Efficacy of *Chaetomium globosum* as Biological Control Agents for Controlling Leaf Blight of Corn

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ABSTRACT

Leaf blight is one of the major problems in the quality and quantity of corn production in Thailand. Nowadays, biological control is used in controlling plant diseases, especially using *Chaetomium globosum* which is the fungus reported to be a potential antagonist of various plant pathogens. This research aimed to identify the species of fungal pathogen causing leaf blight of corn, and evaluate the efficiency of *C. globosum* for controlling fungal corn leaf blight. The results showed that *Bipolaris maydis* was isolated from baby corn leaf blight samples using tissue transplanting method, and morphological characteristics and molecular data derived from sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. The effectiveness of *C. globosum* was evaluated for the inhibition of *B. maydis* under laboratory and greenhouse condition. The results indicated efficacy of *C. globosum* for controlling *B. maydis* caused by Southern corn leaf blight.

Keywords: *Corn leaf blight, Biological method, Chaetomium globosum*

INTRODUCTION

Corn, *Zea mays* L., is one of the most important field crops of Thailand due to the fact that corn has an impact on food and feed industry. The corn yield of Thailand was 4.46 tons per hectare in 2020 (Knoema, 2020). Disease is one of problem that reduce crop yield and also deteriorate product quality. Leaf blight is a problem in tropical and subtropical corn growing areas in the south-eastern the United States of America, parts of Asia and Africa, where yield losses close to 70% have been reported due to the disease. Leaf blight is an important foliar disease of corn and caused by the ascomycete fungi *Bipolaris maydis* (anamorph) or *Cochliobolus heterostrophus* (telomorph) (Manching et al., 2014).

Biological control is consequently considered as an alternative method, which could reduce expenses and is not toxic to the environment and organisms (Cook, 1993). In both agriculture and forestry, the use of fungal biocontrol is an important alternative for protecting crops against weeds, insects and fungal pathogens (Aggarwal et al., 2014). *Chaetomium*, a saprophytic fungus that belongs to Ascomycota and Chaetomiaceae. Several plant pathogens have been reported to be antagonized by *Chaetomium* species. *Chaetomium globosum* has been researched to be a potential biocontrol agent. It is effective against the seedling blight caused by *Rhizoctonia solani* and rice blast caused by *Pyricularia oryzae* (Soytong & Quimino, 1989,). In addition, *C. globosum* can inhibit the growth of *Phytophthora infestans* caused tomato late blight (Mol et al., 2014). Antagonistic fungus, *C. globosum* reported to be actively against various plant pathogens through the production of lytic enzymes and metabolites as control mechanism. Others active metabolites are antibiotic substances such as chaetomin, chaetoglobosin, cochliodinol, chaetosin and prenisatin (Moya et al., 2016).

Therefore, the present study aimed to identify the species of leaf blight of corn using morphological characteristics and sequence analysis and evaluate the efficiency of *C. globosum* for controlling the corn leaf blight.

METHODOLOGY

Isolation of pathogens

Fungal pathogens were isolated from leaf blight samples of baby corn planted in cultural area at Phra Thaen District, Tha Maka District, Kanchanaburi Province, Thailand. Pathogenic fungal

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isolates were cultured potato dextrose agar (PDA) at room temperature for 7 days. Each pure fungal culture was single-spore isolated by the hyphal tip method and maintained on PDA slants.

Antagonistic strain

Chaetomium globosum strain BCC 31359 was isolated from coral in the sea of Thailand and obtained from BIOTEC Culture Collection Laboratory, National Centre for Genetic Engineering and Biotechnology (BIOTEC). Fungal antagonist was grown on PDA at 28°C under a photoperiod of 12: 12 (L: D) h for 14 days.

Morphological based identification

The isolates of fungal pathogen and antagonist were sub-cultured on PDA and incubated at 28°C under a photoperiod of 12: 12 (L: D) h for 14 days. Each pathogenic isolate and antagonist were examined under a light microscope to determine the colonial features and the morphological structures of fungi to confirm species identity and compare morphological characters.

DNA extraction and molecular based identification

The fungal pathogenic isolate was further confirmed as a species by molecular-based identification. On the other hand, fungal antagonist, *C. globosum* strain BCC 31359 was also confirmed by molecular-based identification. DNA extraction of fungal pathogen and antagonist was applied by following the method of Saitoh et al. (2006). Extracted DNA was used as the template in a PCR to amplify the universal primers ITS1/ITS4 of internal transcribed spacer (ITS) regions of rDNA (White et al., 1990). The final concentrations for 50 µl reaction with the following components: 5 pmole of each primer, 2.5 mM MgCl2; 0.2 mM dNTP and 1 unit of Taq DNA polymerase. The cycling parameters were 95 ºC for 1 min followed by 35 cycles of 95 ºC for 30 s, 55 ºC for 1 min, and 72 ºC for 1 min, and a final extension at 72 ºC for 10 min. The PCR products and 0.1 µl/ml Nucleic acid Gel stain, 1,000 X concentrate in DMSO) were subjected to agarose gel electrophoresis using 1.0% (W/V) agarose gel electrophoresis in 1X in a TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) buffer and photographed under UV light.

After amplification, 20 µl of the PCR products were purified and sequenced at SolGent (Solutions for Genetic Technologies) Analysis Services. The ITS1-5.8s-ITS2 sequences of pathogenic isolate and fungal antagonist were examined for homology using the NCBI BLAST program. ITS sequence alignments were operated using MUSCLE and phylogenetic relationships were analysed on the neighbour-joining (NJ) method (Saitou & Nei, 1987) by MEGA 7.

Pathogenicity of pathogen in greenhouse condition

Pathogenicity was examined by artificial inoculation of fungal pathogen onto leaf of corn seedling plants (7-d-old). This experiment was conducted using a completely randomized design (CRD) with 10 replications of each treatment. The pathogenic isolates were inoculated by spraying the leaves and petioles of corn with 10 ml of 1×10^4, 1×10^5 and 1×10^6 spores/ml in 0.1% Tween 20 (v/v). While, the control treatment was sprayed with 10 ml of 0.1% Tween 20 (v/v) only. The plants were placed under the greenhouse at 26 to 32ºC with 65–90% relative humidity (RH) for 14 days. Disease severity ratings were made according to a 0-4 scale using the following scale: 0 = no symptoms, 1 = 1-25%, 2 = 26–50%, 3 = 51–75%, 4 = 75–100% leaf area covered with lesions (Huang et al., 2010). The disease severities from all repeated experiments were statistically analysed using analysis of variance (ANOVA). Significant differences among treatment means were compared using Duncan's multiple range test (DMRT) at $P<0.01$. Statistical analysis was performed by SPSS statistics software (version 16.0, Window).

***In vitro* antagonistic test**

The inhibitory action of *C. globosum* strain BCC 31359 was tested for its ability to suppress the mycelial growth of *B. maydis in vitro* dual culture method on PDA (Rahman et al., 2009). The experiments were conducted in a randomized complete block design (CRD) with five replications of each treatment. A fungal antagonistic disc (0.6 cm diameter) was placed in the PDA and a pathogenic disc (0.6 cm diameter) was placed the opposite site of the PDA. While, the negative control was only pathogen, and the positive control used 50 µl of 1,000 ppm (19%EC) of Triporin [N, N- (piperazine - 1,4-diylbis (trichloromethy) methylene] in 0.6 cm diameter paper disc. Cultures were incubated for 14 days

at 28°C under white fluorescent light with a 12-h photoperiod. Antagonistic activity was determinated after incubation by measuring the radial growth of pathogen in the direction of *C. globosum* mycelia a (R2) and the radial growth of *C. globosum* mycelia in the control plate (R1). The percent inhibition of radial growth (RIRG)= [(R2-R1)/R1] x100. Data was analysed by One-way ANOVA, followed by DMRT.

Antagonism of *C. globosum* to control *B. maydis* under greenhouse condition

Chaetomium globosum strain BCC 31359 was tested for controlling Southern corn leaf blight caused by *B. maydis* under greenhouse conditions using spray method. Fungal pathogen and antagonist were cultured and incubated on PDA at 28°C and 12 h/12 h light/dark a photoperiod under white fluorescent light. Seven-day-old healthy corn seedling plants were with 10 ml of 1×10^5 in 0.1% Tween 20 (v/v) of spore suspension of *B. maydis* using a hand sprayer. The experiment was conducted using a CRD with 10 replications in each treatment and repeated three times. The experiment consisted of six treatments (N= 10 plants). Control plants were sprayed with10 ml of 0.1% Tween 20 (v/v) only (control 1), chemical controls consisted of 10 ml of the following: 1,000 ppm of Triporin in 0.1% Tween 20 (v/v) (control 2) and 10 ml of 1×10^5 in 0.1% Tween 20 (v/v) of spore suspension of *B. maydis* only (control 3). Treatments were inoculated with 10 ml of 1×10^6, 1×10^7 and 1×10^8 spores/ml spore suspension in 0.1% Tween 20 (v/v) of *C. globosum*. All corn plants were grown in a growth chamber with 100 % humidity for 24 h before moving to their natural condition. The disease severity was observed at 15 days after inoculation using the following visual rating scale: 0= no symptoms, 1= 1-25%, 2= 26-50%, 3= 51-75%, 4= 76-100% of leaf area covered with lesions. The disease severity means were compared using DMRT at $P<0.01$ following One-way ANOVA.

RESULTS

Isolation and morphological identification of pathogen and antagonist

Characteristics of corn leaf blight disease showed that leaf lesions are yellow spots surrounded by a reddish-brown border, later enlarged lesions along the length of the leaf. Morphological characteristics of fungal pathogen showed that colony characters were dark grey on PDA (Figure 1A). Conidiophores were pale to dark brown, single and branched. Conidia were mostly curved, canoe-shaped, fusoid or obclavate, and 7.5-12.5 µm×48.3-66.6 µm in size, with similar characters to the species in a previous study (Manamgoda et al., 2014) in Figure 1 B-C. The fungal pathogenic isolates were identified as *B. maydis* based on morphological characteristics. The colony characterization of *C. globosum* strain BCC 31359 was white and turned yellowish green with age. (Figure 2A). Perithecia were dark brown to black. The terminal hairs were branched, undulate, and flexuous. (Figure 2B). The conidia were brown and lemon-shaped, 5-7.5×8-10 µm in size (Figure 2C), with similar characters to the species in a previous study (Abdel-Azeem, 2020).

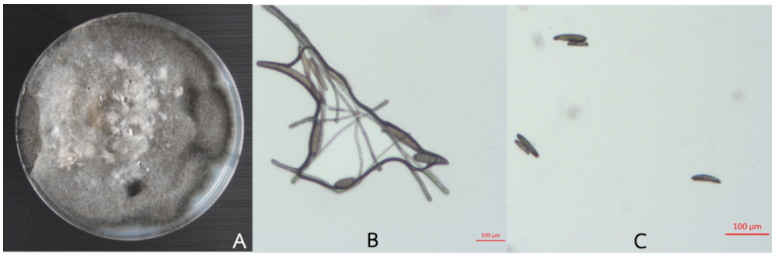

Figure 1. Morphological characteristics of *B. maydis*; dark grey colony(A), conidiophores (B, 400X) and conidia (C, 400X)

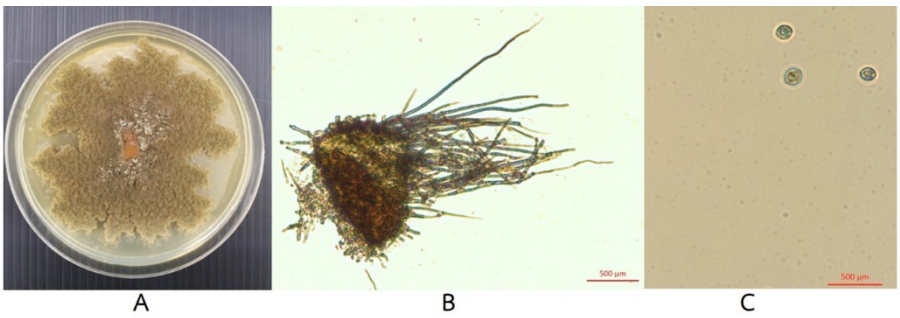

Figure 2. Morphological characteristics of *C. globosum*; colony (A), conidiophores (B, 400X) and conidia (C, 400X)

Molecular based identification using ITS sequence analysis

The morphological characterization and molecular analysis were confirmed the identification of the fungal pathogen as *B. maydis*. The sequence lengths of *B. maydis* were 560 bp. The code number of *B. maydis* obtained from the GenBank database was LC326252 and then the sequence lengths of *B. maydis* were aligned and analysed together with the sequences obtained from the GenBank database (NCBI)."

NCBI-BLAST search results showed the highest sequence similarity with *B. maydis* and accessions number were KC005707, HM195267 and GQ870276), *B. yamadae* strain CBS 127087 (KY905673), *B. yamadae* strain (CBS127087), *B. sivanesaniana* strain BRIP 15847 (KX452456) and *Curvularia lunata* strain JGS10 (GU966505). The similarity coefficient in sequences of *B. maydis* was 99.995% when compared with the sequences of *B. maydis* obtained from the database; this was supported by a 93% bootstrap value (Figure 3). Furthermore, fungal antagonist (LC326517) had nucleotide sequences of 557 bp.

This result of NCBI-BLAST of fungal antagonist when aligned together with the sequences obtained from the GenBank database (NCBI) such as *C. globosum* (KT192346, KU293593 and LT906574), KU597363 (*C. cupreum*), JX280827 (*C. lucknowense* strain CBS 796.71) and *Sordaria fimicola* strain S3 (KX990277) showed that the similarity coefficient in sequences of was 99.99% with *C. globosum* BCC 31359, with a 100% bootstrap value in Figure 4. Therefore, the phylogenetic analysis showed that fungal antagonist could be identified as *C. globosum* based on ITS sequence which was to the same as the morphologically based identification and BIOTEC Culture Collection Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC).

Pathogenicity of pathogen in greenhouse condition

The fungal pathogen, *B. maydis* was proved by pathogenicity test in corn plants under greenhouse conditions. The infected plants showed symptom in corn leaves. Leaf blight disease expressed the leaf lesions to be yellow spots surrounded by a reddish-brown border, later enlarged lesions along the length of the leaf (Figure 5). The concentrations (10^5 and 10^6 spores/ml) of spore suspensions of fungal pathogen had the significantly highest disease severities on corn leaf.

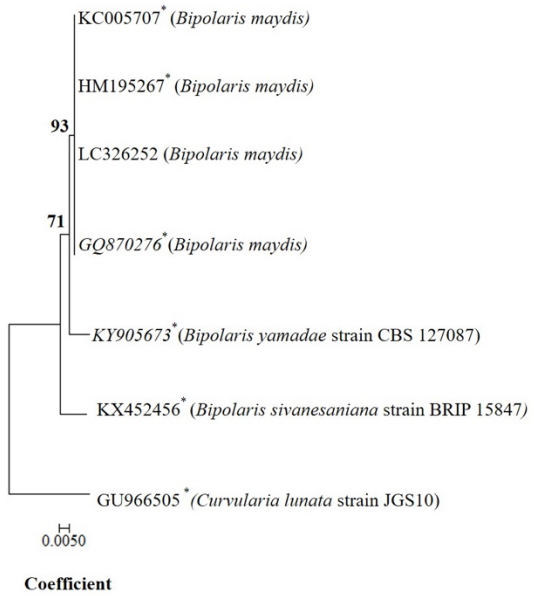

Figure 3 Phylogenetic relationship of identified *B. maydis* (LC326517), six sequences from *Bipolaris* species and one sequence from *Curvularia lunata* using nucleotide of the ITS regions and 5.8S rDNA. Percentage bootstrap support (1,000 replications; ≥ 93%) is shown on branches (*= sequences obtained from the GenBank database).

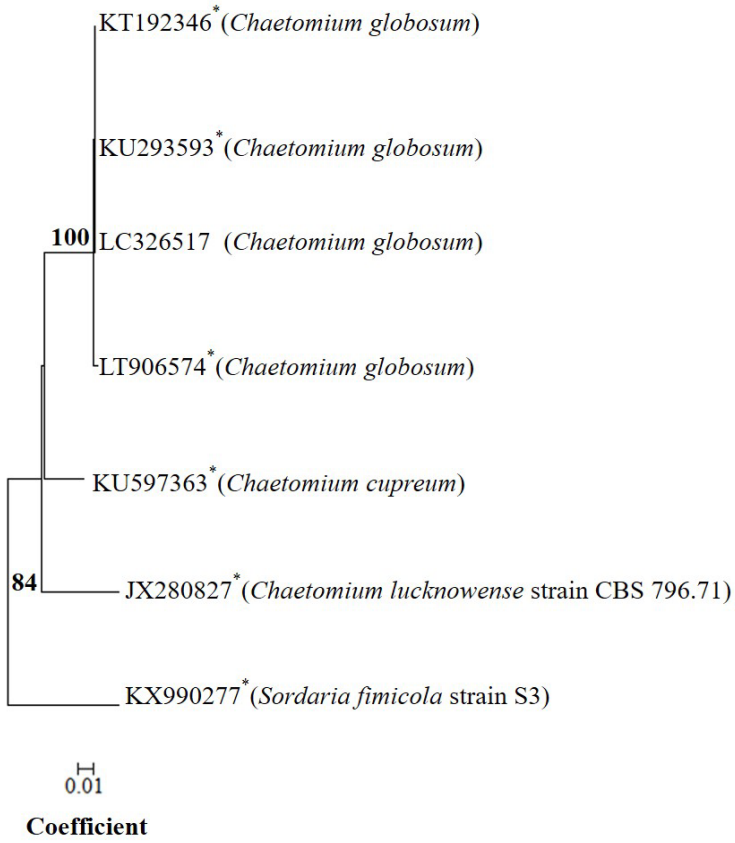

Figure 4. Phylogenetic analysis of the nucleotide sequences of the ITS regions including 5.8S rDNA of identified *C. globosum* (LC326517), five sequences from *Chaetomium* species and one sequence from *Sordaria fimicola* strain S3. Percentage bootstrap support (1000 replications; = 100%) is shown on branches (*= sequences obtained from the GenBank database).

Figure 5. The infected corn plant under greenhouse condition by *B. maydis*; normal leaf corn (A), Leaf blight disease symptom (B)

***In vitro* antagonistic test**

In the antagonism bioassays, an inhibition zone on the PDA plate was noted, and the mycelium of the pathogen stopped growing adjacent to the *C. globosum* of culture. The result showed that *C. globosum* could inhibit the mycelial growth of *B. maydis* in Table 1. The percent inhibition of radial growth of *B. maydis* from *C. globosum* was significantly different from 1,000 ppm of Triporin.

Table 1 The percent inhibition of radial growth of *C. globosum* by *B. maydis* as compared with 1,000 ppm Triporin, same concentration used by farmer

	The percentages of the inhibition of *B. maydis* (%)		
Inoculation (Day)	**Control**	**Triporin**	***B. maydis***
5	0.0^{c}*	31.7^{b}	46.4^{a}
10	0.0^{c}	34.9^{b}	62.0^{a}
15	0.0^{c}	35.3^{b}	72.2^{a}

*Means in the same column followed by a common letter were not significantly different by DMRT (P<0.05).

Antagonism of *Chaetomium* species to control Southern leaf blight under greenhouse condition

Application of *C. globosum* significantly reduced disease severity (P<0.01, Table 2). Analysis of fungal disease progression indicated that 1×10^{7} and 1×10^{8} spores/ml spore suspension in 0.1% Tween 20 (v/v) of *C. globosum* had the significantly the least disease severities on corn seedlings after 15 days-inoculation.

Table 2 The effect of *C. globosum* on Southern leaf blight on corn seedlings under greenhouse conditions

Treatments	**Disease severity***
Control 1	0.0^{d}**
Control 2	0.0^{d}
Control 3	3.8^{a}
1×10^{6} spores/ml spore suspension	2.2^{b}
1×10^{7} spores/ml spore suspension	1.1^{c}
1×10^{8} spores/ml spore suspension	0.8^{c}

*Disease severity was rated using the following scale: 0= 0%, 1= 1-25%, 2= 26-50%, 3= 51-75%, 4= 76-100% leaf blight

**Means in the same column followed by a common letter are not significantly different by DMRT (P<0.01)

DISCUSSION

Samples of corn leaf spot and blight disease could be identified as *B. maydis* from the morphologically based identification which was the same as the based on ITS sequencing identification. Similar result of Gan et al. (2018) identified morphology and ITS sequence analysis of a fungal pathogen isolated from corn leaf blight disease in Fujian province of China. The fungal pathogen showed the species of *C. heterostrophus* (anamorph: *B. maydis*) caused by Southern leaf blight. Morphological characteristics and ITS sequences identification of *C. globosum* BCC 31359 was identified and obtained similar results and reported in Moya et al. (2016). were confirmed analysis species of *C. globosum*.

The inhibition of *B. maydis* growth by *C. globosum* indicated that hyphae of *B. maydis* can be parasitized and lysed by *C. globosum.* This supports the investigations of Hung et al. (2015), who studied *Chaetomium globosum* can be parasitized and lysed hyphae *of Phytophthora cinnamomi and P. nicotianae*. *Chaetomium globosum* could produce various lytic enzymes for degradation of the cell wall of fungi such as chitinase and beta-1,3-glucanase (Sekita et al*.,* 1981). Moreover, other studies indicated that *C. globosum* could produce secondary metabolites such as antibiotics including chetomin, sterigmatocystin and chaetomin. Earlier researchers have also reported *C. globosum* could make chaetoblobosin C which exhibited biocontrol potential against inhibited plant pathogens such as *Colletotrichum gloeosporioides*, *C. dematium*, *Fusarium oxysporum*, *Phytophthora parasitica*, *P. palmivora*, *P. cactorum*, *Pyricularia oryzae*, *Rhizoctonia solani* and *Sclerotium rolfsii* (Chen et al., 2020).

The Efficiency of *C. globosum* for controlling *B. maydis* was reduced under greenhouse conditions, compared with laboratory conditions. Many results were agreed with these findings (Alam et al., 2011). Further studies on *C. globosum* should be further studied to understand other mechanisms of disease suppression and are developed application for controlling *B. maydis* caused Southern corn leaf blight in corn field.

CONCLUSIONS

Leaf blight of corn disease caused by fungal pathogen, *B. maydis* based on morphological characteristics and sequence analysis. *Bipolaris maydis* could infect leaf blight disease symptom in corn leaves. *Chaetomium globosum* was confirmed species based on morphological and molecular identification. *Chaetomium globosum* can be effectively used to control *B. maydis* caused Southern leaf blight on corn. Further study of, development of bio-product of *C. globosum* for controlling *B. maydis* caused by Southern corn leaf blight would be carried out.

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