

Mycoparasitism of *Acremonium strictum* BCP on *Botrytis cinerea*, the Gray Mold Pathogen

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A fungal strain BCP, which parasitizes *Botrytis cinerea* gray mold pathogen, was isolated and identified as *Acremonium strictum*. BCP strain overgrew the colonies of *B. cinerea* and caused severe lysis of the host hyphae. Frequent penetration and hyphal growth of *A. strictum* BCP inside the mycelia of *B. cinerea* were observed under light microscopy. In addition, some morphological abnormalities such as granulation and vacuolation of the cytoplasm were observed in mycelia and spores of *B. cinerea*. In dual culture test, *A. strictum* BCP strongly inhibited the mycelial growth of several plant pathogenic fungi as well as *B. cinerea*. To our knowledge, this is the first report on mycoparasitism of *Acremonium* species on *B. cinerea*.

Keywords: *Acremonium strictum*, *Botrytis cinerea*, mycoparasite, mycoparasitism, biological control, gray mold

Botrytis cinerea, the pathogen of gray mold disease, causes severe damage on vegetables, ornamentals, fruits, and even some field crops throughout the world. The *Botrytis* disease is the most common disease of greenhouse-grown crops [2]. The greenhouse environment optimized for maximal plant growth may also be favorable for the pathogen. Specifically, warmth and humidity, due to water vapor transpired by the plants and the lack of air exchange with the outside, provide ideal conditions for the pathogen. Biological control of fungal pathogen is an attractive alternative to the strong dependence of modern agriculture on chemical fungicides, which cause environmental pollution and development of resistant strains [4, 5, 16, 19, 20, 25, 27]. Moreover, some of the greenhouse conditions that favor the disease also favor the management of the disease with a biological control agent. Mycoparasites, organisms having

parasitic activity against plant pathogenic fungi, have frequently been used in the biological control of plant diseases. *Trichoderma* species, known as a typical mycoparasite of *B. cinerea*, has been commercialized for control of *Botrytis* disease in many countries [11, 12, 15, 29]. The reported mechanisms for the biological control mediated by fungi are mycoparasitism, the production of lytic enzymes or antibiotics, competition for nutrients, and induction of disease resistance in the plant [6, 10, 15]. Recently, *Pythium periplocum* has also been reported as an aggressive mycoparasite of *B. cinerea* [28].

Acremonium species have been known not only as plant endophytes [26] or pathogens of some plants [14, 24], but also as mycoparasites on plant pathogenic fungi. Srivastava *et al.* [36] reported that *A. furcatum* overgrew the colonies of *Aspergillus* spp. and caused frequent hyphal coiling, penetration, and growth inside the conidiophores of some *Aspergillus* species. *A. sordidulum* and *A. alternatum* represent mycoparasitism against *Colletotrichum dematium* F. *truncate* causing anthracnose disease and *Sphaerotheca fuliginea*, cucurbits powdery mildew fungi, respectively [23, 34]. *A. strictum*, parasitizing urediniospores of *Uromyces viciae-fabae*, has also been detected by Simay [33]. In the present study, we isolated *A. strictum* BCP, which parasitizes *B. cinerea*, and examined its mycoparasitism on the plant pathogenic fungi.

Acremonium sp. isolates were obtained from contaminated culture of *B. cinerea*. Among them, a fungal strain, BCP, was selected because of its same morphological and physiological characteristics, and used for further study. The fungus was transferred to potato dextrose agar (PDA; Becton and Dickinson Co.) slant tubes, which were stored at 4°C.

For taxonomic identification, the strain was inoculated on PDA, oatmeal agar (OA; Becton and Dickinson Co.) and malt extract agar (MEA; 2% malt extract and 1.5% agar; Becton and Dickinson Co.) plates, incubated at 20°C, and then the fungus was identified according to the keys provided by Gams (Table 1) [9, 13]. The colony was white, moist to slimy, and pale pink from below on PDA. The

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Table 1. Descriptions of *A. strictum* provided by Gams.^a

Character	Gams (1971)
Ascomata	Absent
Chlamydo-spore	Absent
Sclerotinia	Absent
Colony color	White
Color from below	Pale pink
Morphology	Moist, smooth
Diameter ^b	16–25 mm
Colonial color	Hyaline
Morphology	Cylindrical
Septum	Aseptate
Size	3.3–5.5×0.9–1.8 μm
Phialides	Simple, not verticillate
Phialide base	Hardly chromophilic

^a*A. strictum* was incubated on potato dextrose agar, oatmeal agar, and 2% malt extract agar (MEA) media.

^bMeasured after incubating *A. strictum* on MEA medium for 10 days at 20°C.

fungus formed no ascomata, chlamydo-spore, or sclerotia on OA and PDA media, and its radial growth was slow; it took ten days to cover the entire MEA plate (16 mm in diameter). Conidia were hyaline, aseptate, and cylindrical, and their average size was $(2.1\pm 0.16)\times(0.9\pm 0.07)$ μm. Phialide of the fungus was simple and not verticillate, and the phialide base was hardly chromophilic and granulose (Fig. 1). Based on these characteristics, we identified the BCP strain as *A. strictum*.

Acremonium species have earlier been reported to parasitize other fungi. *A. aranearum* was isolated from rust pustules of *Puccinia graminis* [30]. Furthermore, *A. sordidulum* parasitizing *C. dematium* F. *truncata*, *A. furcatum* parasitic on *Aspergillus* species, *A. alternatum* showing parasitic activity on *S. fuliginea*, and *A. strictum* and *Cephalosporium* (a synonym of *Acremonium*) *acremonium*

as mycoparasites on *U. viciae-fabae* and *Mucor racemosus*, respectively, were also characterized [3, 22, 33, 34, 36]. To our knowledge, this is the first report on *Acremonium* species parasitizing *B. cinerea*, although some *Acremonium* species inhibiting the mycelial growth of fungal pathogen have been reported [17, 22].

To observe the interaction between *B. cinerea* and *A. strictum* BCP, a culture of *B. cinerea* was prepared by incubating the fungus on PDA plates at 20°C for 5 days in the dark and further for 5 days with daily lighting for 12 h. The BCP isolate was inoculated in a flask containing potato dextrose broth (PDB; Becton and Dickinson Co.) and incubated on a rotary shaker at 150 rpm for 7 days at 20°C. The spore suspension of BCP strain was made to the concentration of 1×10^8 spores/ml by centrifuging the culture filtrate. A 30 μl aliquot of the spore suspension was placed in the center of the *B. cinerea* culture and incubated at 20°C for 10 days.

A. strictum BCP overgrew the culture of *B. cinerea* and formed a whitish colony. When change of a fraction of the colony to whitish color was observed under a microscope, this indicated that BCP with its narrow diameter had penetrated into the host hyphae with wide diameter, branched freely within the host cells, coagulated its cytoplasm, and finally tore its hyphae apart, thereby resulting in widespread destruction of the gray mold pathogen (Fig. 2). However, the typical coilings around the host hyphae, as reported by others [3, 31, 36], were not observed in the present study. The mycelia of *B. cinerea* infected by BCP failed to revive when it was transferred to a new PDA plate. Therefore, it is highly likely that the fungus was mycoparasitic on *B. cinerea*. The mode of parasitism observed in the present study seems to be of a necrotrophic or destructive type [1, 32].

As previously reported [3, 36], the penetration of BCP was not accompanied by appressoria, but rather the entrance

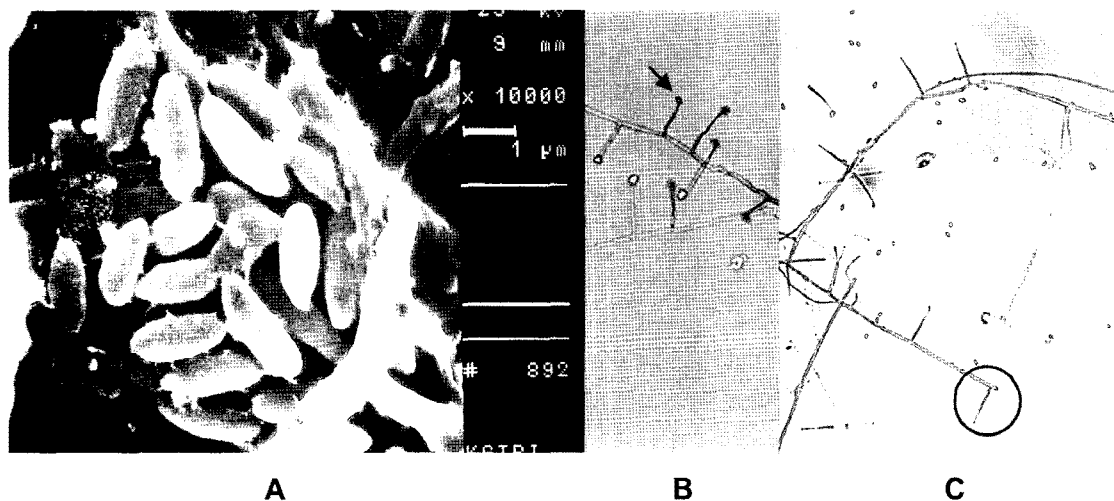


Fig. 1. Microscopic observations of *A. strictum* BCP: A, Conidia; B, Conidia in slimy head; C, Conidiophores are simple and not verticillate.



Fig. 2. Mycoparasitism of *A. strictum* BCP on *B. cinerea*. The mycelia of *A. strictum* BCP (A) grew inside the hyphae of *B. cinerea* (B).

could be found at any point without narrowing or thickening the hyphae of the parasite at the penetration site. Penetration is possible only by disintegrating the host cells by the action of enzymes, antibiotics, or complementary action of both [7]. Therefore, the BCP isolate may produce an antifungal compound and/or lytic enzyme. On the other hand, granulation, vacuolation, and rupture of host hyphae, because of hyphal interference of BCP, have also been reported in parasitism of many hyperparasitic fungi [3, 8, 32].

The relationships between *A. strictum* BCP and the plant pathogenic fungi, such as *Alternaria alternata*, *Botrytis allii*, *B. cinerea*, *Bipolaris maydis*, *Colletotrichum orbiculare*, *Fusarium oxysporum*, *Magnaporthe grisea*, *Phytophthora capsici*, *Pythium ultimum*, and *Rhizoctonia solani* were examined in dual culture tests. A mycelial agar disc (6 mm in diameter) of BCP isolate was cut from a 2-week-old PDA culture grown at 20°C and placed on a new PDA plate (8.5-cm diameter). The plate was incubated at 20°C for 3 days, and the mycelial plug of each pathogenic fungus was then placed at the opposite side of the BCP colony on the same plate. Three replicate plates of each fungus were used. Plates of a fungal pathogen without *A. strictum* BCP were used as controls. After incubation for the next 2–7 days at 25°C, the radial growth of each fungus was measured. The experiment was conducted twice, and the inhibition of fungal growth was determined by comparing with the growth of the control.

A. strictum BCP inhibited the radial growth of the other fungi tested, except for *R. solani* as well as *B. cinerea* (Table 2). However, all the pathogenic fungi did not inhibit the growth of BCP. *M. grisea*, *B. maydis*, and *A. alternata* were the most strongly inhibited, followed by *B. cinerea*, *B. allii*, and *C. orbiculare*. *F. oxysporum*, *P. capsici*, and *P. ultimum* were inhibited to some extent. In the dual culture test, the clear zone that formed because of the inhibition of hyphal growth of the fungus indicates the production of an

Table 2. Inhibitory activity of *A. strictum* BCP against mycelial growth of several plant pathogenic fungi in a dual culture test.^a

Test fungus	Inhibition
<i>Alternaria alternata</i>	+++ ^b
<i>Bipolaris maydis</i>	+++
<i>Magnaporthe grisea</i>	+++
<i>Botrytis cinerea</i>	++
<i>Botrytis allii</i>	++
<i>Colletotrichum orbiculare</i>	++
<i>Fusarium oxysporum</i>	+
<i>Phytophthora capsici</i>	+
<i>Pythium ultimum</i>	+
<i>Rhizoctonia solani</i>	-

^aA hyphal plug of BCP was placed on a PDA plate and incubated at 20°C for 3 days. Then, each pathogenic fungus was inoculated at the opposite site of the BCP colony in the same plate. After further incubating the plate at 20°C for the next 2–7 days, the radial growth of the test fungus was measured. The inhibition of growth of fungus by BCP was determined by comparing with a control.

^b+++ , strongly inhibited; ++ , moderately inhibited; + , weakly inhibited; - , not inhibited.

antifungal compound by the BCP isolate. The antifungal substance produced by BCP has been identified as verlamelin [21]. Therefore, it is possible that the verlamelin plays some roles in the penetration of BCP into the hyphae of *B. cinerea*, followed by host lysis. *Trichoderma* species, as mycoparasites on *B. cinerea* as well as on other pathogenic fungi, are known to produce many antibiotics as a principal mechanism of biocontrol [18, 35, 37]. Since *A. strictum* BCP was not pathogenic on various crops such as rice, wheat, barley, lily, tomato, cucumber, and strawberry (data not shown), *A. strictum* BCP can also be used as a biological control agent for plant diseases caused by *B. cinerea*.

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