.

Table 2
Results of analysis of wheat and flour samples (teliospores per gram).

Sample	1	2	3	4	5	6	7	8	9	10
Wheat	ND	ND	ND	ND	$5.24 \times 10^3 \pm 279$	$1.53 \times 10^4 \pm 1\,412$	ND	$3.66 \times 10^4 \pm 1\,333$	ND	ND
Flour	ND	ND	ND	ND	ND	ND	ND	ND	$9.24 \times 10^3 \pm 565$	$7.57 \times 10^4 \pm 1\,753$

Note: ND, not detected.

Table 3
Comparison of different detection methods.

Methods	Target spore	LOD	Specificity	Detection time
IMBs-ELISA (this paper)	*T. foetida*	300 teliospores per gram	accurate diferentiation of the pathogen from *A. niger*, *A. flavus*, and *P. varioti* Bainier	Less than 2 h
Indirect ELISA[27]	*T. indica*	The detection limit is as small as 5–1 000 teliospores	/	At least 20 h
Dyed latex bead agglutination test for immunodiagnosis[26]	*T. indica*	7.5 mg solubilized teliosporic antigens equivalent to 750 teliospores and suitable for single seed analysis	No cross reactivity was found with teliosporic antigen(s) of spores of *Curvularia lunata*, *Ustilaga tritici*, *Helminthosporium sativum*, *Ustilagi noidea virens* and *Alternaria triticina*	20 min
Trimethylamine-based HS-SPME-GC-MS[16]	*T. foetida*	0.02 μg/kg	/	50 min
Real-time PCR[28]	*T. foetida*	0.4 ng/μL	accurate diferentiation of the pathogen from *T. tritici* and *T. controversa*	At least 20 days

Note: HS-SPME-GC-MS, headspace solid-phase microextraction-gas chromatography-mass spectrometry; /, specificity is not mentioned in the article.

4. Discussion

In recent decades, common bunt disease has mainly been controlled by chemical treatment of seeds[29-30]. As a result of the intensive use of chemical seed treatments, combined with the cultivation of resistant cultivars, the incidence of common bunt had dropped in wheat-growing regions of the world. However, there has been a resurgence of this disease because of worldwide trading of wheat, as well as the increasing use of organic farming methods that use biological treatments rather than chemical treatments, so that wheat crops are now more susceptible to common bunt[31-34]. For instance, the disease has continuously occurred in Zhangjiagang, Changshu, and Zhenjiang, Jiangsu Province, China, from 2015 to 2023, causing serious economic losses to the local governments and farmers. The low frequency of infected kernels in the wheat grains means that highly sensitive detection methods are required to guarantee seed quality. The IMBs-ELISA method was able to detect 300 teliospores per gram infected kernels, making it far more sensitive than organoleptic tests and other methods. Therefore, this IMBs-ELISA method can be applied to the screening of wheat seeds in organic agriculture to ensure seed safety and quality.

5. Conclusion

In this study, we developed and validated an IMBs derived-based sandwich ELISA method utilizing IMBs and HRP for the rapid and sensitive detection of *T. foetida* teliospores. This simple analytical system can quantitatively detect 1 000−10^6 teliospores per gram *T. foetida* teliospores, and the analysis can be completed within 2 h. The advantages of this system are that it is fast and easy to use, as well as being sensitive and accurate. The concept of this method can be easily applied to develop simple biosensors to detect various substances in wheat and flour.

Conflict of interest

The authors have declared that they have no conflicts of interest associated with this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250270.

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