New Strain of *Bjerkandera Adust* as a Potential Biocontrol Agent Against Wheat Scab

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Abstract

Bjerkandera adusta can decompose polycyclic aromatic hydrocarbons including cellulose and lignin, but its roles in inhibiting plant pathogens are unknown. Here, the confrontation culture and greenhouse pot experiments were employed to study the control effect of *B. adusta* M1 on *Fusarium graminearum* and wheat scab. The results indicted the *B. adusta* M1 fermentation broth (FB) could inhibit the growth of *F. graminearum* and decrease the disease index of wheat scab, and the control effects on wheat scab were 93.27% of the chemical fungicide carbendazim. The growth rate was significantly ($P < 0.05$) higher in *B. adusta* M1 than in *F. graminearum*, indicating a strong competitiveness by *B. adusta* M1. The images from the scanning electron microscope showed substantial deformations of the hyphae of *F. graminearum* penetrated by the hyphae of *B. adusta* M1, indicating a strong mycoparasitism by *B. adusta* M1. Similarly, FB increased the activities of leaf catalase, peroxidase and phenylalanine ammonia-lyase related to disease resistance, and decreased the cell membrane permeability and malondialdehyde. So we concluded that *B. adusta* M1 could be a promising fungal strain to control the detriment of *F. graminearum* to field cereal productions.

Introduction

Wheat Scab, a globally fungal disease that is commonly occurred in cereal crops, including wheat, barley, oats and rye, infects the wheat heads, causes the kernel to shrivel and reduces the grain yield (O'Donnell et al. 2004; Starkey et al. 2007). *F. graminearum*, the main pathogenic fungus to induce the wheat scab (Champeil et al. 2004), causes spike rot, stem rot and seedling rot while producing a number of fungal toxins. Among them, the strongest toxin is called deoxynivalenol (DON), which pollutes wheat seeds and various wheat products, causing human and mammals vomiting, diarrhea, dizziness and other acute toxicity, immune function decline and other chronic toxicity, leading to seriously threat to food security, and human and animal health (Ehlin et al. 1997). As a result, wheat scab has been a highly concerned disease in the world (Gilbert and Tekauz, 2000; Song et al. 2018).

At present, except the resistant cultivars and agricultural practices, the prevention and control of wheat scab is mainly depended on chemical fungicides (Brar et al. 2019; Song et al. 2018). For example, the benzimidazole fungicide carbendazim has been widely used to control wheat scab in China (Song et al. 2018). However, the long-term application of chemical fungicides has increased the pathogen resistance and their residue pollution in soil and water environments (Siranidou and Buchenauer, 2001). Therefore, it is important to screen antagonistic microorganisms for avoiding of fungicide resistance, which are not harmful to human, animals and the ecological environment (Schulz et al. 2002).

Numerous antagonistic microorganisms on wheat scab have been recently reported (Schisler et al. 2015). For instance, 3 bacillus strains and 4 yeast strains have demonstrated their effectively control effects on wheat scab across geographical environments of the United States (Khan et al. 2004). In addition, a strain of *Trichoderma harzianum* from wheat rhizosphere has displayed a strong inhibitory effect on wheat scab (Dal Bello et al. 2002). Up to 73% or 100% of *F. graminearum* in wheat stems were decreased

after 90 d or 180 d of the application of both the strains *Gliocladium roseum* strain 016 and 1457 to wheat stubble in Argentina (Palazzini et al. 2013). Both the incidence of wheat scab, and DON toxin were significantly decreased by *Gliocladium roseum* ACM941 in Canada (Xue et al. 2009) and *Cryptococcus magnus* OH182.9 in the United States (Schisler et al. 2013).

B. adusta, a species of wood rot fungus belonging to genus *Bjerkandera* and family *Meruliaceae* in the order *Polyporaceae*. At present, researches about *B. adusta* were mainly focused on cellulose and lignin degradation (Moody et al. 2018; Zavarzina et al. 2018), laccase synthesis (Behrens et al. 2018), organic pollutants such as textile dyes degradation (Andriani and Tachibana, 2016; Behrens et al. 2016) and drug-polluted waste water treatment (Aydin, 2016). However, studies are rarely reported on the biological control of plant diseases by *B. adusta*. We have isolated a strain *B. adusta* M1 from a purple soil (*Eutric Regosol*, FAO Soil Classification System) locating in the Southwest University campus in Chongqing, southwest China, and found that *B. adusta* M1 and its fermentation broth had great bio-control effects on *Didymella bryoniae* and watermelon vine blight in greenhouse experiments (Zhang et al. 2017). However, the biological control of *B. adusta* M1 on wheat scab has not been reported. In this study, the biocontrol effects and mechanism of *B. adusta* M1 against wheat scab were addressed with the confrontation culture and greenhouse pot experiments. The generated results could provide potentially field application of *B. adusta* M1 to control wheat scab in cereals.

Materials And Methods

Materials

Seeds of wheat (*Triticum* spp. cv. Xinmai 18) were commercially from the Mianyang Seed Company, Sichuan, China. The purple soil (*Eutric Regosol*, FAO Soil Classification System) was collected at 5-10 cm depth from a field within the National Monitoring Station of Soil Fertility and Fertilizer Efficiency on Purple Soils, locating in the Southwest University campus (E 106°24'37"; N 29°48'32") Beibei, Chongqing, China. The collected purple soils were thoroughly mixed after the removal of debris, dried at 30°C, then sieved to 2 mm and autoclaved before the pot experiments, and the basic chemical properties were shown in Table 1.

Table 1 Chemical properties of the experimental purple soil

Chemical properties	OM	TN	TP	TK	AN	AP	AK	pH
	g/kg	g/kg	g/kg	g/kg	mg/kg	mg/kg	mg/kg	
Purple soil	10.62	0.84	0.63	21.33	70.43	31.21	117.22	5.52

OM: Organic matter; TN: Total nitrogen; TP: Total phosphorus; TK: Total potassium; AN: Available nitrogen; AP: Available phosphorus; AK: Available potassium.

Two fungal strains were examined: *B. adust* M1 was isolated from the above-mentioned rhizosphere soil in April 2016, and *F. graminearum*, a pathogenic fungus resulting in the wheat scab, was from the Institute of Plant Ecology and Pathology, Southwest University.

A chemical fungicide: carbendazim [50% N-(benzimidazlyl-2) methyl carbamate] was from the Sichuan Guoguang Agrochemical Company, Jianyang, Sichuan, China.

The Potato dextrose agar (PDA) medium was used for cultivating the strains of *B. adusta* M1 and *F. graminearum* (Farias et al. 2010). The basic broth medium (20 g glucose, 10 g NH_4Cl, 1 g $MgSO_4$, 1 g $CaCl_2$, 2 g KH_2PO_4, and 1,000 mL distilled water) was used for obtaining the fermentation broth of *B. adusta* M1 and the spore suspension of *B. adusta* M1 and *F. graminearum*.

Preparation of spore suspension of *F. graminearum*

F. graminearum was inoculated in the PDA plate and cultivated at 28°C for 5 d until the mycelia filled with the plate, and then added 5 mL sterilized basic broth medium into the plate, gently scraped off the conidia with the inoculating spear. The liquid was filtrated with 4 layers of gauzes. Meanwhile, the spore numbers of the filtrate were counted by the globulimeter under ordinary optical microscope microscope, and finally diluted to 4.5×10^5 pcs/mL basic broth medium.

Preparation of FB

B. adusta M1 was inoculated in 250 mL triangular bottle with 100 mL basic broth medium, which was shaken at 32°C and 150 r/min for 5 d, and then centrifuged at 3000 r/min for 1 min. The supernatant was filtrated with 0.22 μm sterile filter, and the filtrate was termed as FB.

The inhibitory effects of *B. adusta* M1 on *F. graminearum*

The inhibitory effect of *B. adusta* M1 on *F. graminearum* was measured by the antifungal test as follows. Both the *B. adusta* M1 and *F. graminearum*, with a size of 5 mm-diameters, were inoculated on the same PDA plate (9 cm × 9 cm), while the two strains were 3 cm apart to form a two-point confrontation. The sole *F. graminearum* that was inoculated on the PDA was the control. After cultivated at 32°C for 5 d, the inhibitory effect of *B. adusta* M1 on *F. graminearum* could be observed. The fungal colony sizes in the control and confronting plates were recorded and percent inhibition was calculated as

$$\frac{A1-A2}{A1} \times 100 \text{ (formula 1)}$$

Where A_1 and A_2 are the areas in mm^2 of the indicator pathogen in the control and treatment, respectively. The tests were performed in three replications.

The inhibitory effects of FB on *F. graminearum*

The antifungal effect of FB on *F. graminearum* was determined by the antifungal test (Campanile et al. 2007) and the relevant inhibition rate was calculated with the above formula 1. Briefly, *F. graminearum* was inoculated on PDA plate (9 cm × 9 cm) with 10%, 20% or 40% FB (the control was different concentration basic broth medium) and cultivated at 32°C for 5 d, and repeated for 3 times. The inhibition rate was calculated with the formula 1.

The competition and mycoparasitism effects of *B. adusta* M1 against *F. graminearum*

Two groups of experiments were conducted. *B. adusta* M1 and *F. graminearum*, both with 5 mm-diameters, was inoculated on the same PDA plate, and the two strains were 3 cm apart, forming a two-point confrontation. One group was used to observe the competition effect of *B. adusta* M1 on *F. graminearum* (only *F. graminearum* was inoculated on the PDA as control group). For another group, a 2 cm × 2 cm foil paper was laid on the surface of the PDA plate between two strains, and the fungal strains were cultivated at 32°C for about 3 d until they intermingled on the foil paper. Then the foil paper was removed to prepare microscope slides and the mycoparasitism effect of *B. adusta* M1 on *F. graminearum* was observed under a scanning electron microscope.

The control effects of FB on wheat scab

Firstly, wheat seeds were immersed in 75% alcohol for 5 min, and treated with 20% sodium hypochlorite for 3 min, and rinsed several times with sterile water, and then cultivated in a constant temperature incubator at 28°C for 3 d to promote their germination. The germinated seeds were sowed in a seedling tray (20 cm × 30 cm) that was filled with aseptic nutrient soil. Three wheat seedlings with two cotyledons were transplanted to the pot (18 cm × 24 cm) with 2 kg sterilized soil. After 30 d of transplanting, the wheat seedlings were irrigated with 10 mL *F. graminearum* spore suspension. After the wheat seedlings got sick, four treatments were examined: The wheat seedlings were irrigated with 10 mL basic broth medium (CK), 10 mL *B. adust* M1 fermentation broth (FB), or 10 mL 0.08 mg/mL chemical fungicide polymyxin carbendazim (CF). Each test was repeated 3 times. After 24 h of treatment, the disease index and control effects were respectively counted once every 5-day for a total of 3 times (this article showed the last statistical results) according to the formula 2 and 3. The disease classification criteria were as follows: Grade 0: no disease spots, or the stem leaves infected the disease are less 1%; Grade 1: there are small disease spots, the stem leaves infected the disease are 1% ~ 25%; Grade 2: there are large disease spots, the stem leaves infected the disease are 26% ~ 50%; Grade 3: there are a large number of disease spots, blight and curly, the stem leaves infected the disease are 51% ~ 75%; Grade 4: there are a large number of disease spots, the stem leaves infected the disease are 76% ~ 90%; Grade 5: the stem leaves infected the disease are more than 90%, or whole plant die of disease (Lian et al. 2017).

$$\text{Disease index} = \frac{\Sigma \text{disease scale} \times \text{number of corresponding leaves}}{\text{maximum disease scale} \times \text{total number of leaves}} \times 100 \text{ (formula 2)}$$

$$\text{Control effect (\%)} = \frac{\text{disease index of control} - \text{disease index of treatment}}{\text{disease index of control}} \times 100 \text{ (formula 3)}$$

Wheat leaves were collected after the last count of plant disease, and the activities of leaf catalase (CAT), peroxidase (POD) and phenylalanine ammonia lyase (PAL) were measured by the (UV) spectrophotometry and guaiacol method (Pandey et al. 2018). The leaf cell membrane permeability and malondialdehyd was analyzed by the electrolyte infiltration and thiobarbital (TBA) colorimetry (Yuan et al. 2005).

Data analyses

The statistical software SPSS 21.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL) was used to analyze the data obtained from our experiments. The significant differences between treatments were compared with the Duncan's multiple range tests at a significance level of $P < 0.05$. The figures and tables were generated with Excel 2017, Oringin 9.0.

Results

Inhibitory effects of B. adusta M1 on F. graminearum

The *B. adusta* M1 inoculation showed significant growth inhibition on *F. graminearum*, which had a significantly ($P < 0.05$) smaller colony radius on the PDA (Table 2), compared to the non-*B. adusta* M1 control, and the inhibition rate of *B. adusta* M1 against *F. graminearum* reached 57.71%.

Table 2
The inhibitory effects of *B. adusta* M1 on *F. graminearum*

Treatment	Radius (cm)	Inhibition ratio (%)
Control	4.47±0.09a	—
B. adusta M1	1.87±0.09b	57.71±1.92
Values are means ± SE. Different small letters indicate significant difference ($P < 0.05$).		

Inhibitory effects of FB on F. graminearum

F. graminearum was inhibited significantly ($P < 0.05$) by FB of different concentrations (Fig. 1), and the inhibition rates with 20% and 40% FB reached 80.76% and 89.17% respectively.

The competition effects of B. adusta M1 on F. graminearum

The growth rate was significantly ($P < 0.05$) higher in *B. adusta* M1 than in *F. graminearum*, indicating a strong competitiveness for nutrition and space by *B. adusta* M1. *F. graminearum* with the *B. adusta* M1 inoculation was inhibited in a small circle on the PDA (Fig. 2b), compared to the non-*B. adusta* M1 control (Fig. 2a).

The mycoparasitism effects of B. adusta **M1 on** F. graminearum

The images under the scanning electron microscopy showed that the hyphae of *B. adusta* M1 directly penetrated the hyphae of *F. graminearum* (Fig. 3), leading to the latter's expansion, deformation and even fracturation.

Control Effects Fb On Wheat Scab

The disease index of wheat scab was significantly ($P < 0.05$) decreased under both FB and CF (Table 3). The treatment with FB had a significant control effect on wheat scab, which was 93.27% of CF.

Table 3
The control effects of different treatments against wheat scab

Treatment	Disease index	Control effects (%)
CK	47.43±2.20 a	—
FB	13.16±0.86 b	72.14±1.42 b
CF	10.60±0.85 b	77.34±1.76 a

CK, the control; FB, *B. adusta* M1 fermentation broth; CF, the chemical fungicide. Values are means ± SE. Different small letters indicate significant difference ($P < 0.05$).

Effects of FB on the activities of enzymes relevant to disease resistance in wheat plant

The activity of CAT, POD and PAL under the FB was significantly ($P < 0.05$) increased by 75.72% (Fig. 4a), 22.87% (Fig. 4b) and 86.76% (Fig. 4c), respectively.

Effects of FB on the cell membrane permeability and malondialdehyd in wheat leaves

Both the cell membrane permeability and malondialdehyde content in wheat leaves under FB was significantly ($P < 0.05$) decreased by 63.65% (Fig. 5a) and 67.12% (Fig. 5b), respectively.

Discussions

Biocontrol fungi can prevent and control plant diseases by diverse mechanisms, such as competition (Schouteden et al. 2015), mycoparasitism (Nasini et al. 2004), inducing plant systems to acquire disease resistance (ISR) (Choudhary et al. 2007), and the production of antimicrobial active substances (Liu et al. 2009) and so on.

Normally, biocontrol fungi grow faster than pathogenic fungi in a same ecosystem, since the former can obtain more water, nutrient and living space (Schouteden et al. 2015). Results from this present study showed that the growth rate was significantly ($P < 0.05$) higher in *B. adusta* M1 than in *F. graminearum*, indicating a strong competitiveness for nutrition and space by *B. adusta* M1 (Fig. 2).

Biocontrol fungi inhibit the growth of the pathogenic fungi by the mycoparasitism is one of important pathways to control plant diseases (Eziashi et al. 2006). The hyphae of mycoparasitic fungi can penetrate the cells of their host fungi to build parasitic relationships, resulting in not only a declined growth, but also deformations, shriveled and even dissolved for the hyphae of their host fungi (Troian et al. 2014). The contribution of biocontrolling plant pathogen by microbial antagonists is one of future disease control strategies in the organic agriculture (Karlsson et al. 2015). Recently, the mycoparasitism of plant pathogenic fungi by *Trichoderma* isolates has been reported (Ojha et al. 2011) and is commercially widely applied to control *Rhizoctonia solani*, *Botrytis cinerea*, *Pythium*, and *Fusarium* (Howell, 2003). In this study, the expansion, deformation and even fracturation of the hyphae of *F. graminearum* deriving from a direct penetration (Fig. 3) of the hyphae of *B. adusta* M1 indicated that *B. adusta* M1 had a strong inhibition in *F. graminearum* by the mycoparasitism.

The induced systemic resistance (ISR) can result in an altered resistance against subsequent challenges by pathogen or parasite (Choudhary et al. 2007). The mechanism of ISR action is mainly through the tissue lignification to enhance cellular mechanical barrier and to produce an apopharmactin, which involves in the enzymes such as PAL, POD, CAT and other catalyzed biochemical reactions (Qiu et al. 2008; Chen et al. 2010), and the activities of these enzymes are positively correlated with plant disease resistance (Arias et al. 2005). For example, Patil et al. (2011) found that the POD activity was increased after plants were treated with non-pathogenic *Fusarium moniliforme* Fu3, Fu4, Fu7, Fu24 and Fu25 strains. In this study, FB increased the activities of CAT, POD and PAL (Fig. 4). The higher activities of CAT, POD and PAL may be favorable to reactive oxygen species elimilation, reducing the damage from pathogen cells, which may confirm the induction of systematic resistance in plants.

Plant cell membrane is an exchange barrier of materials between inside and outside of plant cell. When plant is damaged by adverse abiotic factors such as pathogens, drought and freezing, the permeability of cell membrane will be increased, but the selective permeability of cell membrane will be decreased, which resulting in leakage of the cytoplasm and the increasing of the conductivity of plant cell. The permeability of cell membrane was hence considered as a signal of plant resistances (Plazek and Zur, 2003). Malondialdehyde is the peroxide lipid in plant leaf membrane, and its content reflects the metabolism of oxygen and damage degree of cell membrane, which negatively correlates with the plant pathogenic damage (Akita and Cabuslay, 1990). Therefore, malondialdehyde can be used as one of the biochemical indicators for plant disease resistances. In this study, the application of FB significantly ($P < 0.05$) decreased cell membrane permeability and malondialdehyde content in wheat leaves (Fig. 5), indicated that FB could reduce the damage to wheat leaves by *F. graminearum*, effectively protect cell membrane and improve the disease resistance of wheat.

Studies showed that the fermentation broth of *Coniothyrium minitans* inhibited the growth of some ascomycetes, basidiomycetes, oomycetes and all four Gram-positive bacteria tested (Tomprefa et al. 2009), and the metabolites produced by *Trichoderma* species had strong inhibitory effects against *Ceratocystis paradoxa* (Eziashi et al. 2006). In our research, FB significantly ($P < 0.05$) inhibited the growth of *F. graminearum* (Fig. 1) and decreased the disease index of wheat scab, and the control effects on wheat scab were 93.27% of chemical fungicide carbendazim (Table 3). Taken together, the potential for using *B. adusta* M1 as a biocontrol agent against wheat scab was demonstrated along with the possible mechanisms involved in disease biocontrol.

Declarations

Author contributions Suping Li and Yong Li designed experiments; Suping Li, Xiao Feng and Jingjie Zhang carried out experiments; Suping Li analyzed experimental results; Suping Li, Yong Li and Xinhua He wrote and revised the manuscript.

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Data availability Data are available from the authors.

Ethics approval The current research does not involved human participants and/or animal models.

Competing interests The authors declare no competing interests.

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Figures

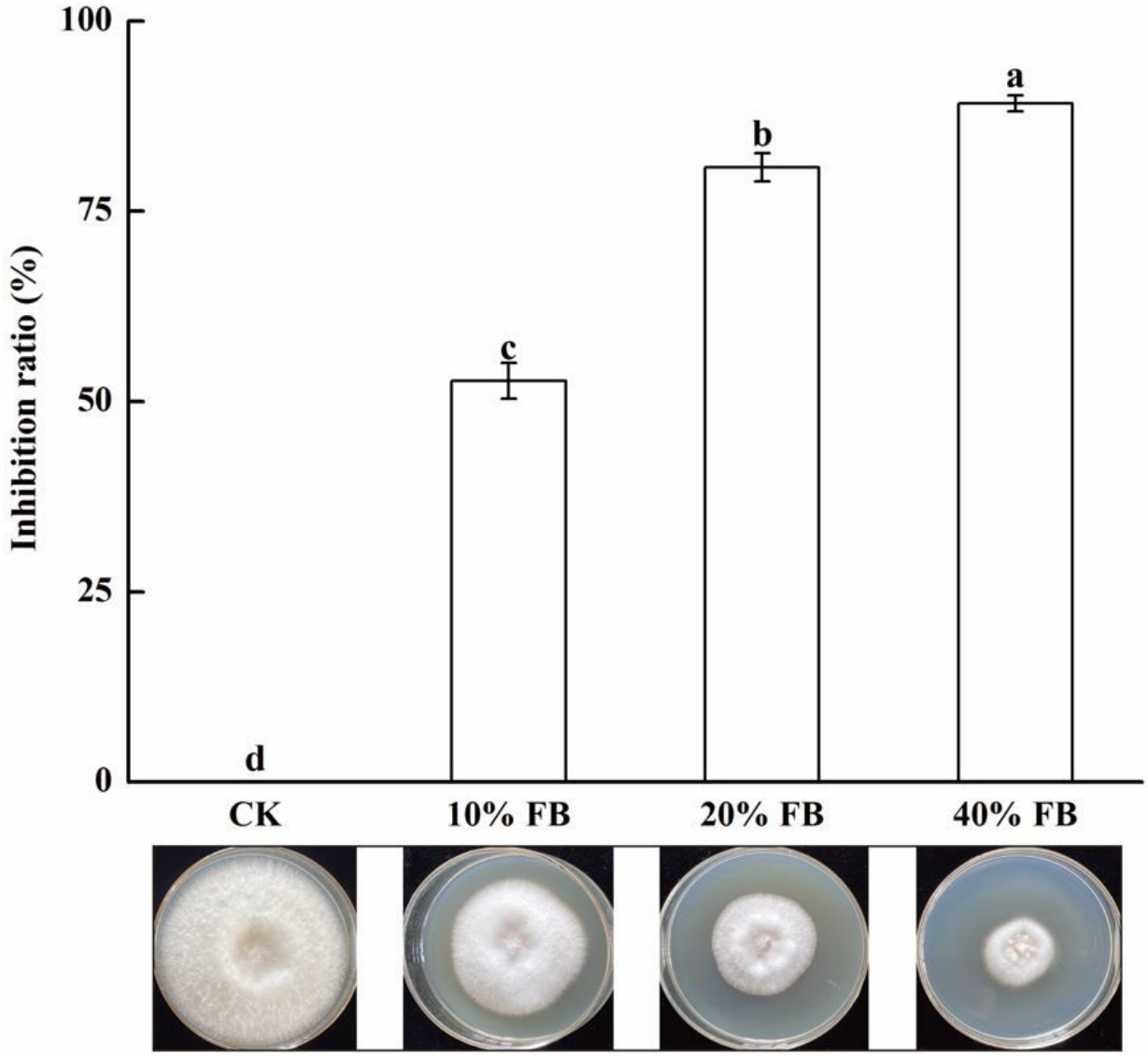

Figure 1

The inhibitory effects of *B. adusta* M1 fermentation broth (FB) on *F. graminearum.* CK, the control; FB, *B. adusta* M1 fermentation broth; CF, the chemical fungicide. Values are means ± SE. Different small letters indicate significant difference ($P < 0.05$).

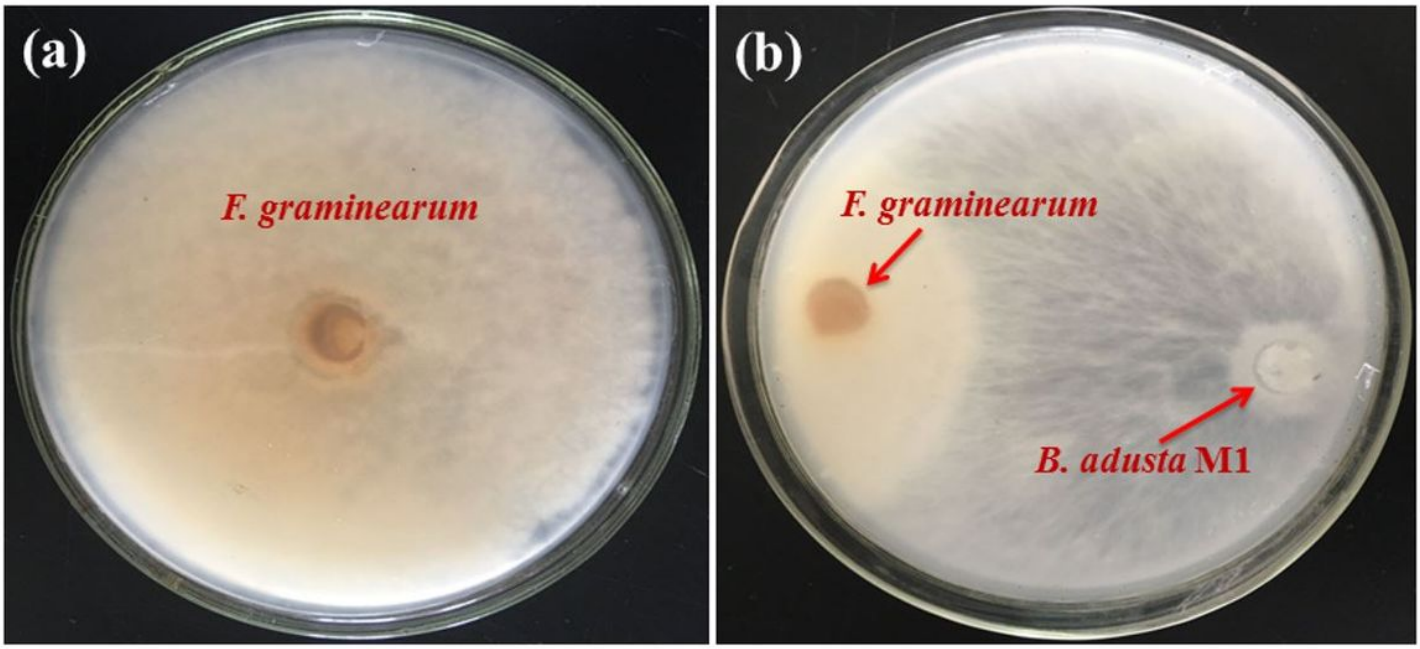

Figure 2

The competition effects of *B. adust* M1 on *F. graminearum*. (a) The plate inoculated only with *F. graminearum*; (b) The plate inoculated simultaneously with *B. adusta* M1 and *F. graminearum*.

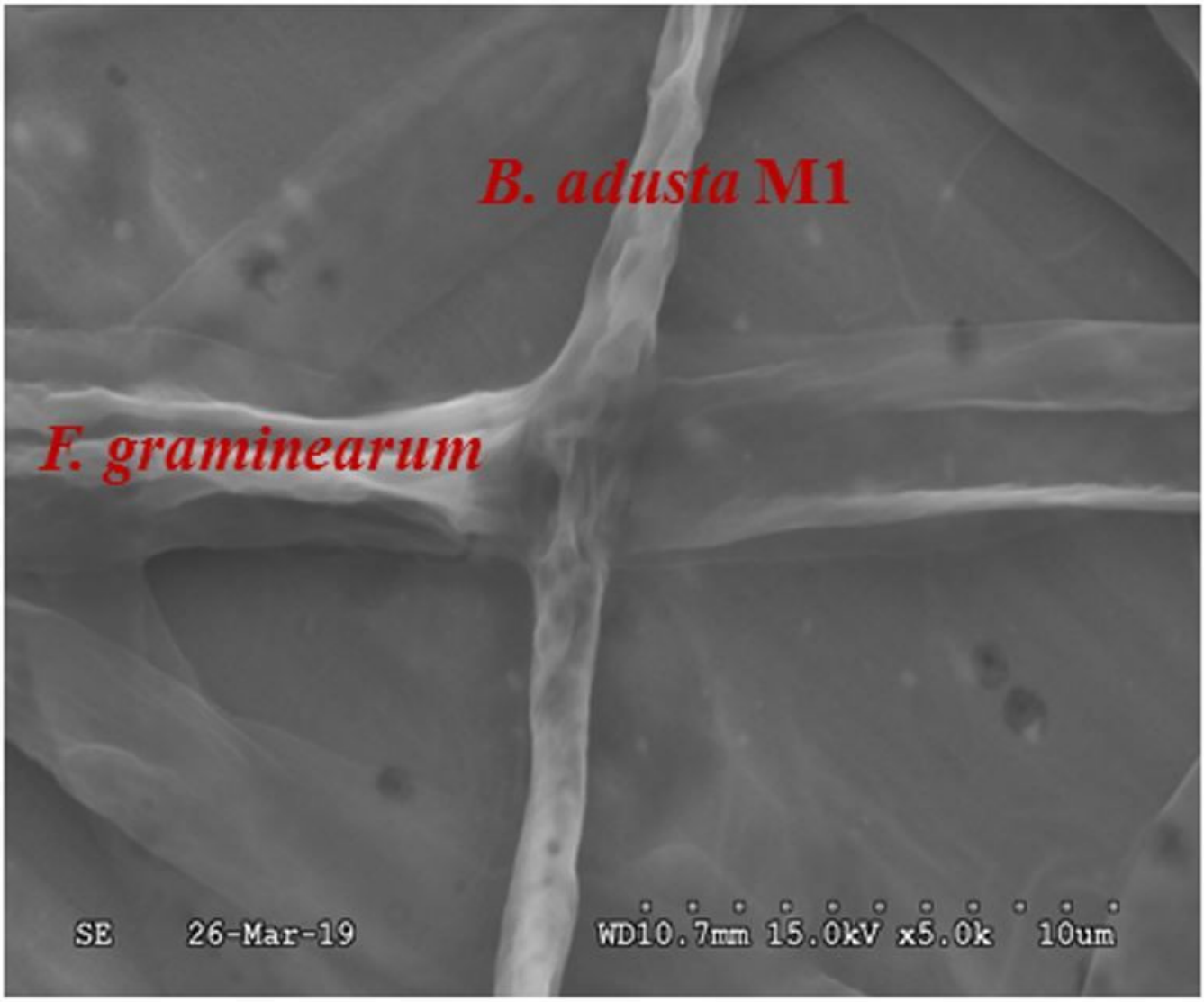

Figure 3

The mycoparasitism effects of *B. adust* M1 on *F. graminearum*.

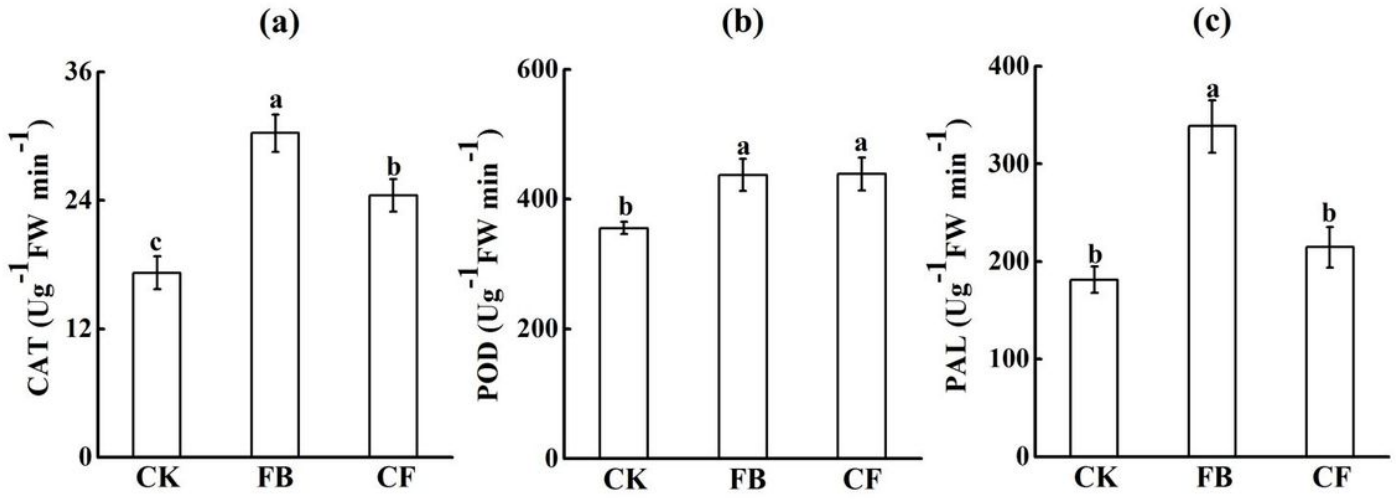

Figure 4

Effects of *B. adusta* M1 fermentation broth (FB) on activities of enzyme related to disease resistance in wheat leaves. CK, the control; FB, *B. adusta* M1 fermentation broth; CF, the chemical fungicide. Values are means ± SE. Different small letters indicate significant difference ($P < 0.05$).

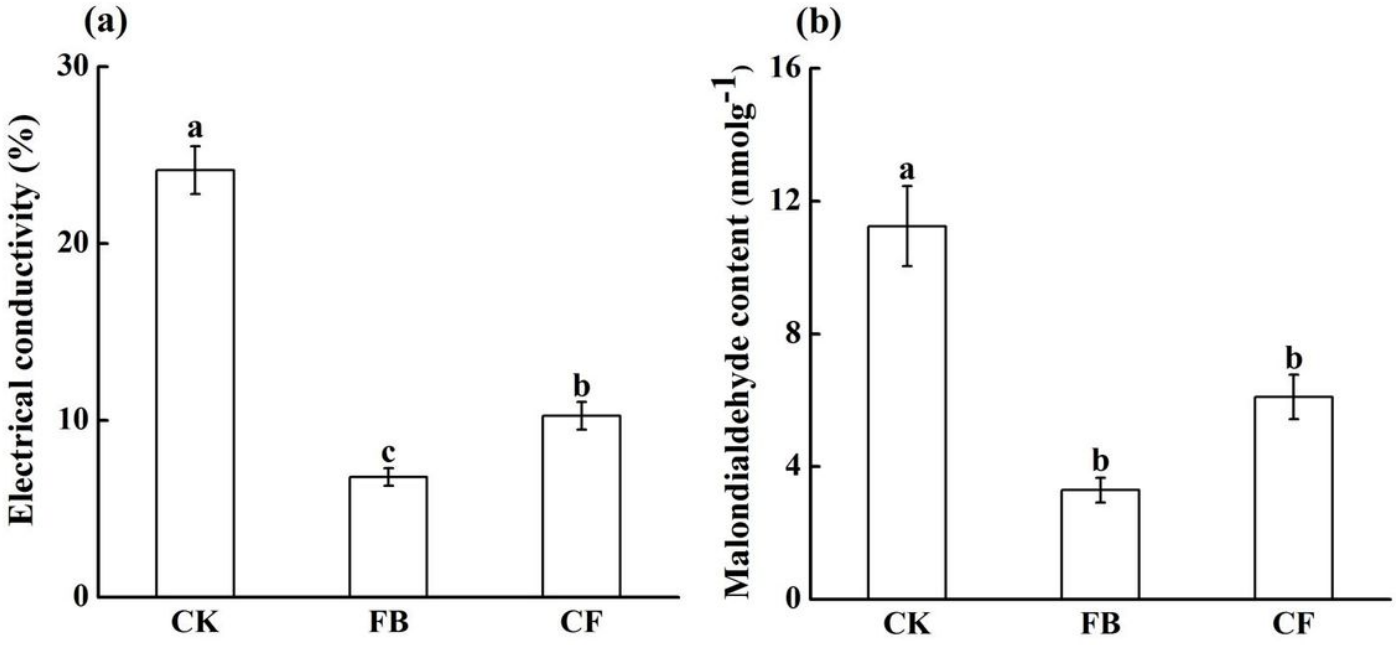

Figure 5

Effects of *B. adusta* M1 fermentation broth (FB) on membrane permeability (a) and malondialdehyde (b) in wheat leaves. CK, the control; FB, *B. adusta* M1 fermentation broth; CF, the chemical fungicide. Values are means ± SE. Different small letters indicate significant difference ($P < 0.05$).