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Toxigenic potential analysis and fumigation treatment of three *Fusarium* spp. strains isolated from Fusarium head blight of wheat

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Abstract

Fusarium Head Blight (FHB) of wheat and small grain cereals caused by *Fusarium graminearum* and other *Fusarium* species is an economically cereal disease worldwide. *Fusarium* infections results in reduced yields and mycotoxin contamination of the grain, and the research on the toxin production and growth control of *Fusarium* is the key to prevent and control of mycotoxin contamination in wheat. In this study, the molecular identification of toxigenic potential and gas fumigation control of typical *Fusarium* strains isolated from FHB-infected wheat were studied. The results showed that the consequences of molecular identification of toxigenic potential were consistent with the actual production of toxins, which can be used for rapid identification of fungal toxicity. And the effects of the gas fumigants were different. Chlorine dioxide could kill *Fusarium* spores and mycelium in a short time (0.5 h) at relatively low concentration (300 ppm), while ozone could only kill *Fusarium* spores and had no obvious inhibitory effect on the growth of mycelium, even at a concentration of 1400 ppm. Taken together, gaseous ClO_2 could significantly inhibit the growth of *Fusarium*, and it's an ideal fumigant used to control this fungal contamination during the postharvest storage of grain.

Keywords: *Fusarium*; toxigenic potential; mycotoxins; chlorine dioxide; ozone; fumigation.

Practical Application: This study investigated the inhibitory effect of two gas fumigants on the growth of *Fusarium*. Among them, chlorine dioxide has a good bacteriostatic effect at low concentrations, which can be used for the prevention and control of Fusarium spp. in food.

1 Introduction

Wheat is one of the three major food species in the world, providing 20% of global calories and protein, and it is also one of the most important food crops in China (He et al., 2001). Wheat suffers from many diseases during the production process, among which fusarium head blight (FHB), also known as scab, is one of the severe fungal diseases that plague the sustainable development of wheat production in China (Choo, 2009). Infected by this disease, grains are light in weight, discolored and degraded in proteins that cause important economic losses through decreased grain yield and reduced grain quality (McMullen et al., 2012; Palacios et al., 2021). Besides, the disease has serious impacts on human and animal health via the contamination of grains with mycotoxins such as trichothecenes, especially deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN) (Bennett & Klich, 2003; Bin-Umer et al., 2011; Lemmens et al., 2005; Tibola et al., 2015).

FHB of wheat is caused by the infection of a complex of different toxigenic *Fusarium* species at wheat heading at flowering (O'Donnell et al., 2013). Among them, *Fusarium graminearum* species complex (FGSC) is considered the most important globally due to its widespread incidence and aggressiveness (Beccari et al., 2019; Goswami & Kistler, 2004; Kazan et al., 2012). However, other pathogens such as *F. culmorum*, *F. avenaceum*, *F. poae* and *F. cerealia* that considered as 'weak' pathogens, can also cause the disease (Aoki et al., 2012; Bottalico & Perrone, 2002; Valverde-Bogantes et al., 2020). After harvest, strong wind blowing by a blower, sieving treatment and other physical methods can be used to remove the small and light proportion of scab grains, while the *Fusarium* and other microorganisms on the grain surface will still be a potential hazard (Machado et al., 2017). The life activities of these harmful microorganisms are the main causes of grain quality loss and spoilage, which interact among themselves, with the grain, and with the environment of the storage facilities, and the mycotoxins continue to be produced during processing, packaging, distribution, and storage of food products at suitable temperature and humidity (Bhatnagar et al., 2006; Ortega et al., 2019; Pereira et al., 2014). The presence of mycotoxins in crops and animal products is a serious problem globally and have a great influence on the people's daily life that calls for global concern (Murshed et al., 2022; Lima et al., 2022). And with the advancement of detection technology and the wide application of rapid detection technology, the occurrence of mycotoxins is well monitored (Anfossi et al., 2016; Pimpitak et al., 2020; Zhou et al., 2020; Shkembi et al., 2022).

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The mycotoxins produced by *Fusarium* species are diversely in structures and actions, and can cause abnormalities in reproductive and embryo development mutations and chromosomal (Alshannaq & Yu, 2017; Awuchi et al., 2021). Furthermore, most mycotoxins are chemically and thermally stable and cannot be destroyed during most food processing operations, including coking, baking, boiling, frying, roasting, and pasteurization. The contaminated foodstuffs and feedstuffs with mycotoxins can have serious consequences to human and animal health (Murshed et al., 2022). Different approaches have been used to remove or degrade the mycotoxins in foods, and the most prominent of these can be categorized into physical, chemical, and biological methods, where biological methods are considered to be more efficient and safer (Assaf et al., 2019; Ismail et al., 2018). Mycotoxin contamination has become an important issue related to the food safety requirement for international marketing of agri-food commodities for human and animal consumption (Costa et al., 2019; Silva et al., 2022). Therefore, the research on the minimize of microbial contamination is the key to the prevention and control of mycotoxin contamination in grain.

The use of post-harvest technologies, such as irradiation that kills microorganisms directly, and modified atmosphere storage that reduce the O_2 content around the grain, can contain microbial development, consequently reducing conservation problems. While, limited by the feasibility of use, and different fungal responses, these two methods are not effective in grain storage (Santis et al., 2021; Mannaa & Kim, 2017). In recent years, numerous studies have shown that chlorine dioxide (ClO_2) and ozone (O_3), two strong oxidizing gases, can be used in gaseous or aqueous form to sanitize food and for food storage (Cao et al., 2018; Cao et al., 2022; Horvitz & Cantalejo, 2014; Lee et al., 2019; Park et al., 2021; Sun et al., 2017; Venta et al., 2010; Zhang et al., 2019). ClO_2 is a rapid and effective fungicide, which is active against bacteria, yeasts, and molds, and it is legally permitted in China to be used for fruit and vegetables sanitization in water (Li, 2010; Yang et al., 2015). And O_3 is recognized by the US Food and Drug Administration as an antimicrobial agent for the treatment, storage and processing of foods, and has been widely applied in food processes to eliminate or reduce bacteria and fungi (Ali et al., 2014; Santis et al., 2021; Kim et al., 1999; Ong & Ali, 2015; Werlang et al., 2022).

Taking all this into account, the objectives of this study were: (i) to isolate and identify *Fusarium* strains from FHB of wheat, (ii) to determine the toxigenic potential of the strains and verify their capability to produce mycotoxins in vitro, (iii) to study and compare the effects of gaseous ClO_2 and O_3 fumigation on *Fusarium* growth in lab condition.

2 Material and methods

2.1 Sample collection and isolation of Fusarium

In this experiment, about 10 kg wheat samples to be warehoused were collected from Anhui province, China. The collected samples were kept in sterile plastic bags during transport to the laboratory. Then, on the same day, according to the national standard of the People's Republic of China GB 1351-2008 'Wheat', we selected the FHB symptomatic wheat grains in the collected samples and classified it into pink and white. The classified samples were cleaned with sterile water, the washing liquid was coated on potato dextrose agar (PDA) supplemented with streptomycin (25 mg/L) to discourage bacterial contamination. Part of the washed FHB wheat was directed planted on PDA, and the other part was crushed and coated with sterile water. The plates were incubated in the dark at 28 °C for 3-5 days. Fungal isolated were transferred singly to PDA plates and subcultured at least twice to obtain pure cultures.

2.2 Identification of pathogenic fungi

Morphological identification of the isolates was carried out on the basis of criteria according to the descriptions in Burgess et al. (1994) and Nirenberg (Leslie & Summerell, 2006). For molecular identification of isolates obtained from diseased wheat, the genomic DNA was extracted from fungal mycelia grown in complete medium at 25 °C for 7 days using Plant DNA Isolation Mini Kit (Vazyme Biotech Co., Ltd) according to the manufacturer's instructions. The internal transcribed spacer gene (*ITS*) and translation elongation factor 1-alpha (*EF-1α*) gene were amplified using the primer pairs listed in Table 1 (O'Donnell et al., 1998; White et al., 1990). Polymerase chain reaction (PCR) was carried out in a 50.0 μL reaction system contain 25.0 μL 2 × Rapid Taq Master Mix (Vazyme Biotech Co., Ltd), 10.0 μM of each primer (Synthesized by Chengdu Youkang), 100 ng of DNA template, and make up ddH_2O to 50 μL. Reactions were programmed for 94 °C for 10 min, followed by 35 cycles of 94 °C 40 s, 55 °C 45 s, and 72 °C 1 min, and a final extension at 72 °C for 10 min (Kim et al., 2009; Sang et al., 2013). The sequences of nucleotide alignments obtained were analysis with BLAST, the GenBank database (National Library of Medicine, 2022) and with a specific database of the genus *Fusarium*, CBS-KNAW Fungal Biodiversity Centre's *Fusarium* MLST database (Fusarium MLST, 2020), and the strains were confirmed to species level. Phylogenetic trees were constructed by neighbor-joining method and the evolutionary analysis were conducted using MEGA5 software package (Kumar et al., 2018; Saitou & Nei, 1987).

Table 1. Primers used in the molecular identification of isolated strains.

Locus	Primers		Target Fragment (bp)	Reference
	Designation	Sequences (5'-3')		
ITS	ITS1	TCCGTAGGTGAACCTGCGG	560	White et al., 1990
	ITS4	TCCTCCGCTTATTGATATGC		
EF1-α	EF1	ATGGGTAAGGAAGACAAGAC	680	O'Donnell et al., 1998
	EF2	GGAAGTACCAGTGATCATGTT		

2.3 Spore preparation

Activated Fusarium strains were inoculated into CMC liquid sporulation medium and culture at 25 °C, 180 rpm for 5 days. The culture medium was filtered by single-layer Miracloth filter. The spore suspensions were then centrifuged at 5000 rpm for 15 min, and the supernatants discarded. This wash procedure was carried out three times with sterile saline added with 0.1% sterile Tween 80 (Merck, Australia). The initial concentration of spore suspension was determined by the measurement of hemocytometer. The final spore concentrations were adjusted to yield a final count of 10^6 spores/mL with sterile saline.

2.4 Detection of toxigenic genes

Based on the molecular identification of fusarium isolates, the mycotoxin-producing genes that responsible for the biosynthesis of DON (*Tri5*), ZEN (*PSK*), and FB (*FUM1*) were detected by using the corresponding specific primers listed in Table 2 (Baird et al., 2008; Lysøe et al., 2006; Niessen et al., 2004). The PCR system was the same as above, while the reactions were programmed for 94 °C for 10 min, 5 cycles of 94 °C 40 s, 52 °C 45 s, and 72 °C 1 min, followed by another 35 cycles of 94 °C 40 s, 55 °C 45 s, and 72 °C 1 min, and a final extension at 72 °C for 10 min. All the PCR products generated were resolved on 1.2% agarose gels. The gels were stained with Ultra GelRed (Vazyme Biotech Co., Ltd) and visualized under UV light.

2.5 Mycotoxin production by Fusarium strains isolated in vitro

In this experiment, 1 kg of corn, wheat, and brown rice samples with full and intact grains and free from mold and rot were selected, added with sterile water to adjust the water activity to 30%, and sealed in the refrigerator at 4 °C overnight. The next day, the samples were crushed with knife mill (KN 295 Knifetec, Foss Analytical). After mixing, 20 g of the crushed samples were taken and dispensed into 250 mL conical flasks, and three replicates of each sample were prepared. Sterilized with the autoclave (HIRAYAMA, Japan) at 121 °C for 20 min. 5 mL diluted spore suspension was added to each conical flask, and 5 mL sterile water was added to the control group. The cells were incubated in the dark at 25 °C, 60% RH for 7 days. The cultures were handshake daily to disperse the fungus throughout grain, and to avoid clump (Palacios et al., 2021). The grain cultures were dried at 50 °C, and stored at 4 °C until mycotoxin analysis.

2.6 Mycotoxins analysis

The content of fumonisin was determined by rapid quantitative method of colloidal gold technology (Ling et al., 2015). The stirps were purchased from SiTechno, China, and determined according to the manufacturer's instructions.

Deoxynivalenol was extracted according to the second method of the national standard of the People's Republic of China GB 5009.111-2016 (People's Republic of China, 2016b), with some modifications. A volume of 50 mL ddH_20 was added to the conical flask containing 20 g samples, soaked overnight in refrigerator at 4 °C, and shacked at 25 °C, 200 rpm for 30 min the next day, filtered for later use. The filtrate was diluted with water 4 : 6 to 10 mL, and loaded to the immunoaffinity column at the speed of 1-2 drops per second. After passing, the immunoaffinity column was washed with 20 mL of water at the same speed, and all the effluent was discarded and the column was dried. Eluted with 1.5 mL methanol, add 0.5 mL ddH_2O to the eluate, mixed and filtered into the sample bottle with a 0.22 μm filter membrane. The quantitatively analyzed was performed using an Agilent 1290 Infinity II LC (Agilent Technologies, Santa Clara, CA, USA). Reserve-phase column chromatography was performed using C18 (YMC, Kyoto, Japan). The mobile phase consisted with 20% methanol in distilled water, the column temperature was maintained at 35 °C, the injection volume was 50 μL, and the detection wavelength was set to 218 nm.

Zearalenone was extracted according to the first method of the national standard of the People's Republic of China GB 5009.209-2016 (People's Republic of China, 2016a), followed by some modifications. The total volume of 50 mL extraction solvent [Methanol:H_20, 80:20 (*v/v*)] was added to the conical flask with 20 g sample, soaked overnight in refrigerator at 4 °C, and shacked at 25 °C, 200 rpm for 30 min the next day, filtered for later use. The filtrate was diluted with supersaturated saline 4 : 16 to 20 mL and loaded to the immunoaffinity column at the speed of 1-2 drops per second. After passing, the immunoaffinity column was washed with 20 mL water at the same speed, and all the effluent was discarded and the column was dried. The eluent was collected by elution with 2 mL methanol, and filtered into the sample bottle with 0.22 μm filter membrane. ZEN was analyzed by the above-mentioned instrument and column with a mobile phase consisted acetonitrile:water:methanol (46 : 46 : 8, *v/v/v*) at 0.5 mL/min. The column temperature was maintained at 25 °C, the injection volume was 50 μL, and detected with a fluorescence detector (274 nm excitation, 440 nm emission).

Table 2. Primers used in detection of toxigenic genes.

Primer	Sequence (5'-3')	Size (bp)	Tm (°C)	Types of Toxicity	Reference
Tri5-F	ACTTTCCCACCGAGTATTTT	525	53	DON	Niessen et al., 2004
Tri5-R	CAAAAACTGTTGTTCCACTGCC				
PSK-F	AGATGGCCATGGTGCTTCGTGAT	480	55	ZEN	Lysøe et al., 2006
PSK-R	GTGGGCTTCGCTAGACCGTGAGTT				
Fum-F	GTCGAGTTGTTGACCACTGCG	846	58	FB	Baird et al., 2008
Fum-R	CGTATCGTCAGCATGATGTAGC				

2.7 Fumigated with two strong oxidizing gases

To examine the effect of the two strong oxidizing gases on the growth control of the *Fusarium* strains on the medium, spotted 20 µL of spore suspension on the center of the PDA plate supplemented with streptomycin. Next, these spore-inoculated plates were treated with gaseous fumigant directly to study the effect on spore germination. For the study on the inhibition of mycelial growth, the plates should first be cultured at 28 °C for 24 h. The gas-treated plates were further incubated at 28 °C. The mycelial length was measured once a day, and the numbers of colony-forming units (CFUs) representing spore germination were counted after 2 days incubation.

The gaseous ClO_2 was generated by a ClO_2 commercial generator (WAERTE, China), and gaseous O_3 was generated by a O_3 commercial generator (DAHUAN, China), and principles were shown in Figure 1. The test was carried out in a modified laboratory drying tank (Figure 2), divided into a control group (no fumigation) and a treatment group [fumigated with ClO_2 (300 ppm) or O_3 (400 ppm or 1400 ppm)]. The treated groups were exposed for 30 min, 60 min, 90 min and 120 min. The gaseous fumigant enters the bottom of the from the air inlet, and the redundant air was let out from the top of the glass reactor (Savi et al., 2014; Sun et al., 2017). For safety issues, the exhaust gas was neutralized with saturated aqueous sodium thiosulfate (Jones et al., 2006; Ma et al., 2017).

A

$$2ClO_2 + H_2O \rightarrow HClO_2 + HClO_3$$

$$\frac{(HClO_2)(HClO_3)}{ClO_2} = 1.2\times 10^7 (20℃)$$

B

$$3O_2 \Leftrightarrow 2O_3$$

Figure 1. The principles of the gaseous fumigant generated. (A) Gaseous ClO_2 was generated by the mixing of Solution A and Solution B, that mentioned in the manufacturer's instructions. (B) Ozone gas was produced by ionizing oxygen in the air.

2.8 Scanning electron microscopy analysis of the isolated Fusarium

A small piece of *Fusarium* mycelium fumigated by ozone and chlorine dioxide was scraped and placed in 1.5 mL EP tube. The sample was fixed with 500 µL 4% paraformaldehyde. The precipitate was collected by centrifugation, washed twice with PBS, 5 min each time, and washed once with 4% (w/v) sucrose solution for 5 min, dehydrated with a series of gradient alcohol, 30%, 50%, 70%, 80%, 90%, 95%, 100%, 10 min each gradient. After adding 100% alcohol to resuspend, a small number of suspended droplets were absorbed and added to the glass. The glass was gently adhered to the conductive adhesive, dried at the critical point, and vacuum sprayed. Finally, observed its morphologic changes by scanning electron microscope (FEI, USA).

3 Results

3.1 Isolation and identification of Fusarium spp.

From the observed experimental results, there were more *Fusarium* in pink FHB wheat and more Aspergillus in white FHB wheat, and the overall bacterial phase difference was not obvious. The washing and coating method was used to isolate the fungi on the surface of wheat, and the grinding method was used to isolate the fungi inside wheat (Figure 3). As a result, the latter method was more suitable for isolating *Fusarium* spp.

Three typical strains of Fusarium were isolated in this experiment, named N1, N2, and N3. For the phylogenetic analysis of the isolates, sequence of the *ITS* region and *EF1-α* gene, respectively, were analyzed. The amplified fragments had the expected size (Table 1, Figure 4). The amplified results were sent for sequencing, and the sequences were aligned against the GeneBank (NCBI, nucleotide blast) and MLST database showed 95% to 100% homology to previously described *Fusarium* spp.

A phylogenetic tree was constructed based on the ITS and *EF1-α* gene from the isolated strains (Figure 5). This analysis revealed that N1 was identical to *Fusarium graminearum*, N2 to *Fusarium asiaticum*, and N3 to *Fusarium culmorum*.

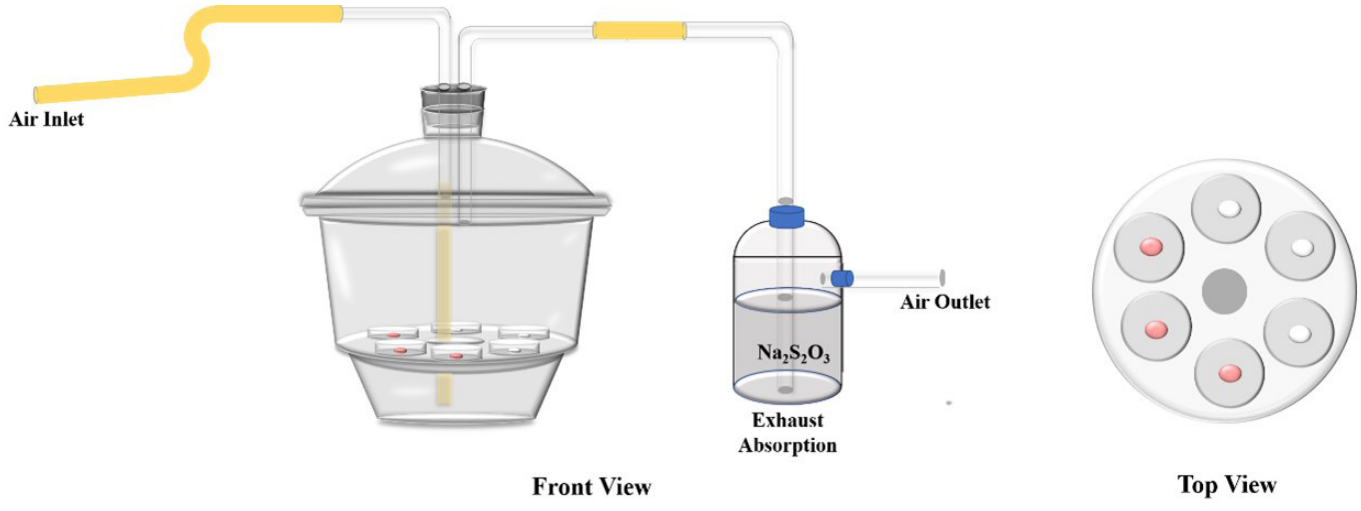

Figure 2. Schematic diagram of simple gaseous fumigant treatment system.

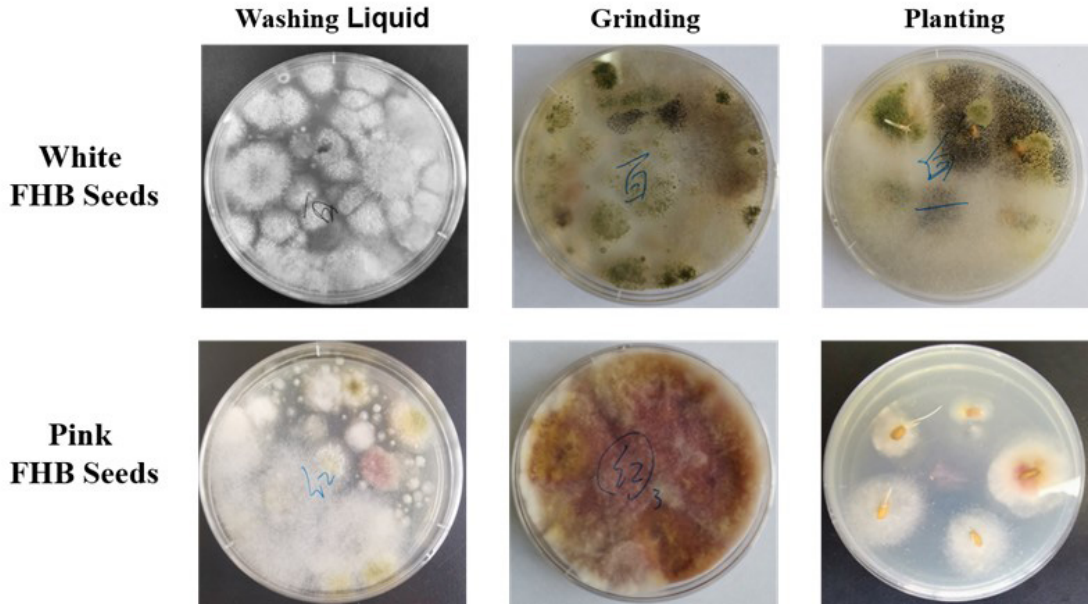

Figure 3. Isolation of *Fusarium. spp*. Pink FHB seeds contain more *Fusarium*, while white ones contain more *Aspergillus*. The grinding method was more suitable for isolation of *Fusarium*.

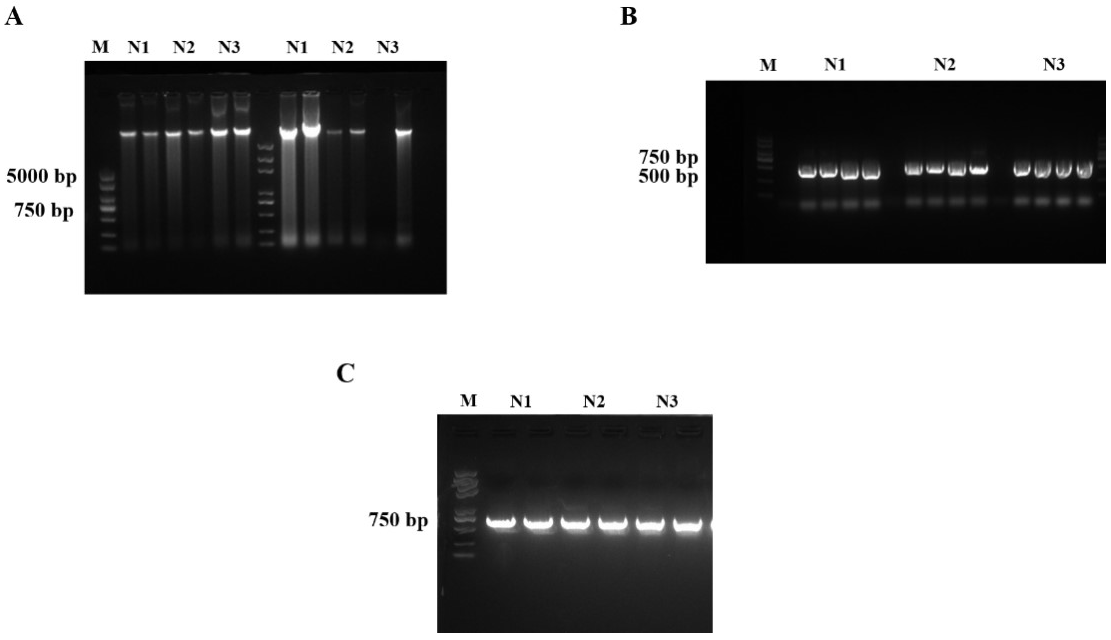

Figure 4. Molecular identification of the *Fusarium* spp. isolates. (A) Analysis of total DNA extracted from the isolates. Molecular identification of Fusarium species by PCR amplification of Internal Transcribed Spacer (ITS) (B), and the translation elongation factor-1 alpha (*EF1-α*) (C). Primer sets and the reference to the sequences used are given in Table 1.

3.2 Detection of toxigenic genes and toxigenic capacity in vitro

The PCR-based detection of the genes associated with mycotoxin biosynthesis were carried out. It can be seen from the genetic results that strain N2 did not contain any of these three toxin-producing genes, and both N1 and N3 contained the *PSK* gene and *Tri5* gene, thus indicating that these isolates can potentially produce ZEN and DON under the suitable conditions (Figure 6).

The background contents of the mycotoxins (Table 3) and the mycotoxins produced by three strains cultured on three natural grain mediums (Table 4) were analyzed based on immunoaffinity

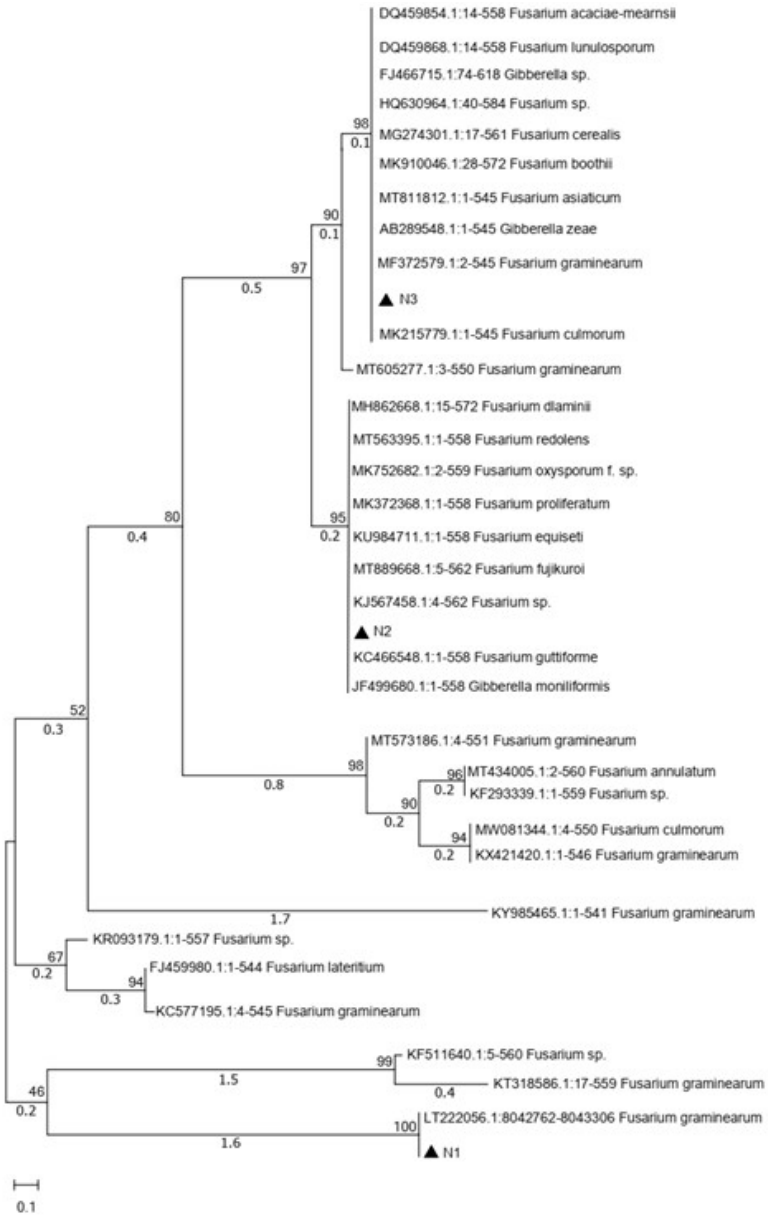

Figure 5. Phylogenetic tree of isolated *Fusarium* spp. strains and related members of the genus *Fusarium*. The tree was constructed using the neighbor-joining method based on ITS and *EF1-α* gene sequences. The bar represents a genetic distance of 0.1.

Table 3. Background content of mycotoxins in the grain mediums.

Culture medium type	Mycotoxins (μg/kg ± SD)[a]		
	DON	FBs	ZEN
Wheat	136.37 ± 8.7	951.71 ± 38.3	ND
Corn	234.21 ± 10.2	1642.71 ± 50.4	ND
Paddy	ND	787.67 ± 28.5	ND

[a]Average value of three replicates. ND: not detected (blow detection limit).

chromatography purification-HPLC. Among them, N1 and N3 were able to produce ZEN and DON in vitro but not FBs, and N2 strain did not produce any of the three toxins, that were agreed with the molecular identification of the key mycotoxin-producing genes. In addition, the two strains produced different amounts of mycotoxins on three different natural mediums.

3.3 Effect of gaseous fumigants against Fusarium strains isolated

The effect of the gaseous fumigants against *Fusarium* strains isolated was tested on PDA. The gaseous ClO_2 and O_3 were generated by commercial ClO_2 generator and O_3 generator, respectively.

Inhibition the spore germination of Fusarium

Plates inoculated with spores were placed into the reactor under sterile conditions and fumigated with ClO_2 at a concentration of 300 ppm or O_3 at a concentration of 400 ppm under different exposure times. The germination of fungal spores was completely suppressed for 30 min treatment of the fumigation gas. It can also

be clearly seen from the microscopic results that the fumigated conidia cells changed from transparent to turbid, the structure of the outer coats were destroyed and the spore lost its activity (Figure 7).

Inhibition the mycelial growth of Fusarium

Plates incubated at 28 °C for 36 h were placed into the reactor under sterile conditions and fumigated. The mycelium length was measured at the same time every day once the experiment started. The experimental results showed that O_3 had a small inhibitory effect on mycelium (Figure 8), even when fumigated at 1400 ppm for 2 h (data not shown) there was no particularly significant inhibition. The same conclusion can be seen from the results of scanning electron microscopy that the surface of the mycelia after fumigation was smooth as that of the control group.

While, the effect of ClO_2 on mycelial growth was different from that of O_3, the mycelium did not continue to grow the next four days after fumigation for 0.5 h. The results of scanning

Table 4. Mycotoxigenic capacity of isolated *Fusarium* in the grain mediums.

Isolate	Culture medium type	Mycotoxins (μg/kg ± SD)[a]		
		DON	FBs	ZEN
Control	Wheat	112.29 ± 7.2	925.37 ± 29.6	ND
	Corn	198.34 ± 8.9	1429.52 ± 46.3	ND
	Paddy	ND	736.21 ± 22.9	ND
N1	Wheat	5520.6 ± 258.4	930.25 ± 27.3	41148. 0 ± 499.2
	Corn	33309.6 ± 492.5	1572.41 ± 43.7	34103.2 ± 372.1
	Paddy	16808.4 ± 312.6	802.37 ± 25.2	38572.1 ± 403.7
N2	Wheat	143.1 ± 8.5	961.37 ± 28.2	ND
	Corn	212.42 ± 9.1	1433.61 ± 44.6	ND
	Paddy	ND	745.70 ± 32.1	ND
N3	Wheat	47125.0 ± 529.1	922.11 ± 25.4	12458.2 ± 294.6
	Corn	7796.7 ± 297.3	1407.24 ± 43.1	9663.1 ± 287.3
	Paddy	11379.0 ± 302.2	792.41 ± 24.7	4872.7 ± 215.9

[a]Average value of three replicates. ND: not detected (blow detection limit).

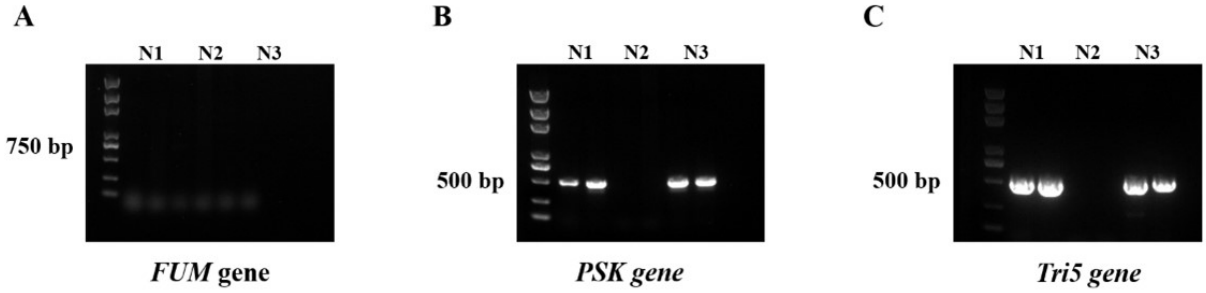

Figure 6. Molecular detection of toxigenic genes. (A) None of the three strains were detected to have the *FUM* gene for biosynthesis FBs. (B) *PSK* gene was detected in N1and N3. (C) The detection of *Tri5* gene in N1 and N3 was also positive.

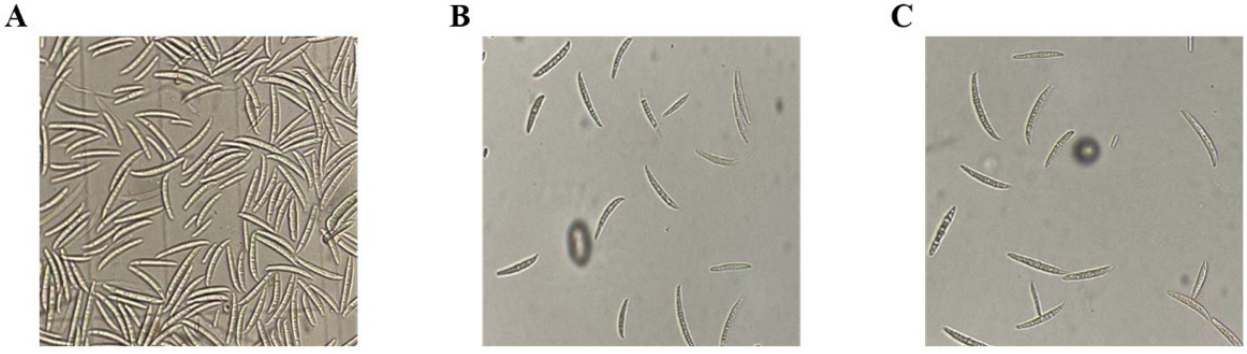

Figure 7. Inhibition of spore germination. (A) Observation of spore morphology of N1 strain under microscope, sickle-shaped with smooth surface, and transparent cells with obvious transverse septa. The spore morphology was observed after 30 min of treatment with O_3 (B) and ClO_2 (C). The surface was uneven, the cells were turbid, and the intracellular septum disappeared.

electron microscopy also showed that the surface of mycelium became rough and shrunk, and mycelium aging or death occurred (Figure 9). Therefore, we believe that chlorine dioxide has a significant inhibitory effect on the mycelial growth of *Fusarium*.

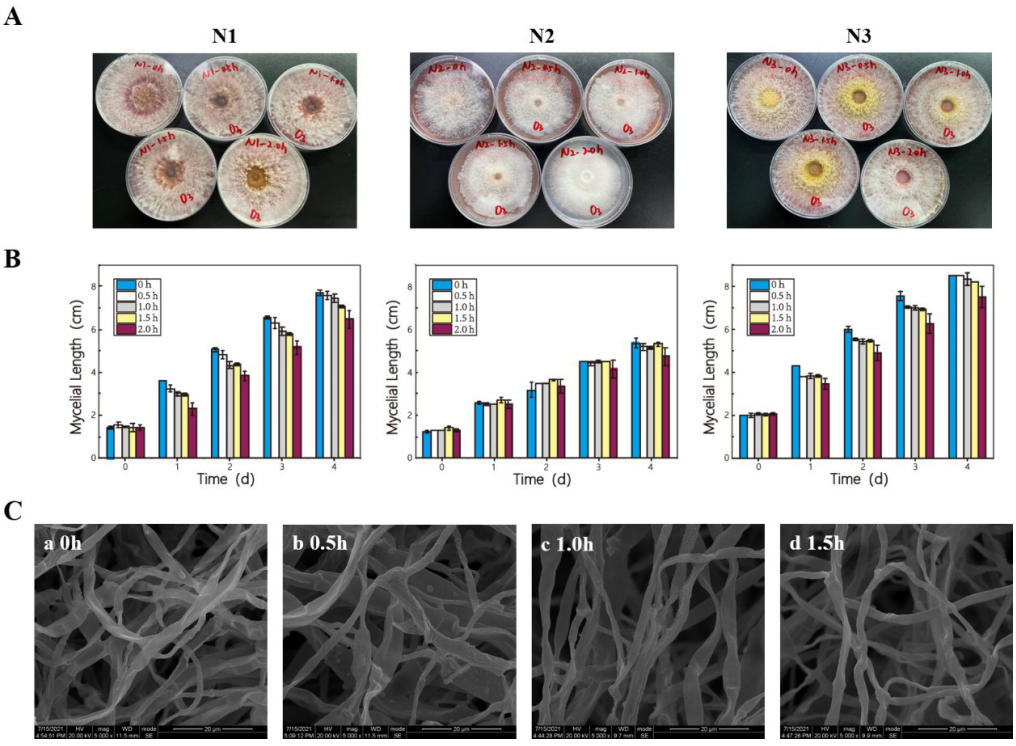

Figure 8. The effect of O_3 on mycelial growth. (A) Mycelial growth results of plate cultured for 4 days after O_3 fumigation. (B) Measurement results of mycelial length during cultivation. (C) Scanning electron microscopy of the hyphae of N1 post-treated with O_3 for different times.

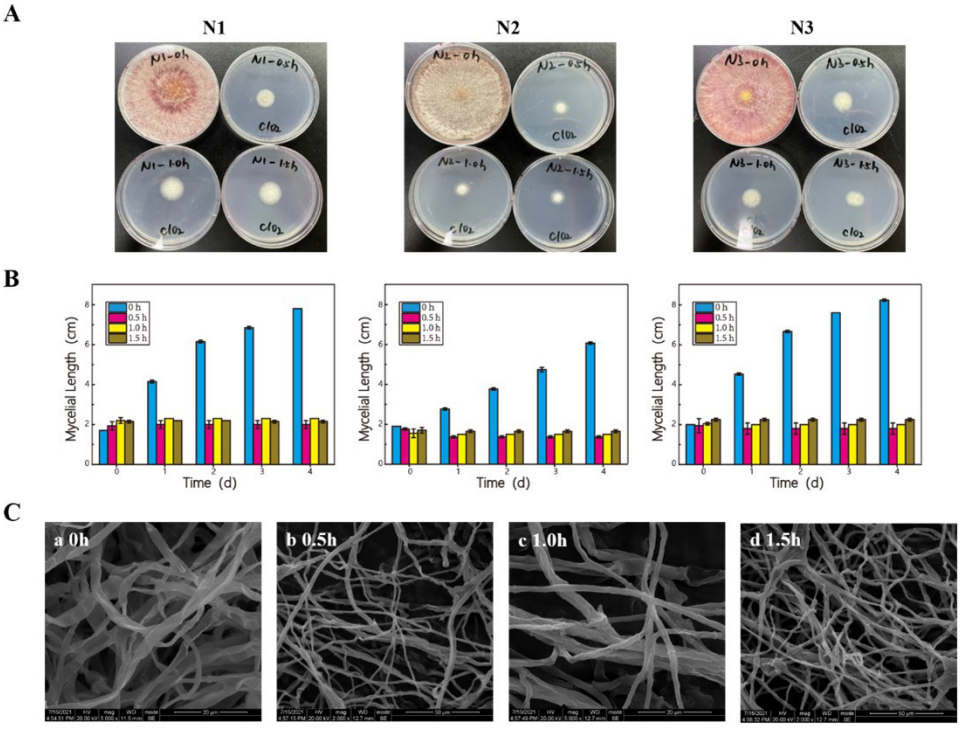

Figure 9. The effect of ClO_2 on mycelial growth. (A) Mycelial growth results of plate cultured for 4 days after ClO_2 fumigation. (B) Measurement results of mycelial length during cultivation. (C) Scanning electron microscopy of the hyphae of N1 post-treated with ClO_2 for different times.

4 Conclusion and discussion

In this study, we isolated three typical Fusarium spp. named N1, N2, and N3 from the FHB infected wheat and identified them as *Fusarium graminearum*, *Fusarium asiaticum*, and *Fusarium culmorum*, respectively, using phylogenetic analysis of the internal transcribed spacer (ITS) and the elongation factor 1-α gene (*EF1-α*). Furthermore, we detected the toxigenic genes of the isolated strains and their toxigenic capacity in vitro. The results of the FBs detection with rapid quantitative method of colloidal gold technology and DON and ZEN assays based on immunoaffinity chromatography purification-HPLC were agreed with the molecular identification of key mycotoxin-producing genes. Finally, we tested the ClO_2 and O_3 for their effects on the control of *Fusarium* growth in vitro under different exposure times. Both ozone and chlorine dioxide that produced by commercial gas generator could kill fungal spores in a short time, and the fumigation-treated spores were not colonized even after 7 days of continuous incubation, the results of the plate culture were consistent with the results of microscopic. However, their inhibitory effects on mycelial growth of *Fusarium* spp. were different. Low concentration of chlorine dioxide (300 ppm) treatment for 0.5 h can significantly inhibit the growth of mycelium, and no longer grow after culture. However, high concentration ozone (1400 ppm) treatment for 2 h has no obvious inhibitory effect on mycelial growth. The growth data of mycelium were consistent with SEM results.

In addition, gaseous ClO_2 has been widely used to control food-borne or post-harvest microbial contamination on fruits and vegetables (Bhagat et al., 2011; Du et al., 2002; Yuk et al., 2006). It was also documented that ClO_2 fumigation is effective in killing phosphine-susceptible and resistant strains of stored product insect species and without serious chemical residues on stored rice (E et al., 2018). Yu et al. (2020) pointed out that AFB1 can be decomposed by gaseous ClO_2 successfully into products that are non-toxic to human (Yu et al., 2020). This has important guiding significance for the application of ClO_2 in mycotoxin degradation.

Taking together, the results in this study showed that the PCR-based identification of genes associated with mycotoxin biosynthesis can be used for rapid prediction of fungal toxicity, and ClO_2 gas is effective in the growth control of *Fusarium* spp. as a main contaminant in wheat at a low concentration. Therefore, gaseous ClO_2 can be used as a potential green fumigation agent for mold and insect control, and toxin degradation during grain storage.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Jin Wang, Yuxi Gu and Jinying Chen conceived and designed the experiments; Jin Wang and Yuxi Gu performed the experiments; Jin Wang, Yuchong Zhang, Zilong Liao, Xiaoxue Shan, Li Li and Chen shuai analyzed the data; Ji Wang, Jinying Chen and Linhong He wrote and revised the paper.

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