Antimicrobial activity of fungal endophytes against causal agents of cassava diseases

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Abstract

Cassava is an important crop grown worldwide for food and industries. Plant diseases result in decreased yield and quality of products. Instead of harmful chemical treatment, a friendly alternative method to treat crop diseases becomes necessary. This study aims to identify fungal endophytes from cassava as potential biocontrol agents against cassava diseases. Fungal endophytes were isolated from cassava cultivar Rayong-11 stem. Four fungal isolates were obtained. Based on sequence of internal transcribed spacer region of rDNA, these four fungal isolates showed highest similarity to *Colletotrichum* sp., *Fusarium* sp., *Phomopsis* sp. and *Diaporthe* sp. Co-culture assay showed that two of the four isolates, R11-01 and R11-04., possessed an antifungal activity against mycelial growth of two strains of *Colletotrichum gloeosporioides*, causal agents of anthracnose disease in guava and cassava. R11-01 also showed inhibitory activity against *Xanthomonas* sp., bacteria causing bacterial blight disease in cassava. Currently, culture filtrates of the antagonists are being examined for their effects on each pathogen.

Keywords: endophyte, *Colletotrichum gloeosporioides*, antimicrobial activity

Introduction and Objectives

Colletotrichum gloeosporioides is known as pathogenic fungus causing anthracnose disease in many crops such as avocado, almond, guava, and cassava (1). The fungus produces one-celled, ovoid to oblong, or dumbbell shaped conidia, approximately 10-15 µm in length and 5-7 µm in width. Anthracnose is one of the major important economic diseases of cassava. Disease dispersion occurs easily and rapidly under wet and warm conditions by spreading of fungus spores (2). Anthracnose disease in cassava is found to be a multiple infection with bacterial blight disease caused by bacteria *Xanthomonas campestris* (3). This study aims to evaluate antagonistic activity of endophytic fungi isolated from cassava plant against *Colletotrichum gloeosporioides*, causal agent of anthracnose disease in cassava and guava, and against *Xanthomonas* sp., causal agent of bacterial blight disease in cassava.

Materials and Methods

2.1 Pathogen

Colletotrichum gloeosporioides (C-1060), causal agent of anthracnose disease in guava, was kindly provided by Prof.Wattanalai Panbangred, Department of biotechnology, Faculty of science, Mahidol University. *Colletotrichum gloeospoioides* (C-DOA), the fungus causing anthracnose in cassava, and *Xanthomonas* sp., bacteria causing bacterial blight disease in cassava, were kindly provided by Dr. Rungsri Charaensathapon, Field Crops Research Institute, Department of Agriculture.

2.2 Fungal endophyte isolation

Stem of cassava cultivar Rayong-11 was cut and surface sterilized by soaking in 70% alcohol and 10% bleach. After being washed with sterile distilled water, stem was cut into small pieces and put on water agar. Fungal isolates were obtained by hyphal tip collection.

2.3 Molecular identification of fungal isolates

Genomic DNAs of the fungi were obtained by using Qaigen DNeasy Plant mini Kit. Polymerase chain reaction (PCR) was performed by using ITS1 and ITS4 universal primers (white *et al*, 1990). PCR fragments were submitted to sequencing (Macrogen, Korea). Sequences were blast with GenBank database.

2.4 *In vitro* antagonistic assay

Antimicrobial activity of the fungal isolates was evaluated when fungal endophytes were cultured on PDA for 14 days. Agar blocks of *C. gloeospoioides* C-1060 and C-DOA were pre-cultured on the center of PDA plates for three days before endophyte agar blocks were

inoculated at 1 cm away from the edge of *C. gloeospoioides* colony. Plates were incubated at 30°C. Alteration of *C. gloeosporioides* growth was recorded. For antibacterial activity test, ten colonies of *Xanthomonas* sp. were grown in LB broth for 5 hours. Bacterial culture was spread over LB agar plates, and then endophyte agar blocks were placed on the agar surface. Bioassay plates were incubated at 37°C, and then diameter of clear zone was recorded.

Results and Discussion

Molecular identification of fungal endophytes

Four different fungal isolates were obtained from cassava Rayong-11 stem**.** Based on sequence of internal transcribed spacer region of rDNA, R11-01 showed highest similarity to *Fusarium* sp. TA26-30 (JF819150.1) and *Fusarium* sp. P6-16 (GU723434.1) with 100% identity. R11-02 showed highest similarity to *Phomopsis* sp. 40GP/S (GQ352478.1) with 99% identity. R11-03 showed highest similarity to *Diaporthe* sp. SAB-2009a strain Q1983 (FJ799938.1) with 97% identity. R11-04 showed highest similarity to *Colletotrichum lini* (JF923836.1) with 94% identity.

Assay of antimicrobial activity against phytopathogens

Among the four fungal endophytes tested for antifungal activity against two strains of *Colletotrichum gloeosporioides*, C-1060 and C-DOA, R11-01 and R11-04 showed inhibitory activity against both pathogens in which R11-01 exhibited the greatest distance from pathogen colonies (Table1).

Table1. Distance from pathogen colony

Isolate	Distance from pathogen colony (mm.)	
	C-1060	C-DOA
R11-01	+++	+++
R11-02	-	-
R11-03	-	-
R11-04	+	++

+: x ≤ 1 mm, ++: 1 mm< x ≤2 mm, +++: x > 2 mm
x = diameter of clear zone

For antibacterial activity assay, only R11-01 showed inhibitory effect against *Xanthomonas* sp. in which it produced clear zone of 10 mm diameter.

Two endophytes (*Fusarium* sp. R11-01 and *Colletotrichum* sp. R11-04) showed antimicrobial activity against selected phytopathogens. Many *Fusarium* species have been reported to produce bioactive secondary metabolites including antimicrobial compounds. For example, extracellular metabolites of *Fusarium* sp. were active against gram-positive bacteria, gram-negative bacteria and fungi (4). In addition, fusapyron and deoxyfusapyrone from *Fusarium semitectum* also inhibited growth of *C. gloeosporioides* (5). Most *Colletotrichum* sp. was reported to be host-specific phytopathogen. However, some endophyte strains were found to produce bioactive metabolites (6).

Currently, R11-01 and R11-04 are being analyzed for the effects of their metabolites against phytopathogens.

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