Ecological Control Effect of Two Mating Types of Beauveria bassiana on the Asian Corn Borer (Postprint)

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Abstract

Utilizing interspecific interactions to suppress agricultural pest outbreaks represents an important strategy in biological control. To investigate the mutualistic relationship between two mating types of endophytic Beauveria bassiana and maize, and the ecological effects of the resulting symbiont on Asian corn borer control, we employed maize as the host plant, conducted root drenching with B. bassiana spore suspension, and constructed two mating type (MAT1-1-1 type, B5; MAT1-2-1 type, B2) B. bassiana-maize symbionts in a greenhouse. We subsequently examined the symbiont' s effects on maize growth, oviposition preference and larval development of the Asian corn borer, and on the biological characteristics of B. bassiana. The results demonstrated that endophytic colonization of B. bassiana could be detected via detached leaf culture, ITS gene, and mating type gene MAT detection. The MAT1-2-1 type B2 strain exhibited a high colonization detection rate, whereas the MAT1-1-1 type B5 strain demonstrated a colonization advantage in mixed inoculation. Recovered B. bassiana showed no significant alterations in colony diameter or virulence, yet their spore production increased significantly, with the most pronounced enhancement observed in strains derived from the B5 treatment group. Maize plants inoculated with B. bassiana displayed superior above-ground growth rate, biomass, and underground root biomass compared to the control group, with root dry weight exhibiting a marked increase and above-ground plant dry weight also showing relative improvement. The MAT1-1-1 type strain B5 contributed substantially to promoting above-ground height growth of symbiont maize plants, while the MAT1-2-1 type strain B2 contributed significantly to increasing underground dry weight of symbiont maize plants. Overall, endophytic colonization by B. bassiana exerted a greater influence on maize underground root biomass than on above-ground plant biomass. In oviposition preference assays, oviposition quantity of Asian corn borers in all treatment groups was significantly reduced

compared to the control group. The symbiont manifested a significant repellent effect on Asian corn borer oviposition, with the MAT1-2-1 type strain B2 showing a pronounced repellent effect, while the MAT1-2-1 type strain B5 exhibited a weaker repellent effect. In artificial larval inoculation experiments, the survival rate of recovered Asian corn borer larvae in treatment groups was significantly lower than that in the control group, with the B5 group displaying the lowest survival rate at merely 38.33%; the pupation rate in treatment groups did not differ significantly from the control group, but the pupation rate of recovered larvae in the B5 group was significantly lower than that in the B2 group and the control group, at only 34.77%, indicating that the MAT1-1-1 type B5 strain produced the most conspicuous inhibition of corn borer larval development. These results indicate that different mating types of B. bassiana vary in endophytic colonization efficiency, exhibit significant improvement in spore production following endophytic colonization, and the two mating type strains display synergistic effects when applied in combination; both mating type strains can form symbionts with maize through endophytic colonization and promote maize plant growth, demonstrating that a mutualistic symbiont has been established between B. bassiana and maize. Although the symbiont' s potential in repelling Asian corn borer oviposition, inhibiting larval survival, and reducing pupation rates differs, it contributes to sustainable ecological control of the Asian corn borer. This also proves that symbiont establishment effectively enhances the ecological adaptability of maize, providing a novel approach for implementing Asian corn borer management by exploiting the endosymbiotic nature of B. bassiana.

Full Text

Preamble

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Ecological Control of the Asian Corn Borer, *Ostrinia furnacalis* (Guenée), by Two Cloned *Beauveria bassiana* Strains

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Abstract

Utilizing interspecies interactions to suppress agricultural pest outbreaks represents an important approach in biological control. To investigate the mutualistic relationship between two mating-type strains of endophytic *Beauveria bassiana* and corn, and the ecological effects of this symbiosis on Asian corn borer (ACB) control, we established endophytic symbioses by root drenching with *B. bassiana* spore suspensions. The symbiosis effects on corn growth, oviposition preference of ACB adults, larval development, and the biological characteristics of recovered *B. bassiana* were evaluated. Through in vitro leaf culture, both ITS and mating-type genes confirmed successful endophytic colonization. While colony diameter and virulence of recovered *B. bassiana* showed no significant changes, sporulation quantity increased significantly. The EN-B5 strain (recovered from the B5 treatment) showed the most pronounced improvement. Inoculated corn plants exhibited superior above-ground growth rate, biomass, and root biomass compared to controls, with root dry weight showing particularly significant increases. The B5 strain (MAT1-1-1) contributed more to above-ground plant height enhancement, while the B2 strain (MAT1-2-1) contributed more to underground biomass increase. Overall, endophytic colonization affected corn root biomass more than above-ground biomass. In oviposition choice tests, all treatment groups showed significantly fewer eggs than the control, demonstrating clear deterrent effects. The B2-corn symbiosis showed the strongest oviposition deterrence. In larval inoculation experiments, survival rates in all treatment groups were significantly lower than controls, with the B5 group showing the lowest recovery survival rate (38.33%). Pupation rates in treatment groups were also significantly lower than controls, with the B5 group showing the lowest rate (34.77%). These results indicate that different mating-type strains of *B. bassiana* differ in endophytic colonization efficiency, with synergistic effects when applied in combination. Both mating-type strains formed mutualistic symbioses with corn, promoting plant growth and enhancing ecological adaptation. This symbiosis contributes to sustainable ecological control of ACB through oviposition deterrence, larval survival suppression, and reduced pupation rates, offering a novel strategy for ACB management using endophytic *B. bassiana*.

Keywords: *Beauveria bassiana*; mating types; *Ostrinia furnacalis*; ecological control

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Introduction

In natural and agricultural ecosystems, interspecific relationships are complex. Entomopathogenic fungi play crucial roles in regulating insect populations, a characteristic that can be exploited for biological pest control. Previous studies have shown that some entomopathogenic fungi can form endophytic symbioses with plants either naturally or through artificial inoculation, inducing physiological and biochemical changes that promote plant growth, enhance stress

resistance, and improve ecological adaptability. These fungi also directly or indirectly affect pathogen and insect development, profoundly influencing the biological structure of tripartite ecosystems.

Beauveria bassiana, one of the most extensively studied entomopathogenic endophytic fungi, can affect plant metabolic processes and exert dual control over insect populations and plant diseases. Bing and Lewis pioneered research on *B. bassiana* endophytism for pest control, demonstrating that foliar application significantly reduced European corn borer (*Ostrinia nubilalis*) damage. They also showed that stem-base injection allowed the fungus to invade and spread through vascular tissues, suggesting that endophytic *B. bassiana* might control borers through toxic metabolite secretion rather than spore infection. However, few studies have investigated the mechanisms and applications of endophytic *B. bassiana* for Asian corn borer control. Our laboratory previously established a molecular identification system for *B. bassiana* mating types and demonstrated that different mating-type strains could colonize corn plants, with MAT1-1-1 and MAT1-2-1 strains showing mutation sites that revealed impacts on fungal evolution from both plants and insects. This study examines the ecological control effects of symbioses formed between corn and two mating-type strains of *B. bassiana*.

Materials and Methods

1. Test Strains and Insects

The test *B. bassiana* strains were BbOFDH1-5 (B5, MAT1-1-1) and BbDPSD2 (B2, MAT1-2-1), both isolated and maintained in our laboratory. Asian corn borers were also reared in our facility.

2. Corn Planting and *B. bassiana* Inoculation

Corn seeds were surface-sterilized with sodium hypochlorite, rinsed with sterile water, and sown in sterilized substrate (soil:nutrient soil = 1:1, v/v, autoclaved at 121°C for 1.5 h). At the three-leaf stage, seedlings were root-drenched with *B. bassiana* spore suspension at 1×10^5 spores/mL (20 mL per plant). Treatments included B5, B2, B5+B2 (mixed inoculation), and a sterile water control.

3. Isolation and Identification of Endophytic *B. bassiana*

3.1 Endophyte Isolation Fresh, healthy plants were selected from each treatment group. Leaves were surface-sterilized and cultured on PDA plates (60 mm) at 26°C, 180 rpm for 3-4 days to isolate endophytic fungi.

3.2 Molecular Identification Mating-type genes (MAT1-1-1/MAT1-2-1) were amplified and analyzed following Li et al. [21]. ITS fragments were amplified following White et al. [23], separated by agarose gel electrophoresis,

and sequenced. Sequences were compared to original strains using DNAMAN software.

3.3 Colony Diameter and Sporulation Recovered and original strains were cultured on PDA plates at 26°C, RH 90%. Colony morphology was observed after sporulation. Colony diameter was measured on day 10 using the cross method. Sporulation quantity was determined by the dilution method: 0.1 g spore powder was suspended in 100 mL sterile water with 0.01% Tween-80, vortexed, and counted using a hemocytometer. Sporulation quantity = (average spores per grid $\times$ 4 $\times$ 10^6) / (3.14 $\times$ 0.16).

3.4 Virulence Testing Second-instar ACB larvae were used for bioassays. Strains recovered from different treatments were randomly selected. Non-agar semi-artificial diet [24] was mixed with spore suspension (1 g spores/L, 10^5 CFU/mL) and placed in 24-well plates (one larva per well). Controls received diet with 0.01% Tween-80. Plates were incubated at 26°C, 60-80% RH, 14:10 L:D. Larval mortality was recorded daily for 10 days.

4. Effects of *B. bassiana*-Corn Symbiosis on Corn Growth

Plant height was measured at 35, 63, and 100 days post-inoculation. Dry biomass (above-ground and roots) was determined at 100 days after drying at 65°C for 36 h.

5. Effects on ACB Oviposition and Development

5.1 Oviposition Preference At the 10-leaf stage, plants were covered with nylon mesh cages (80 $\times$ 70 $\times$ 80 cm). Each cage contained one plant from each treatment group (B5, B2, B5+B2) and a control, arranged equidistantly. Ten newly emerged ACB adults were released per cage. After one week, egg masses were counted on each plant. Oviposition response index (ORI) = (Nt - Nc)/N, where Nt = egg masses on treatment, Nc = egg masses on control, N = total egg masses.

5.2 Larval Survival and Pupation At the tasseling stage, healthy plants from each treatment were selected. Ten second-instar larvae were inoculated into each plant. After 2-3 days, plants were dissected to record survival, weight, and pupation rates until adult emergence.

6. Data Analysis

Data were analyzed using DPS 15.10 and Microsoft Excel. Plant height, biomass, larval survival, and pupation rates were analyzed using completely randomized design. One-way ANOVA was used for variance analysis, with Duncan' s new multiple range test for multiple comparisons. Corrected mortality (%) = (treatment mortality - control mortality)/(100 - control mortality) $\times$ 100%.

Results

1. Establishment of *B. bassiana*-Corn Symbiosis and Biological Characteristics of Recovered Strains

Endophytic *B. bassiana* was detected on PDA plates from all inoculated treatment groups, showing white to grayish-yellow powdery colonies that expanded over time. No *B. bassiana* colonies were detected in the control group [Figure 1: see original paper].

Molecular identification of recovered strains (designated EN-B5-01, EN-B5-02, EN-B5-03 from B5 treatment; EN-B2-14, EN-B2-17, EN-B2-19 from B2 treatment; EN-B5+B2-23, EN-B5+B2-25, EN-B5+B2-27 from mixed treatment) confirmed successful symbiosis establishment. The B2 treatment showed higher endophytic detection rates than B5, while B5 demonstrated greater success during co-inoculation. ITS and mating-type gene amplification confirmed that recovered strains originated from the inoculated strains (100% homology) [Figure 2: see original paper]. The MAT1-2-1 strain appeared dominant in mixed infections, suggesting potential ecological advantages.

Recovered strains showed more vigorous vegetative growth than original strains, with most colonies being pure white and thick. Colony diameters varied significantly: EN-B5-3, EN-B2-19, and EN-B5+B2-23 showed superior growth ($ $40.5 cm). Sporulation quantity improved significantly in all recovered strains, with EN-B5, EN-B5+B2-23, and EN-B5+B2-25 producing 8.68×10^6 spores/mL. Virulence against second-instar larvae remained high, with B5+B2 combination showing 71.52% corrected mortality at 5 days and 83.98% at 10 days, indicating synergistic effects .

2. Effects on Corn Biomass

Inoculated plants showed significantly greater height than controls at all growth stages (35, 63, and 100 days) [Figure 3: see original paper]. Above-ground dry weight was significantly higher in B5 and B5+B2 treatments compared to controls ($F = 18.573$, $P < 0.001$), while B2 alone showed no significant difference. Root dry weight was significantly increased in all treatment groups, with B5+B2 showing the greatest enhancement ($F = 3.295$, $P = 0.0217$) [Figure 4: see original paper]. These results indicate that endophytic colonization affects root biomass more than above-ground biomass, with MAT1-1-1 enhancing plant height and MAT1-2-1 promoting root growth.

3. Effects on ACB Oviposition

ACB females laid significantly fewer eggs on treated plants than controls. The B2 treatment had the fewest eggs (0.20 ± 0.20 masses per plant) with an ORI of -0.500, indicating strong deterrence. B5 and B5+B2 treatments also showed

significant deterrence (ORI = -0.400 and -0.233, respectively) . The deterrent effect ranking was B2 > B5+B2 > B5.

4. Effects on ACB Larval Survival and Pupation

Larval weights did not differ significantly between treatments and control. However, survival rates were significantly lower in all treatment groups: B5 (38.33%), B2 (62.50%), and B5+B2 (65.00%) compared to control (80.83%) ($F = 0.425$, $P < 0.001$). Pupation rates were also significantly reduced, particularly in B5 (34.77%) compared to control (41.33%) ($F = 0.025$, $P = 0.260$). The B5 treatment showed the strongest inhibition of both survival and pupation, followed by B5+B2 and B2 .

Discussion

Beauveria bassiana is an important entomopathogenic fungus widely used in China to control the Asian corn borer, with annual application areas exceeding 2 million hectares and producing significant ecological benefits by reducing chemical pesticide use. However, changing climate and agricultural practices necessitate new control strategies.

This study is the first to examine the ecological control potential of *B. bassiana*-corn symbioses using mating-type markers (MAT1-1-1 and MAT1-2-1), which are essential for sexual reproduction in this fungus. Molecular markers provided rapid, reliable identification of endophytic strains and confirmed that recovered fungi originated from inoculated strains. The dominance of MAT1-2-1 in mixed infections suggests potential ecological advantages that warrant further investigation.

Endophytic colonization induced phenotypic plasticity in *B. bassiana*, with recovered strains showing enhanced sporulation despite non-significant changes in colony diameter and virulence. Different mating-type strains differentially affected plant growth: MAT1-1-1 enhanced above-ground height while MAT1-2-1 promoted root biomass, with mixed inoculation showing synergistic effects. This demonstrates that endophyte genotype influences host plant responses.

The symbioses effectively deterred ACB oviposition and reduced larval survival and pupation rates. The B2-corn symbiosis showed strongest oviposition deterrence, while B5 showed greatest impact on larval development. These effects may involve toxic metabolite production, nutrient competition, or altered plant volatiles, as observed in other endophyte-herbivore interactions.

In conclusion, different mating-type strains of *B. bassiana* can establish mutualistic symbioses with corn, promoting plant growth and enhancing pest resistance through multiple mechanisms. The synergistic effects of combined strains offer new strategies for sustainable ACB management. This approach improves corn' s ecological adaptability while reducing pest populations, representing a

promising direction for biological control that merits further development of application methods and breeding technologies.

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Note: Figure translations are in progress. See original paper for figures.