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ORGINAL PAPER

Antagonistic Effects of *Bacillus cereus* Strain B-02 on Morphology, Ultrastructure and Cytophysiology of *Botrytis cinerea*

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Abstract

The study on antagonistic mechanism of biocontrol strains gives the premise and basis for efficient and stable biological control. This study aimes to overcome of biocontrol agent in aspects of complicated and diversified mode of action, short-lasting and unstable efficacy in the production processes. This study elucidated the antagonistic mechanism of *Bacillus cereus* strain B-02 on *Botrytis cinerea* by detecting changes in morphology, ultrastructure and physiology in affected hyphae of *Botrytis cinerea*. Which provided certain theoretical and practical significance for biological control of gray mould caused by *B. cinerea*. *B. cereus* strain B-02 isolated from tomato rhizosphere mightily suppressed gray mold in tomato caused by *B. cinerea*. Spore germination and hyphal growth of *B. cinerea* were inhibited by *B. cereus* strain B-02. Changes of cell morphology such as distortion, shrinking and swelling were observed by SEM. TEM observation further indicated the ultrastructural alterations of hyphae, including mitochondrion reduction, un-membranous inclusion in cytoplasm, considerable thickening of cell walls, and electronic density enhancement. LSCM observation revealed the fluorescence intensity of nucleus DNA, mitochondrion DNA and reactive oxygen radical in treated hyphae were all stronger than control and the difference was significant ($P < 0.01$). These results indicated that the antagonistic effects of *B. cereus* strain B-02 on *B. cinerea* were likely due to a combination of abnormal synthesis of nucleus DNA and mitochondrion DNA and multifarious ultrastructural alterations in hyphal cell.

Key words: *B. cereus*, *B. cinerea*, affected hyphae, antagonistic effect

Introduction

Gray mould, caused by *B. cinerea* Pers. Ex. Fr., is a severe and constant threat to field and greenhouses-grown tomatoes in many countries worldwide (Emine *et al*., 2010). Chemical control has become increasingly difficult due to the development of resistant *B. cinerea* strains as well as the increasing worldwide concern about pesticide use due to environmental problems (Saligkarias *et al*., 2002; Trotel-Aziz *et al*., 2008). Biological control using natural antagonistic microorganisms has been extensively studied, and some fungi, and bacteria have been demonstrated to be effective against gray mold (Jae *et al*., 2006). A great number of reports indicate that certain bacterial strains are beneficial for the growth of plants, these are called plant growth-promoting rhizobacteria (PGPR). An important trait of these bacteria is their ability to maintain a stable relationship with the associated plant species (Smith and Goodman, 1999; Miethling *et al*., 2000). Microorganisms isolated from the rhizosphere are not only non-exotic, thereby presenting no risk of proliferation of a new microorganism in the environment but also may be better adapted to that plant and therefore provide better control of diseases than organisms originally isolated from other plant species (Trotel-Aziz *et al*., 2008). Biocontrol bacteria may protect plants against pathogens by bacteriostatic mode and bactericidal mode.

In tomato, much of research reported on the use of the genus *Bacillus* to control gray mold, mainly including *Bacillus subtilis*, *Bacillus polymyxa*, *B. cereus*, *Bacillus megaterium* and *Bacillus pumilis* (Asaka *et al*., 1996; Kavitha *et al*., 2005). Some strains of the genus *Bacillus* have been reported as potential candidates for biological control of fungal pathogens (Dik *et al*., 1999; Du *et al*., 2004; Chen *et al*., 2004; Wang and Yang, 2004; Touré *et al*.,2004; Qi *et al*., 2005; Li *et al*., 2005; Tang *et al*., 2005; He *et al*., 2006; Li and Jiang, 2006; Yin *et al*., 2007). The genus *Bacillus* depends on a wide variety of traits, such as the production by the strain of various antibiotics and cell wall degrading enzymes (protease, lipase, chitinase, and glucanase) to control pathogen (Tang *et al*., 2005; Li and Jiang, 2006). Recently, a commercial biofungicide Serenade, which contains

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a *Bacillus subtilis* strain (QST 713), was reported to be effective against various pathogenic fungi (Trotel-Aziz *et al*., 2008). Previous studies reported that the biocontrol bacterium, *B. cereus* or its metabolite, can inhibit hyphal growth of *B. cinerea* by alterations in the hyphal morphology, and the formation and germination of *B. cinerea* spore. Recent studies have shown that fermentation broth treatment of biocontrol bacteria not only is effective in halting pathogen growth (Pusey and Wilson, 1984; Janisiewicz, 1987; El-Ghaouth *et al*., 1998; Jijakli and Lepoivre, 1998; Ippolito *et al.,* 2000), but also results in marked morphological changes, structural alterations, and molecular disorganisation of the fungal cells (Roze and Line, 1998; Kamisaka *et al*., 1999; Wang *et al*., 2000; Yazgana *et al*., 2001; Lin and Li, 2003; Hagelina *et al*., 2004; Mendo *et al*., 2004; Sun *et al*., 2005; Xie *et al*., 2005; Manteca *et al*., 2005; Leiter *et al*., 2005). The objectives of this study were to (i) evaluate inhibitory effects of *B. cereus* strain B-02 on spore germination and hyphal growth of *B. cinerea*; (ii) illustrate ultrastructural alterations occurring in hyphal cells of *B. cinerea* during interaction with *B. cereus* strain B-02 by scanning electron microscope (SEM) and transmission electron microscope (TEM) to elucidate the biocontrol mechanism; and (iii) visualize the molecular standard of nucleus DNA, mitochondrion DNA and reactive oxygen radical in the affected hyphae of *B. cinerea* by laser scanning confocal microscope (LSCM) to predict the possible target of *B. cereus* strain B-02 on *Botrytis cinerea*.

Experimental

Materials and Methods

Microorganisms. The pathogen *B. cinerea* used as a cell material for ultrastructural and cytophysiological study was isolated from infected tomato fruit in Zibo, China. The bacterium, *B. cereus* strain B-02, was originally isolated from the tomato rhizosphere and had very strong inhibitory activity against *B. cinerea*.

Identification of *B. cereus* strain B-02. *B. cereus* strain B-02 was identified through the analysis of morphological and biochemical characteristics and 16S rDNA sequence in our previous experiment (Li *et al*., 2007). Morphological characteristics of *B. cereus* strain B-02 were represented after gram staining, spore staining and imaged by Leica TCS-SP2 LSCM. Characteristics of *Bacillus* from "Bergey's Manual of Determinative Bacteriology" was used to identify the biochemical characteristics of *B. cereus* strain B-02. Genome DNA of *B. cereus* strain B-02 was extracted according to CTAB method and amplified by primers 63F and 1494R. *B. cereus* strain B-02 homology was analyzed in NCBI by 16S rDNA sequence. We chose a strain with the strongest inhibitory activity in our isolation experiment by inhibition zone method for the subsequent experiment.

Pretreatment of *B. cereus* strain B-02 fermentation broth. *B. cereus* strain B-02 was inoculated into 50 mL LB in a conical flask at 30°C, 140 r/min for 36 h. The supernatant was filtered by microporous membrane (0.22 μm, Φ25 mm) after centrifuging of *B. cereus* strain B-02 fermentation broth at 4°C, 8000 r/min for 10 min. According to cylinder plate method *B. cereus* strain B-02 filtrate was used to process *B. cinerea* hyphae in the subsequent experiment. *B. cinerea* grown on PDA supplemented with the same proportion of sterile distilled water was used as a control. Each plate was replicated 3 times.

Preparation of *B. cinerea* hyphal cell and spore suspension. Interactions between *B. cinerea* and *B. cereus* strain B-02 were studied by well test. Plates supplemented with *B. cereus* strain B-02 fermentation broth at 1%, 5%, 10% and in the center of the plates inoculated *B. cinerea* agar disks (Φ6 mm) were allowed to grow at 28°C in the dark for 3~5 d, after which time *B. cinerea* hyphae grown on PDA were harvested for SEM, TEM and LSCM. For comparisons, controls supplemented with the same proportion of sterile distilled water were harvested from PDA plates with only *B. cinerea* for SEM, TEM and LSCM visualization, respectively. Each treatment was replicated 3 times and the experiment was repeated twice. *B. cinerea* spores were removed from 2-week-old PDA culture, and suspended in 5 mL of sterile distilled water containing 0.05% (v/v) Tween 80. The spore suspension was filtered through 4 layers of sterile cheese cloth to remove adhering hyphae and the concentration was adjusted using a hemocytometer prior to use (Li *et al*., 2009).

Observation of *B. cinerea* hyphal growth and spore germination. To assess the effects of *B. cereus* strain B-02 fermentation broth on hyphal growth of *B. cinerea*, *B. cinerea* agar disks (Φ6 mm) was placed in the center of PDA plates containing *B. cereus* strain B-02 fermentation broth at 1%, 5%, 10% at 28°C in the dark. Hyphal growth was determined by measuring the colony diameter after 3~5 d of inoculation. Controls were supplemented with the same proportion of sterile distilled water. Each treatment was replicated 3 times and the experiment was repeated twice. The effects of *B. cereus* strain B-02 fermentation broth on spore germination of *B. cinerea* were assessed according to Zhao (2003). Mixture containing 400 μL LB fluid medium and 200 μL *B. cinerea* spore suspension was added 200 μL *B. cereus* strain B-02 fermentation broth at original concentration and cultivated at 28°C in the dark (Figure 1). The experiment was replicated 3 times.

SEM specimen preparation and observation. Sample preparation and SEM observation were done as described previously by Chen *et al.* (2007). Briefly, hyphal samples excised from the 5-day-old culture of *B. cinerea* treated with sterile distilled water and 10% *B. cereus* strain B-02 fermentation broth were fixed in 2.5% glutaraldehyde at 4°C for 2 h, washed in phosphate buffered saline (PBS) for 4 times (15 min each), soaked in 1% osmium tetroxide at 4°C for 2.5 h, dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, and 100%) (10 min each), metathesized in isoamyl acetate for 25 min, hexanenitrile-dried and sputter-coated with gold in vacuum. Micrographs were taken by a Hitachi H-800 SEM. This experiment was repeated 3 times and each treatment was also replicated at least 3 times.

TEM specimen preparation and observation. High-resolution images of hyphal cellular changes in 10% *B. cereus* strain B-02 fermentation broth treated samples were obtained by a Hitachi H-800 TEM. Sample preparation was according to Chen *et al.* (2004). Hyphal samples excised from the 5-day-old culture of *B. cinerea* were fixed in 3% glutaraldehyde at 4°C for 2 h. The samples were then thoroughly rinsed with 0.1 mol PBS (pH7.2) for 3 times (10 min each), post-fixed with osmium tetroxide at 4°C for 1 h and rinsed with PBS again. Samples were then dehydrated in a graded ethanol series (50%, 70%, and 90%) and 100% acetone for 3 times (10 min each), embedded with Epon 812 and polymerized at 60°C for 24 h. Ultra-thin sections (80 nm thickness) were cut with a diamond knife (LKB-V, LKB Company, Sweden) and stained with 2% uranylacetate for 30 min. Then samples were washed with PBS and sections were stained with lead citrate for 30 min. The control group was treated with the same procedure. This experiment was repeated 3 times and each treatment was also replicated at least 3 times.

LSCM specimen preparation and observation. Hyphal samples excised from the 5-day-old culture of *B. cinerea* treated with *B. cereus* strain B-02 fermentation broth at 1%, 5% and 10% were successively stained with 0.1% AO (Sigma) for 30 min, 10 μg/mL Rhodamine 123 for 40 min, 5 μg/mL DCFH-DA for 1 h and rinsed with PBS (pH 7.4) (Kamisaka *et al.*, 1999; Manteca *et al.*, 2005; Leiter *et al.*, 2005). Samples of DNA and reactive oxygen signed were excited by the 488 nm excitation wavelength with Ar ion laser, the excitation wavelength of samples mitochondrion signed was 514 nm. Hyphae were dealt from the control group by the same method. This experiment was repeated 3 times and each treatment was also replicated at least 3 times. Representative images taken by Leica TCS-SP2 LSCM were presented here. The means and standard deviations of hyphal cellular fluorescence intensity were calculated. T-test was used to analysis the difference between experimental group and control group.

Results

Identification of *B. cereus* strain B-02. In this study we isolated five strains in total having inhibitory activity against *B. cinerea*. The inhibitory activity of *B. cereus* strain B-02 was the strongest (Table I). Therefore *B. cereus* strain B-02 was used in the following experiment. Values in Table I like ± 1.5 mean standard deviation (SD). So are values in Table III. Observation of morphological characteristics revealed that *B. cereus* strain B-02 was rod shape, had flagella, could move, had elliptical heatproof spora, were Gram-positive under the light microscope and LSCM (Fig. 1). The biochemical characteristics of *B. cereus* strain B-02 were described in the Table II. *B. cereus* strain B-02 was

Table I
Inhibition of the five *Bacillus* strains on *Botrytis cinerea*

Strain	Inhibition zone (mm)	Inhibitory rate (%)
B-02	17.0 ± 1.5	81.6a
B-04	14.0 ± 2.0	60.9b
B-01	11.0 ± 1.5	55.2b
B-07	9.0 ± 2.0	44.1c
B-03	6.0 ± 1.0	43.5c

Note: χ^2 test, 5% significance of difference

Table II
Partial biochemical characteristics of the five *Bacillus* strains

Strain	B-01	B-02	B-03	B-04	B-07
Shape	rod	rod	rod	rod	rod
Spore	+	+	+	+	+
Gram staining	+	+	+	+	+
Moveability	+	+	+	+	+
15°C cultivation	+	+	+	+	+
50°C cultivation	+	+	+	+	+
pH5.7 cultivation	+	+	+	+	+
Anaerobic cultivation	+	+	+	+	–
Catalase	+	+	+	+	+
Glucose	++	++	++	++	+
Sucrose	++	++	++	++	+
Lactose	–	–	–	–	–
Fructose	+	+	+	+	+
Xylose	–	–	–	–	+
Arabinose	–	–	–	–	+
V.P experiment	–	–	–	–	+
M.R experiment	+	+	+	+	+
Indole experiment	–	–	–	–	–
H_2S experiment	+	+	+	+	++
Gelatin liquefaction	+	+	+	+	–
Amylolysis	+	+	+	+	–

Note: +, positive; –, negative

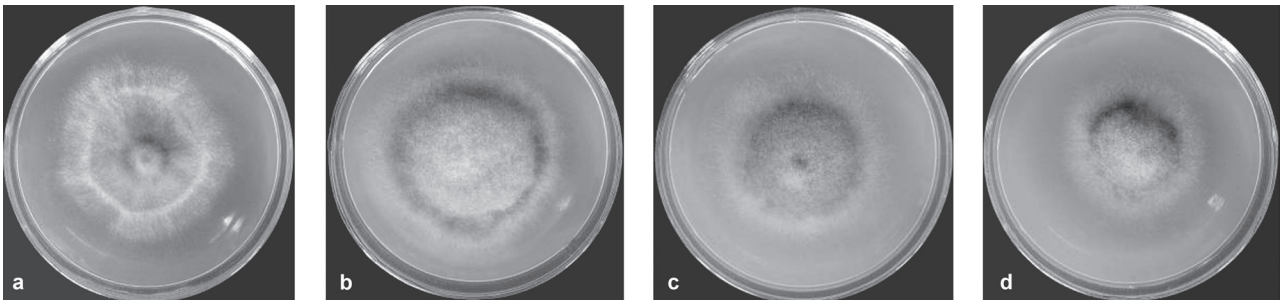

Fig. 1. The comparison of antagonistic activity of *Bacillus cereus* strain B-02 fermentation broth (0.22 μm) in different dilute multiple (a) control, (b) strain B-02 fermentation broth in dilute multiple of 1:100, (c) strain B-02 fermentation broth in dilute multiple of 1:20, (d) strain B-02 fermentation broth in dilute multiple of 1:10

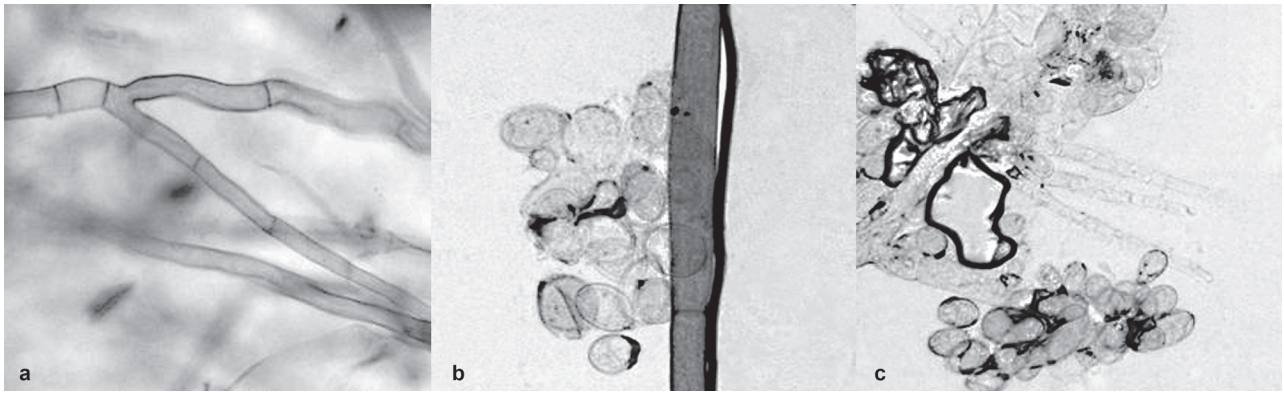

Fig. 2. Effect of *Bacillus cereus* strain B-02 fermentation broth on the germination of *Botrytis cinerea* spores

99% homologous to *B. cereus* by 16S rDNA sequence analysis. In the evolutionary, the phylogenetic tree of the five strains also indicated that *B. cereus* strain B-02 closed to *B. cereus* (Fig. 2). All the evidence, including analysis of morphological characteristics, biochemical characteristics and 16S rDNA sequence, showed that *B. cereus* strain B-02 belonged to *B. cereus*.

Effects of *B. cereus* strain B-02 fermentation broth on hyphal growth and spore germination of *B. cinerea*. Table III showed that *B. cereus* strain B-02 fermentation broth at different concentrations inhibited markedly hyphal growth of *B. cinerea*. The inhibitory rate of *B. cinerea* treated with 10% *B. cereus* strain B-02 fermentation broth was up to 33.7%. When *B. cereus* strain B-02 fermentation broth was diluted to 100 times, its inhibitory action almost disappeared. Fig. 3 showed that the control group, normal hyphal morphology, the rapid speed of hyphal growth, flat and off-white colony, compared with the experimental group. The experimental group was just the reverse. The higher the concentration of *B. cereus* strain B-02 fermentation broth was, the heavier the hyphal colour was.

B. cinerea spores untreated with *B. cereus* strain B-02 fermentation broth germinated normally and *B. cinerea* hyphae were slim and uniform after 12 h by the light microscope (Fig. 4a). Even after 48 h the germination of *B. cinerea* spores was still normal. *B. cinerea* spores treated with *B. cereus* strain B-02 fermentation broth germinated partially and the germinal germ tube appeared distorted after 12 h (Fig. 4b and 4c).

Table III
Inhibition of *Bacillus cereus* strain B-02 fermentation broth on *Botrytis cinerea*

Dilute multiple	2d		3d		4d		7d	
	MD	IRM	MD	IRM	MD	IRM	MD	IRM
10	21.0 ± 2.2	33.7a	33.6 ± 1.4	28.5a	42.6 ± 0.9	25.3a	50.8 ± 2.7	24.8a
20	23.8 ± 2.6	21.0a	37.0 ± 3.9	19.7a	43.4 ± 0.8	24.2a	53.8 ± 2.3	19.8a
100	28.1 ± 1.6	2.2b	44.4 ± 1.8	0.7b	54.6 ± 1.1	0.8b	65.3 ± 1.5	0.42b
CK	28.6 ± 1.4		44.6 ± 1.2		55.0 ± 1.2		65.5 ± 0.8	

MD (mm): Colony diameter; IRM (%): Inhibitory rate
Note: χ^2 test, 5% significance of difference

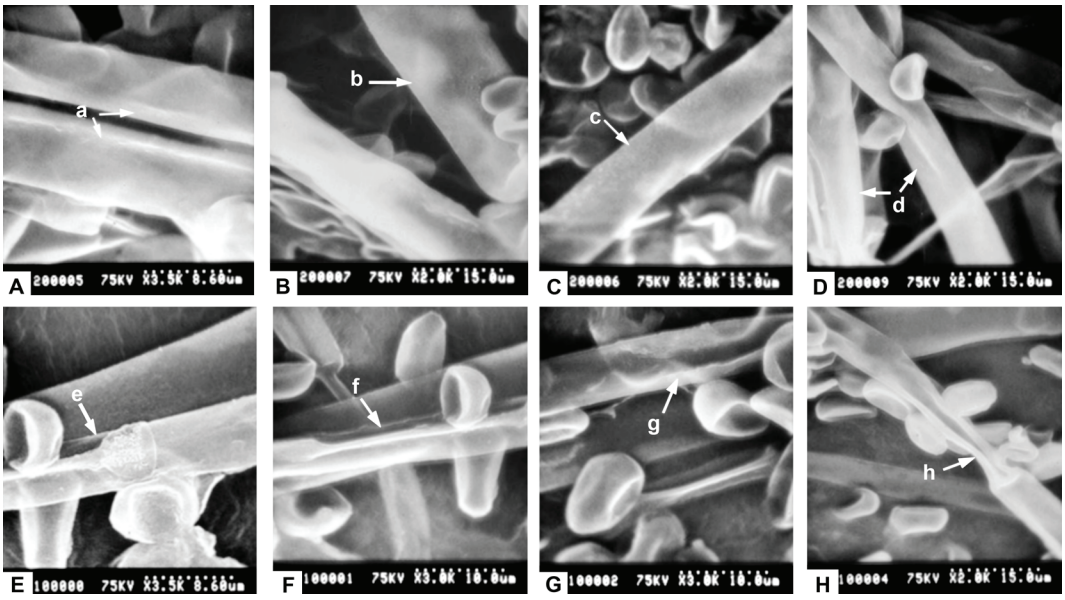

Fig. 3. Scanning electron micrographs of *Botrytis cinerea* hypha treated and untreated with *Bacillus cereus* strain B-02 fermentation broth (×5000) a, b, c, d: untreated normal hypha (arrows)
e, f, g: strongly destroyed hypha (arrows); h: distorted and collapsed hypha (arrow)

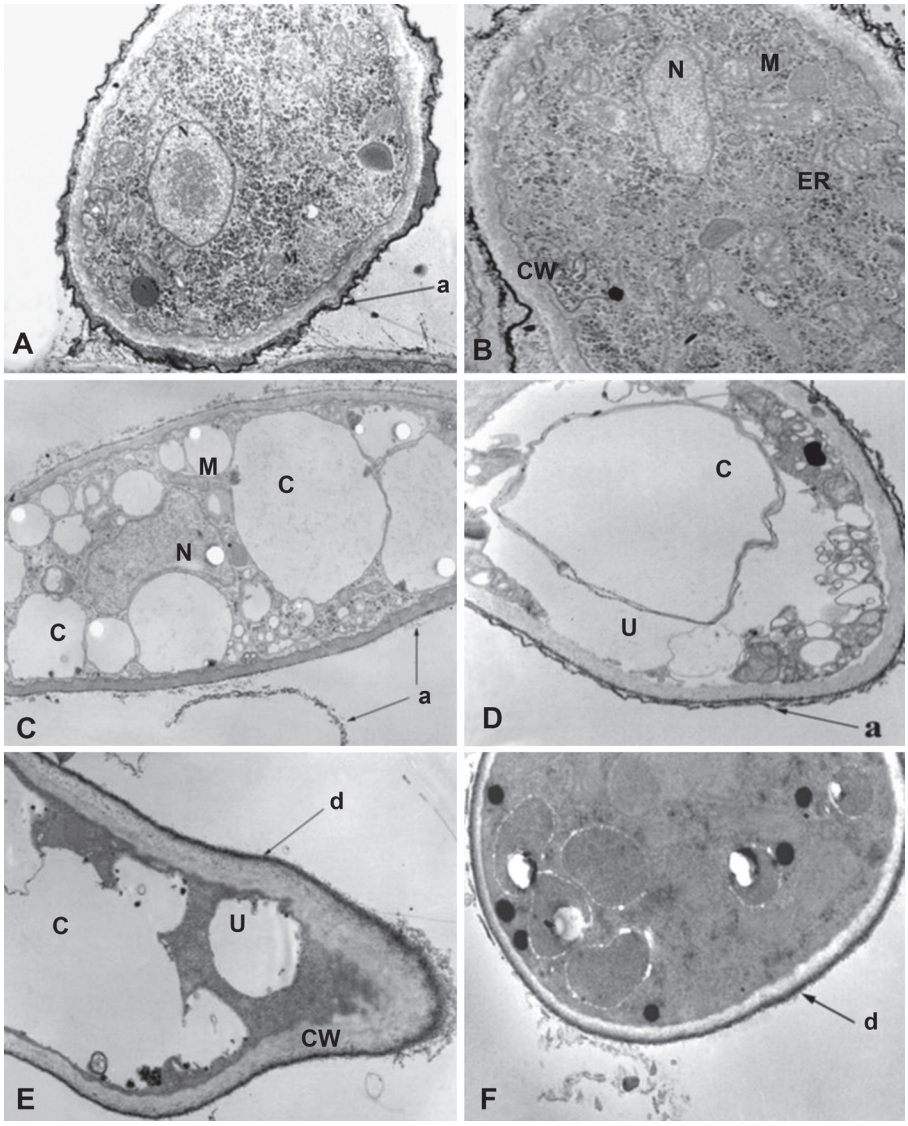

Fig. 4. Untreated hyphal cell configuration of *Botrytis cinerea* under transmission electron micrographs (×5000) A, B: normal cell section
M: mitochondrion; N: nucleus; ER: endoplasmic reticulum; CW: cell wall; a: continuous outer surface layer around the hyphal cell wall (CW). Treated hyphal cell configuration of *Botrytis cinerea* under transmission electron micrographs (×5000) C, D, E, F: The treated hyphal cell configuration was destroyed and there was a lot of un-membrane material in cell. M: mitochondrion; N: nucleus; C: vacuole; U: un-membrane material; a: continuous outer surface layer around the hyphal cell wall (CW); d: heavily stained material

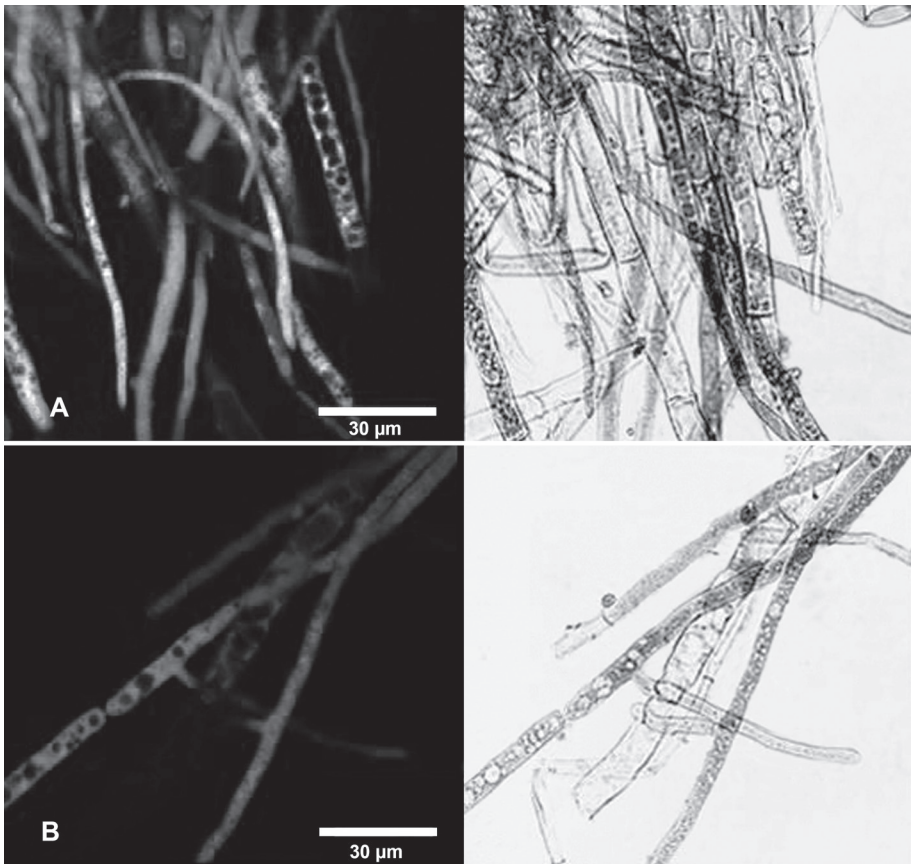

Fig. 5. Laser scanning confocal micrographs (LSCM) of *Botrytis cinerea* hypha treated and untreated with *Bacillus cereus* strain B-02 fermentation broth (×1000)
(A) untreated hyphal cell DNA configuration under LSCM; (B) treated hyphal cell DNA configuration under LSCM. Bars = 30 μm.

Effects of *B. cereus* strain B-02 fermentation broth on hyphal ultralstructure of *B. cinerea*. The external features of *B. cinerea* hyphae untreated with *B. cereus* strain B-02 fermentation broth were slim, uniform and smooth by the SEM (Fig. 5A, 5B, 5C, and 5D). The treated hyphae were destroyed strongly and the external structures were incomplete (Fig. 5E, 5F, and 5G). Part of the treated hyphae were even distorted, malformed and collapsed (Fig. 5H). The cell nucleus and mitochondrion of the untreated hyphal cell were normal, uniform and clear by the TEM (Fig. 6A and 6B). There was a continuous outer surface layer around the hyphal cell wall and no extravasation outer the hyphal cell wall (Fig. 6A).

Part of the treated hyphal cell mitochondrion disappeared crista and its number decreased. The cell nucleus was not obvious and the unknown un-membranous material appeared in cell (Fig. 6C and 6D). Comparing with the untreated hyphal cell wall (Fig. 6A and 6B), the treated hyphal cell wall was thickened irregularly (Fig. 6E). There was a lot of un-membrane material in cell (Fig. 6D and 6E). Heavily stained material occurred out of the treated hyphal cell wall (Fig. 6E and 6F) and the electronic density of partial treated hyphal cell increased (Fig. 6F).

Effects of *B. cereus* strain B-02 fermentation broth on the hyphal cell DNA content of *B. cinerea*. The DNA fluorescence intensity of the treated hyphae with *B. cereus* strain B-02 fermentation broth at different concentration was obviously lower than that of the untreated hyphae by LSCM (Fig. 7A and 7B). The fluorescence intensity values of the treated hyphal cell DNA were 80.90 ± 15.71, 109.74 ± 25.64, 119.8 ± 27.11 with the different concentration of *B. cereus* strain B-02 fermentation broth ($\times 10$, $\times 20$, $\times 100$), respectively. The fluorescence intensity value of the untreated hyphal cell DNA was 153.89 ± 24.52. All the values of experimental group were lower than that of the control group and the difference was significant between the two groups ($P < 0.01$). The reduction of the treated hyphal cell DNA content showed that the normal synthesis of DNA was disturbed.

Effects of *B. cereus* strain B-02 fermentation broth on hyphal cell mitochondrial membrane potential of *B. cinerea*. Comparison of the control group and experimental group showed that the fluorescence intensity of the control group was stronger than that of the experimental group and the control group was more uniform staining than the experimental group by LSCM (Fig. 8A and 8B). The fluorescence intensity values of mitochondrion DNA in the treated hyphal cells were 72.70 ± 27.03, 82.18 ± 26.49, 97.40 ± 29.89 with the different concentration of *B. cereus* strain B-02 fermentation broth ($\times 10$, $\times 20$, $\times 100$), respectively. The fluorescence intensity value of the control group was 108.35 ± 28.59 and the difference was significant between the two groups ($P < 0.01$). So *B. cereus* strain B-02 fermentation

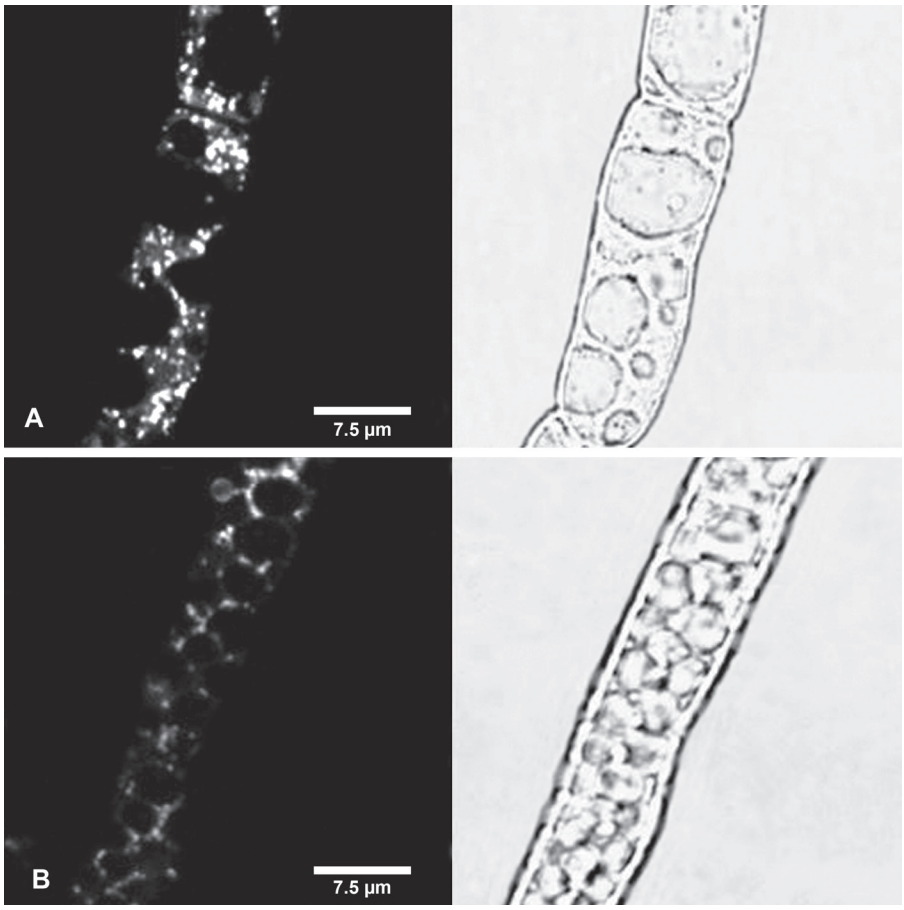

Fig. 6. Laser scanning confocal micrographs (LSCM) of *Botrytis cinerea* hypha treated and untreated with *Bacillus cereus* strain B-02 fermentation broth (×1000)
(A) untreated hyphal cell mitochondrion configuration under LSCM; (B) treated hyphal cell mitochondrion configuration under LSCM. Bar, 7.5 μm

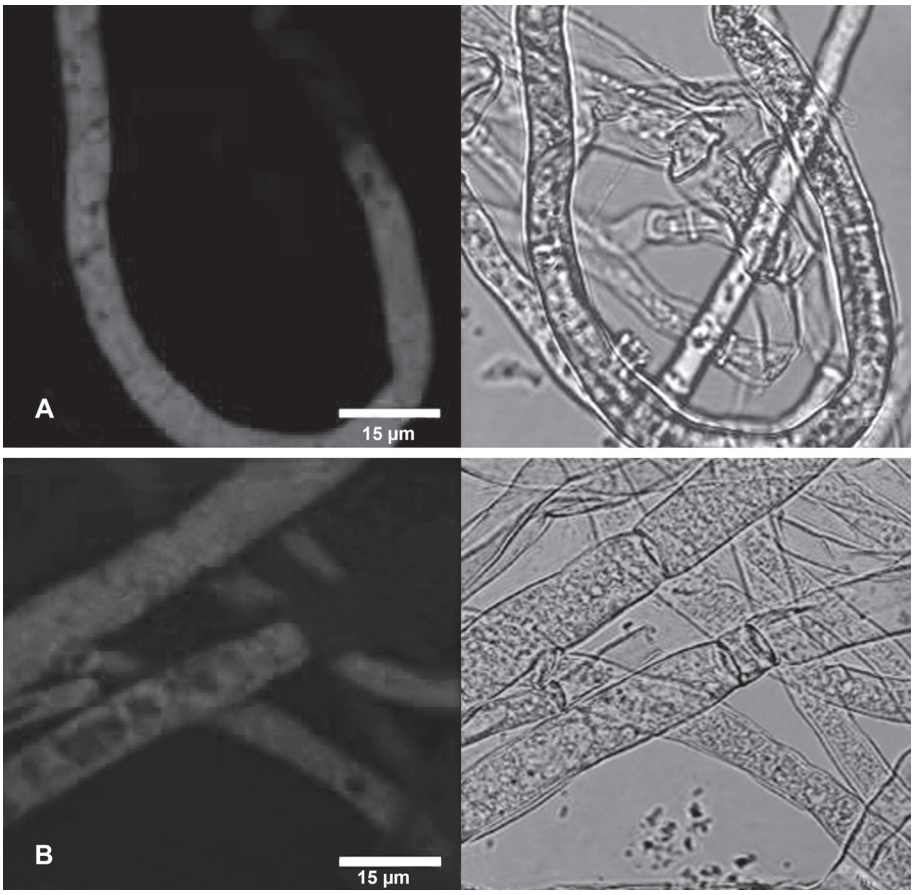

Fig. 7. Laser scanning confocal micrographs (LSCM) of *Botrytis cinerea* hypha treated and untreated with *Bacillus cereus* strain B-02 fermentation broth (×1000)
(A) Fluorescence micrograph of untreated hypha showing reactive oxygen standard (ROS); (B) Fluorescence micrograph of treated hypha showing ROS. Bar, 15 μm

broth could result in the reduction of *B. cinerea* hyphal cell mitochondrial membrane potential.

Effects of *B. cereus* strain B-02 fermentation broth on hyphal cell reactive oxygen standard of *B. cinerea*. The fluorescence intensity of the hyphal cell reactive oxygen standard was obviously lower than that of the control group by LSCM (Fig. 9A and 9B). The fluorescence intensity values of the treated hyphae were 44.45 ± 13.49, 50.01 ± 19.89, 53.67 ± 15.19 with the different concentration of *B. cereus* strain B-02 fermentation broth ($\times 10$, $\times 20$, $\times 100$), respectively. The fluorescence intensity value of the control group was 75.42 ± 17.78 and the difference was significant between the two groups ($P < 0.01$). The reason why the hyphal cell reactive oxygen standard of the experimental group was higher than that of the control group was that the hyphal metabolism and its growth speed became slowly caused by the increase of the hyphal cell reactive oxygen standard.

Discussion

The antagonistic mechanism was studied at the level of cell morphology and cell physiology. Certain concentration of *B. cereus* strain B-02 fermentation broth resulted in abnormal germination of *B. cinerea* spores and distorted hyphae. The configuration of hyphal cell treated with *B. cereus* strain B-02 fermentation broth was destroyed and there were a lot of un-membranous material and vacuoles in cell. Fluorescence intensity of the treated cell and that of the control group was different significantly. Heavily stained material occurred out of the treated hyphal cell wall. We inferred that the membrane permeability of the treated cell was influenced and then the cytoplasm leaked and gathered as a result (Huang *et al.*, 2007). The *B. cinerea* hyphal cell treated with *B. cereus* strain B-02 fermentation broth didn't collapse or die directly. The changes of the treated hyphal cell may be a result of indirect effects or comprehensive effects of multiple factors by *B. cereus* strain B-02 fermentation broth. The relationship between the effects of the chemical bactericide (*e.g.* triadimenfon) to hyphal external feature was related to its action mechanism closely. The main action mechanism of chemical bactericide was to make hyphae swollen or distorted, and influence the formation of fungal cell wall (Huang *et al.*, 2007; Chen *et al.*, 2007). It was obvious that the effects of *B. cereus* strain B-02 fermentation broth on the hyphal feature and ultrastructure were different from that of the chemical bactericide. The treated hyphal cell configuration was changed especially in cell nucleus, mitochondrion, and so on. Which provided morphological basis for the study on antagonistic mechanism of beneficial microorganisms against fungal pathogen.

Mitochondrion, a sensitive organelle in cell, was damaged quite easily and revealed the damaged degree of cell. It was related to the production of oxygen radical and cell apoptosis. The mitochondrion was abnormal, so was the whole cell. Mitochondrial membrane potential, the main part of mitochondrial electrochemistry gradient, reflected the configuration of inner mitochondrial membrane. Mitochondrial membrane potential decrease indicated the configuration of inner mitochondrial membrane was changed and the permeability of it increased (Cortopassi and Wong, 1999). Studies also showed that the descent of mitochondrial membrane potential was one of the important indicators in early cell apoptosis and indicated the cell would access the irreversible process of cell apoptosis. The mitochondrial membrane potential and DNA content in this study decreased markedly. Which indicated *B. cereus* strain B-02 might cause the alteration of mitochondrial membrane configuration and hyphal cell apoptosis in further.

Reactive oxygen, including superoxide radical, hydrogen peroxide, hydroxy radical, and so on, was one of the main factors inducing cell apoptosis (Shi *et al.*, 2002). Above 95% reactive oxygen radical from respiratory chain of mitochondrion in our bodies played an important role in cell signal regulation. Nevertheless, excessive reactive oxygen would attack the mitochondrion by oxidation (Factor *et al.*, 2000). The cause and effect relationship between the reactive oxygen and mitochondrion was a common mechanism of cell apoptosis inducing factors. In this study the reactive oxygen standard of treated hyphal cell decreased obviously. Because the normal physiological activity of hyphae was damaged and the reactive oxygen standard measured might be of the damaged hyphae.

From the above, the synthesis of DNA, mitochondrial membrane potential and reactive oxygen quantity of *B. cinerea* hyphae were greatly influenced by *B. cereus* strain B-02 fermentation broth. So we draw a conclusion that *B. cereus* strain B-02 against *B. cinerea* may act on DNA and mitochondrion firstly. Further the physiological and biochemical process of hyphal cell is influenced and even cell apoptosis is induced. Which lead to hyphal growth slowly eventually. HPLC-MS, which would certainly have provided new and interesting information, concerning the presence of bacteriocins, toxins and other metabolites produced by *B. cereus* in the experimental conditions. What metabolites of *B. cereus* strain B-02 fermentation broth might be responsible for the changes produced. So the chemical analysis of *B. cereus* strain B-02 fermentation broth will be performed on the supernatant met by HPLC-MS in future.

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