Bjerkandera adusta M1 inhibits *Fusarium oxysporum* and prevents the wilt incidence in *Brassica napus*

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Highlights

- *B. adusta* M1 inhibits the growth of *Fusarium oxysporum* f. sp. *conglutinans*
- *B. adusta* M1 & its FB decreases the incidence and disease index of fusarium wilt
- Control effects on fusarium wilt are equal to or greater than fungicide carbendazim
- *B. adusta* M1 improves the activity of relevant leaf enzymes against fusarium wilt
- Mycoparasitism & possible antifungal compounds from *B. adusta* M1 prevent fusarium wilt

Abstract

Background: *Bjerkandera adusta* degrades polycyclic aromatic compounds, such as cellulose and lignin, with the production of laccase and peroxidase. However, its effect on plant disease is unknown.

Results: In this study, both the confrontation culture and greenhouse pot experiments were carried out to address the biocontrol mechanisms of *B. adusta* M1, which was isolated from a unique purple soil (Eutric Regosol), on the growth of pathogenic *Fusarium oxysporum* f. sp. *conglutinans* (FOC) and incidence of fusarium wilt in *Brassica napus*. Results showed that the hyphal growth rate of *B. adusta* M1 was significantly greater than that of FOC, indicating a strong competitiveness by *B. adusta* M1. In addition, the *B. adusta* M1 fermentation broth significantly inhibited the growth of FOC hyphae by 62.79±1.80%, which was greater than an inhibition rate of 40.63±1.68% by the chemical fungicide carbendazim. The image from a scanning electron microscope showed the hyphae of FOC directly was penetrated by the *B.* adusta M1 hyphae, indicating a strong mycoparasitism by *B. adusta* M1. Besides, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth reduced the incidence and disease index of the fusarium wilt in *Brassica napus* leaves, and the control effects of different treatments against fusarium wilt were 57.09% and 47.67%, respectively, which were comparable better to 46.11% of the chemical fungicide carbendazim. Furthermore, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth increased the activity of superoxide dismutase, catalase, peroxidase and phenylalanine ammonia-lyase, which related to an enhancement of disease resistance. Similarly, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth decreased the cell membrane permeability and malondialdehyde contents, thereby reducing the cell membrane damage from the pathogenic fungus.

Conclusion: In summary, results from this present study demonstrated that both the *Bjerkandera adusta* M1 and *B. adusta* M1 fermentation broth inhibited the growth of

FOC and decreased the incidence and disease index of the fusarium wilt disease in *Brassica napus*. Therefore, *B. adusta* M1 could be applied as a potential biocontrol fungus to against the fusarium wilt disease.

Keywords: Biocontrol; carbendazim; disease-resistant enzymes; fermentation broth; fungicide

1. Background

Fusarium oxysporum, a globally saprophytic fungus, can survive in soil for years or decades[1,2]. The fungus invades the host roots and colonizes in the xylem tissues to cause yellowing or wilting disease termed fusarium wilt[1].

Brassica napus is an important economic oil crops grown in China, Canada, Britain and Germany etc[3]. Brassica crops are severely infested by various fungal pathogens, particularly *Fusarium oxysporum* f. sp. *conglutinans* (FOC), which causes the fusarium wilt. That lead to serious loss of quality and quantity of the oilseed brassica plants[4,5]. The symptoms of the fusarium wilt disease include yellowing, wilting, stunting, and necrosis of leaf tissues and suppressed root development appeared in irregular-shaped patches, and finally led to the death of whole plant[2,6, 7]. Since fusarium wilt was observed in Beijing in 2001[8], the disease has presented increasingly severe damage to the production and quality of *Brassica napus* as well as economic losses in northern China[1].

At present, the control methods for fusarium wilt are mainly based on breeding disease-resistant varieties[7,9] and crop rotation[10], whereas soil fumigation with broad-spectrum biocides such as methyl bromide could pose great threat to the ecological environment[11]. In addition, the transgenic strains has showed enhanced resistances to *F. oxysporum* with delayed disease development by the Host-Delivered RNA interference (HD-RNAi) technology[9], but it is still not applied in the field.

The extensive use of chemical pesticides such as copper sulfate solution, carbendazim and thiophanate-methyl suspension to control the fusarium wilt has

posed threat to human health and environment, and resulted in pathogen resistance [12,13]. In this context, biological control, the potential of applying beneficial microorganisms to control the fusarium wilt could provide an alternative to the use of chemical pesticides[14,15]. However, limited biological control methods have been developed to prevent *Brassica napus* from fusarium wilt disease. Nevertheless, studies have shown that non-pathogenic *Fusarium oxysporum* inhibited spore germination and induced resistances by competing for nutrient and space, which might prevent the infection of FOC[3].

Bjerkandera adusta, a white rot fungus belonging to the family *Polyporaceae*, generally parasitizes on plant branch, fallen or dead trees[16,17]. At present, studies on *Bjerkandera adusta* primarily focus on the production of laccase, lignocellulose decomposition[18], and degradation of organic pollutants such as daunomycin and humic acids[19]. At present, limited information is available for the role of *B. adusta* in the biological control of plant diseases, particularly the fusarium wilt disease. The *B. adusta* M1 has been isolated from a purple soil (Eutric Regosol, FAO Soil Classification System) locating in the Southwest University campus in Chongqing, China, and the biocontrol effects of *B. adusta* M1 and *B. adusta* fermentation broth on Didymella bryoniae or watermelon vine blight was greater than those of carbendazim[20]. However, no studies have addressed the biocontrol effects of *Bjerkandera adusta* on the fusarium wilt disease, particularly for the vegetable oil crops including *Brassica napus*. The present study explored the effects and mechanisms of *B. adusta* M1 on controlling the fusarium wilt in the *Brassica napus* under both the laboratory culture and greenhouse pot experiments. The generated results would provide some theoretical and practical bases on the biocontrol of *B. adusta* M1 to the fusarium wilt disease in the field.

2. Results

2.1. Competition of *B. adusta* M1 on FOC

In the confrontation culture test, the growth rate of *B. adusta* M1 was greater than that of FOC. The hyphae of two fungi begin to contact each other after inoculation for 4 days, until 8th day the growth of FOC was obviously inhibited by *B. adusta* M1 (Fig. 1).

2.2. Inhibitory effects of the *B. adusta* M1 fermentation broth on FOC

Inoculation of *B. adusta* M1 fermentation broth or carbendazim significantly inhibited the growth on FOC, being indicated by a smaller colony radius on the plate (Table 1). Besides, the inhibitory effect on FOC was greater under the *B. adusta* M1 fermentation broth than under the chemical fungicide carbendazim.

2.3. Mycoparasitism effects of B. adusta M1 on FOC

The image from the scanning electron microscopy showed that the hyphae of *B. adusta* M1 were directly interspersed and pierced (Fig. 2) that of FOC, indicating that the latter might be expanded and even deformed.

2.4. Control effects of *B. adusta* M1 on FOC

The incidences of the fusarium wilt treated with the *B. adusta* M1 fermentation broth was significantly decreased (Table 2). Additionally, the disease index of the fusarium wilt were significantly decreased under the *B. adusta* M1, *B. adusta* M1 fermentation broth and carbendazim, though the *B. adusta* M1 showed a higher decrease effect than others treatments.

2.5. The activities of enzymes related to disease resistances

Compared to the basic broth media, the activity of SOD or CAT under the *B. adusta* M1 and *B. adusta* M1 fermentation broth was significantly increased by 25.92% and 22.38% or 78.80% and 82.28%. While the activity of POD or PAL was significantly increased by 46.64% or 44.23% under *B. adusta* M1 than under basic

broth media (Fig.3).

2.6. The cell membrane permeability and malondialdehyde contents

By contrast with the control, electrical conductivity was significantly decreased by 48.68% and 33.71% under the *B. adusta* M1 and the *B. adusta* M1 fermentation broth (Fig. 4a). Besides, a considerable decrease of 71.04% in the malondialdehyde contents was observed under the *B. adusta* M1 than under the control (Fig. 4b).

3. Discussion

Bjerkandera adusta, was a white rotting fungi growing on branch and fallen wood. Recent studies have focused on its enzymatic properties and application, such as laccase synthesis[21], lignocellulose decomposition[17], biodegradation of synthetic dyes[16,22] and the wastewater treatment[23]. In contrast, few reports have been done if *B. adusta* could control the fusarium wilt disease. The strain of *Bjerkandera adusta* M1 could effectively inhibit Didymella bryoniae[20]. Both the *B. adusta* M1 and *B. adusta* M1 fermentation broth showed a significant inhibition on the growth of FOC, and a better inhibition effect than the chemical fungicide, carbendazim (Fig. 1 vs Table 2). Besides, both the *B. adusta* M1 and *B. adusta* M1 fermentation broth decreased the incidence and disease index of fusarium wilt, and their control effects were superior or equivalent to that of carbendazim (Table 2). The above results showed that *B. adusta* M1 could be a biological fungus possessing the ability to control fusarium wilt disease, though this remains to be tested in field trials.

A biocontrol fungi could activate various mechanisms to cope with the threats from pathogens, including competition[24], secretion of antimicrobial substance [25], induced systemic resistance[26], and mycoparasitism[27].

In general, after the two fungi came into contacts, the biological *Trichoderma* spp. expressed inhibitory effect on the growth of the pathogenic fungus, *Serpula lacrymans* through space and nutrient competition[24,28]. In our results, the growth rate of *B. adusta* M1 was higher than that of FOC by competing for nutrient and space,

147 and the growth of FOC was obviously inhibited by *B. adusta* M1 (Fig. 1).

148 The mycoparasitism possesses an important role in the suppression of pathogenic
149 fungi by biocontrol fungi[27,29]. The mechanisms of mycoparasitism of *Trichoderma*
150 mainly refer to the process of contact, infection, recognition, entanglement,
151 penetration and degradation against plant pathogenic fungi[30]. *Trichoderma* would
152 establish a parasitic relationship with a pathogenic fungus after successful recognition,
153 and show substantial parasitic ability in the penetration of pathogenic fungal mycelia,
154 and the production of cell wall degrading enzymes including beta-1,6-glucanase and
155 protease, could finally lead to death of pathogenic fungus[31, 32]. In addition,
156 microbial antagonists, such as *Trichoderma harzianum* and *T. asperellum*, are widely
157 applied because of their ability to control plant fungal diseases that were induced by
158 *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*[14,29,33]. In the
159 present study, the *B. adusta* M1 hyphae had directly penetrated the FOC hyphae,
160 which might cause the latter to swell, deform and even rupture, showing a strong
161 mycoparasitism (Fig. 2).

162 The induced systemic resistance (ISR), typically results in an adapted and
163 efficient defense response to pathogens under both biotic and abiotic stress
164 [13,34]. A biocontrol fungus could induce plant response to pathogenic fungus by
165 secreting resistant secondary metabolites, which involves the activity of SOD,
166 CAT, PAL and POD enzymes, thus enhances plant disease resistance[13,35,36]. For
167 instance, after the inoculation with *T. asperelloides* T203, the defense system was
168 activated with the increase of PAL in cucumber[37]. The activity of SOD, POD, CAT
169 and PAL was significantly increased under 1.5 mL methyl jasmonate, and the disease
170 resistance was then improved[38]. In the present study, the *B. adusta* M1
171 significantly improved the activity of leaf SOD, CAT, POD and PAL, and the *B.*
172 *adusta* M1 fermentation broth significantly increased the activity of leaf SOD
173 and CAT (Fig. 3), indicating that both the *B. adusta* M1 and *B. adusta* M1
174 fermented broth could increase the plant's resistance on fusarium wilt with the

increasing activity of defense enzymes.

Plant cell membrane is regarded as an important channel for material exchange between cells and the external environment, and it exerts vital role during plant growth and development[39]. The electrolyte of plant cell is leaked under stress including low temperature and water deficit, leading to a decrease of osmotic potential but an increase of the conductivity of plant cell membrane[40,41]. Malondialdehyde (MDA), an excessive product of membrane lipid peroxidation on the plant cell, and its content is directly related to damage degree of plant cell membrane[39]. In general, the cell membrane permeability or malondialdehyde content was associated with plant resistances[42]. In the current experiment, a significant decrease of both the leaf malondialdehyde content and the conductivity was observed under both the *B. adusta* M1 and *B. adusta* M1 fermentation broth (Fig. 4). As a consequence, the decrease of structural damage of plant cell membrane was related to an increase of disease resistance by *Brassica napus*.

The extracellular proteins of 110 and 17 kDa produced by *Trichoderma* viride TvMN7 could inactivate RS toxin, a carbohydrate compound containing mainly alpha-glucose and mannose, which produced by the rice sheath blight pathogen, *Rhizoctonia solani*[43]. Observation from the confocal laser scanning microscope indicated that, the metabolite of *Pseudomonas chlororaphis* PCL1391, phenazine-1-carboxamide, inhibited the infection of *Fusarium oxysporumon* on tomato[44]. In the present study, the *B. adusta* M1 fermentation broth significantly inhibited the growth of FOC (Table 1), thus the brassica plants become more resistant on fusarium wilt infected by FOC (Table 2). As a result, the *B. adusta* M1 exerted great role in controlling the fusarium wilt disease.

4. Conclusion

Our results indicated that both the *B. adusta* M1 and *B. adusta* M1 fermentation broth inhibited the growth of FOC, and decreased the incidence and disease index of

the fusarium wilt disease, indicating that *B. adusta* M1 might be a potential biocontrol fungus against the fusarium wilt disease.

5. Materials and Methods

5.1. Materials.

Brassica napus was (*Brassica napus* L. cv. Xinde Miscellaneous Oil No. 9) purchased from the Mianyang Special Research Seed Industry Co., Ltd, Sichuan, China. The growth media is a purple soil (Eutric Regosol, FAO Soil Classification System) from the National Key Field Station for Purple Soil Fertility and Fertilizer Monitoring Base, locating in the Southwest University campus (E 106°24′37″; N 29°48′32″) Beibei, Chongqing, China. The collected purple soil at 5-10 cm depth were thoroughly mixed after the removal of debris, dried at 30°C and sieved over 2mm, and then autoclaved for 2 h. The basic soil chemical properties were shown in Table 3.

Two fungus strains: *Bjerkandera adust* M1 was isolated from the above-mentioned rhizosphere soil (5-10 cm depth) in April 2016. *Fusarium oxysporum* f. sp. *conglutinans* (FOC) was donated by the Institute of Plant Ecology and Pathology of Southwest University.

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

The potato dextrose agar (PDA) medium was used for cultivating both the *B. adusta* M1 and FOC[45], and it contained 200 g shelled potato, 20 g dextrose, 15~20g agar, 1000 mL distilled water. The basics broth medium (20 g dextrose, 10 g NH_4Cl,1 g $MgSO_4$,1 g $CaCl_2$,2 g KH_2PO_4,1000 mL distilled water) was used for preparing the fermentation broth of *B. adusta* M1 and FOC.

5.2. Methods

5.2.1. Preparation the spore suspension of *B. adusta* M1 and FOC

B. adusta M1 and FOC were respectively inoculated on the PDA plate, cultured at 28 ℃ for 5 d, subsequently added 5 mL sterilized water and then gently scraped off the conidia with an inoculating spear. The spore suspension was transferred to a sterile 50 mL triangle bottle and thoroughly shaken, then filtered twice through eight layers of gauze. The concentration of filtrate was immediately determined by a Globulimeter (The Qiujing Biochemical Reagent Instrument Co., Ltd, Shanghai, China) under a SMART Biological Microscope (Auto Optical Instrument Co., Ltd, Chongqing, China), and then diluted to 4.5×10^5 pcs/mL water.

5.2.2. Preparation of fermentation broth of *B. adust* M1

Five mycelial discs (5mm diameter) of *B. adust* M1 inoculated on 100 mL basic broth medium inside a 250 mL triangle bottle were shaken for 5 days (150r/min and 28 ℃) and centrifuged (3,000 r/min for 1 min). The filtrate from the supernatant (0.22 mm sterile filter) was then used as the fermentation broth of *B. adusta* M1.

5.2.3. Determination of inhibitory effect of *B. adusta* M1 fermentation broth on FOC

The antifungal test was applied to determine the fungistatic effect of *B. adusta* M1 fermentation broth on the growth of FOC, and the following formula 1 was used to calculate the related inhibition rate[46]. The process was as follows: inoculated a mycelial disc of FOC on a PDA plate containing 3 mL *B. adusta* M1 fermentation broth, and 3 mL 0.4 mg/mL carbendazim as chemical fungicide treatment. After 5 days of culture at 32 ° C, the radius of the FOC colony was determined.

The inhibition rate (%) = (colony radius of control group - colony radius of treatment group) / colony radius of control group × 100% (1)

5.2.4. Competition and mycoparasitism of *B. adusta* M1 against FOC

Two experiments were carried out to address competition and mycoparasitism of *B. adusta* M1 against FOC. Both *B. adusta* M1 and FOC mycelial discs with a diameter of 5 mm were inoculated on the same PDA plate, but separated by 3 cm each

other to form a two-point confrontation. One group of these PDA plates was applied to observe the competitive effect of *B. adusta* M1 on FOC, cultivated for 8 days in the dark at 32 °C, then observed and taken pictures. In another group of these PDA plates, a 2 cm × 2 cm aluminum foil was placed between the two strains on the surface of each PDA plate, incubated at 32℃ for about 48 h until the two strains were in contact with each other on the aluminum foil. The mycelial sample from the interactive regions was prepared as a microscope slide[47] then the mycoparasitism effect of *B. adusta* M1 on FOC was observed under a scanning electron microscope (Nikon Co., Ltd, Shanghai, China).

5.2.5. Effects of *B. adusta* M1 on fusarium wilt

After *Brassica napus* seeds were soaked in 75% ethyl alcohol for 5min, in 20% sodium hypochlorite for 3 min, rinsed with the sterile water several times, and then cultivated at 28 °C for 3 d in an incubator. The germinated seeds were sowed in a seedling tray (18 cm × 24 cm) containing sterilized soil media. Three seedlings with two cotyledons were transplanted to a flowerpot (18 cm × 24 cm) containing 2kg sterilized soil media. The *Brassica napus* seedlings were irrigated with 10 mL FOC spore suspension after 20 d slow growth without nutrient supplements.

After *Brassica napus* seedlings had been infected by *B. adusta* M1 (after inoculation for five days), to test the control effects of *B. adusta* M1 on fusarium wilt under four treatments (3 replicates each) : (1) 10 mL basic broth media (Control); (2) 10 mL *B. adust* M1 spore suspension; (3) 10 mL *B. adusta* M1 fermentation broth; (4) 10 mL chemical fungicide carbendazim (0.08 mg/mL). After 24 h treatment, the incidence and disease index of *Brassica napus* and the control effects of *B. adusta* M1 on fusarium wilt were investigated every five days according to the following respective formulas[48].

Incidence (%) = the number of plants infected / total number of plants × 100% (2);

Disease index = ∑ (the number of leaves at all levels × relative series) / (total number of leaves × maximum series) × 100 (3);

Control effects = (disease index of control group - disease index of treatment group) / disease index of control group × 100% (4).

5.2.6. Effects of *B. adusta* M1 on disease resistance of *Brassica napus*

Brassica napus seedlings were sowed and managed as described as 5.2.5. After growth for 20 days, three treatments (3 replicates each) were respectively done: (1) 10 mL basic broth media (Control); (2) 10 mL *B. adust* M1 spore suspension; (3) 10 mL *B. adust* M1 fermentation broth. After 24h treatments, the above-mentioned *Brassica napus* seedlings were irrigated with 10 mL of FOC spore suspension. After 22 days treatment, the activity of leaf superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and phenylalanine ammonia lyase (PAL) in *Brassica napus* seedlings was respectively measured by the nitroblue tetrazole method and guaiacol method, using a spectrophotometry or UV spectrophotometer[49]. In addition, the cell membrane permeability and malondialdehyd content in *Brassica napus* leaves were detected by an electrolyte infiltration[50] and a thiobarbital (TBA) colorimetry[51], respectively.

5.2.7. Statistical analysis

Data (means ± LSD, $n = 3$) were subjected to one-way analysis of variance (ANOVA)and significant differences between treatments were compared by the least significant differences at $P < 0.05$. All significance tests and correlation analyses were done using the SPSS 10.00 software, and the mean performed using a SPSS 11.0 software (SPSS Inc., Chicago, IL, USA).

Abbreviations

B. adusta M1: Bjerkandera adusta M; FB: B. adusta M1 fermentation broth; Fusarium oxysporum f. sp. *conglutinans*: FOC

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Authors ' contributions

Xiao Feng designed the study and wrote the manuscript. Xiao Feng, Suping Li, Yi-Fan Lu performed the experiments. Xin-Hua He and Yong Li modified this manuscript. All authors reviewed and approved the manuscript.

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Ethics approval and consent to participate

Ethical approval is not applicable in the case of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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