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Controlling Anthracnose of Passion Fruit by Antagonistic Yeast

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Abstract The purpose of this study was to survey, isolate and collect of the yeast fungi from fruits and vegetable crops. The collected yeasts are used for controlling *Colletotrichum boninense* a causal agent of passion fruit anthracnose, in the laboratory and in the greenhouse. Seventeen isolates of *C. boninense* are isolated from various locations of passion fruit farms in Chiang Mai. Of these three isolate are carbendazim resistance. The results shown that 49 isolates of the yeasts are isolated from 28 samples of fruits and vegetable crops. Using dual culture agar plating technique for screening the antagonistic activity against the growth of *C. boninense* which resistance to carbendazim found that 10 isolate of the yeasts are able to inhibit the growth of this fungus, of these, isolate BMF1 give the maximum inhibiting at 67.69 percent. This BMF1 will be used for the further experiment to control the passion fruit anthracnose in the greenhouse. *C. boninense* was first described from *Crinum asiaticum* var. sinicum (*Amaryllidaceae*) collected in the Bonin Islands, Japan (Moriwaki *et al*. 2003). According to these authors, the species was associated with a variety of host plants in Japan, including *Clivia miniata* (*Amaryllidaceae*), *Cucumis melo* (*Cucurbitaceae*), *Cattleya* sp., *Cymbidium* sp. and *Dendrobium kingianum* (*Orchidaceae*), *Passiflora edulis* (*Passifloraceae*) and *Prunus mume* (*Rosaceae*).

Keywords: Passion Fruit, Anthracnose disease, *Colletotrichum boninense*, phylogen

Introduction

Passion fruit is a fruit in New Thailand. Currently, the government and the private sector are promoting the cultivation of passion fruit. Passion fruit is a plant that is important. Economic, because the fruit is sour. And fragrant Water extracted from a high acidity. Therefore, to use in the beverage industry with carbonate by the diluted or mixed with any other fruit to taste more mellow.

C. boninense (in its wide sense prior to our research) has frequently been identified as a pathogen causing fruit and leaf anthracnose, as well as an endophyte of a range of host plants worldwide, especially belonging to *Amaryllidaceae*, *Orchidaceae*, *Proteaceae* and *Solanaceae*. For example, *C. boninense* was found to be associated with diseases of *Leucospermum* and

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Protea cynaroides Teleomorph developed on SNA. Ascomata ovoid to obpyriform, medium to dark brown, 170–210 × 110–140 µm, glabrous, ostiolate, neck hyaline to pale brown, wall 5–10 µm thick, outer layer composed of flattened medium brown angular cells, 5–10 µm diam. Interascal tissue composed of paraphyses, hyaline, septate, branched at the base, disintegrating quickly, 38–60µm long, base 3–4.5 µm diam, apically free, the apex rounded. Asci cylindrical to clavate, 58–74 × 11–16 µm, 8-spored. Ascospores arranged biseriately, hyaline, smooth-walled, aseptate, cylindrical to narrowly fabiform, straight or rarely very slightly curved, both sides rounded, (13.5–)15–17(–18.5) × 5–6 µm, mean ± SD = 16.0 ± 1.1 × 5.6 ± 0.4 µm, L/W ratio = 2.9.

The objective of this study was to explore the collection and isolation of yeasts subpoenaed from fruits and vegetables. Test enmity of yeast that collected the fungus *Colletotrichum boninense* causes anthracnose of passion fruit. And test the ability of yeast antagonistic to control anthracnose Track passion fruit in the laboratory.

Materials and methods

Sample Collection and Isolation

Bring plant samples showing symptoms of the two under a stereo microscope. The direct isolation. But without the spore samples to be incubated inside a box plant diseases that increase the humidity to stimulate sporulation. Leave at room temperature The drop of distilled water on a sterile agar PDA (Potato Dextrose Agar) 1-2 drops of sterile needles at the kickoff of the spores on the surface plant was put on drip water. then spread plate and check the germination of spores after 6 or 24 hours (depending on the type of fungus) isolated single spore.

Morphological characteristics

The study of fungi, such as spore, conidiophore, fruiting body and the germination of spores, etc., stored in lactic acid. These structures by bringing to light under the microscope. In addition, the study of colonies of fungi on agar PDA (Potato Dextrose Agar), such as skin color, the growth of the colony. To study the fungi collected the study is fungal in any group. (Crous *et al.,* 2007, 2009, 2011)

Pathogenicity tests

Pathogenicity tests were done by inoculating detached leaves and fruits of spore with 5 mm. mycelial agar discs of seven-day-old cultures of each of the five isolates. Inoculated leaves and fruits controls treated with

sterile agar discs. After inoculation leaves and fruits were kept in moistened plastic boxs at 25°C.

DNA sequencing and sequence analyses

The mycelia of 3 *Colletotrichum* isolates were grown in PDA broth at room temperature (24 °C) for one week.

1. DNA extraction

Genomic DNA was extracted from fresh mycelia using E.Z.N.A. Forensic DNA Isolation Kit (Omega Bio-Tek), following the manufacturer's manual.

2. PCR: ITS

The internal transcribed spacer (ITS) region was amplified in a 50-ml reaction volume containing 1X buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, 0.2μM of each primer (ITS5 and ITS4), and 1 U Taq DNA polymerase. The PCR temperature profile began with an initial denaturation at 96ºC for 2 min, followed by 35 cycles of 96ºC for 1 min, 53ºC for 1 min and 72ºC for 1:30 min. The final extension was carried out for 10 min at 72°C.

3. Gel Electrophoresis and Sequencing

PCR product was checked by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet (UV) transilluminator. The PCR product was sent to be sequenced for both directions on an automated DNA sequencer (Macrogen Inc., Korea)

4. Sequence analyses

The nucleotide sequences obtained from all primers were assembled using Cap contig assembly program, an accessory application in BioEdit (Biological sequence alignment editor) Program (http://www.mbio.ncsu.edu/ BioEdit/BioEdit.html). The sequences were compared with nucleotide sequences on Genbank, CBS or suitable database. Sequences were compared with *Colletotrichum* sequences available in the EMBL/GenBank database.

Phylogenetic analyses

Phylogenetic analysis of the ITS region was performed with the isolates of *C. boninense* and three additional *Colletotrichum* picies in Clade 1 (obtained from Biotec, DNA data bank, Table 1) using *C. gloeosporioides* as out-group to determine the phylogenetic positions.Sequences were aligned using the Clustal-X program (Thompson *et al*., 1997). A

phylogenetic tree was constructed with the MEGA7, using the Neighbor-joining method with 1000 bootstrap replications.

Table 1. Identity of the ITS sequences available in GenBank used in phylogenetic analysis.

***Colletotrichum* spicies**	**Accession No.**	**ITS**	**Country**
C.karstii	CBS125388	JQ005185	Panama
C.karstii	CBS861.72	JQ005184	Brazil
C.karstii	CBS129927	JQ005206	Thaiand
C.phyllanthi	CBS 175.67	JQ005221	India
C.annellatum	CBS129826	JQ005222	Colombia
C.petchii	CBS378.94	JQ005223	Italy
C.petchii	CBS379.94	JQ005224	Italy
C.petchii	CBS118774	JQ005225	China
C.petchii	CBS118193	JQ005227	China
C.petchii	CBS125957	JQ005226	Netherlands
C.novae-zelandiae	CBS128505	JQ005228	New Zealand
C.novae-zelandiae	CBS130240	JQ005229	New Zealand
C.boninense	CBS123755	JQ005155	Japan
C.boninense	CBS123756	JQ005154	Japan
C.boninense	MAFF306162	JQ005155	Japan
***Colletotrichum* spicies**	Accession No.	ITS	Country
C.boninense	CBS128549	JQ005156	New Zealand
C.boninense	CBS128506	JQ005157	New Zealand
C.boninense	CBS128546	JQ005158	New Zealand
C.boninense	CBS128547	JQ005159	New Zealand
C.boninense	CBS112115	JQ005160	Australia
C.boninense	CBS129831	JQ005161	Australia
C.boninense	CBS128526	JQ005162	New Zealand
C.oncidii	CBS129828	JQ005169	Germany
C.oncidii	CBS130242	JQ005170	Germany
C.beeveri	CBS128527	JQ005171	New Zealand
C.colombiense	CBS129817	JQ005173	Colombia
C.colombiense	CBS129818	JQ005174	Colombia
C.brassicicola	CBS101059	JQ005172	New Zealand
C.hippeastri	CBS125377	JQ005230	China
C.hippeastri	CBS125376	JQ005231	China
C.hippeastri	CBS241.78	JQ005232	Netherlands
C.parsonsiae	CBS128525	JQ005233	New Zealand
C.brasiliense	CBS128528	JQ005234	Brazil
C.brasiliense	CBS128501	JQ005235	Brazil
C.dacrycarpi	CBS130241	JQ005236	New Zealand
C.constrictum	CBS128503	JQ005237	New Zealand
C.constrictum	CBS128504	JQ005238	New Zealand
C.gloeosporioides	CBS112999	JQ005152	Italy

Results and Discussion

Anthracnose disease can occur in all areas are planted with fruit high humidity. (Cedeno *et al*., 1993; Lutchmeah, 1993; de Goes, 1998; Wolcan and Larran, 2000) Symptoms on the leaves of is small incision is about 2-3 ml larger turning brown in the center of the lesion. Symptoms on the branch found is dark brown approximately 4-6 mm enlarged to show signs of canker. Flowers and fruits were infected. It is seen as brown spots. The wound is gray to muddy brown lesions if there is a large drop of water on the surface of a muscular build acervulus wound. When humidity is high, a group of orange conidia is created in which the top dieback symptoms acervulus will not prosper. The rot and die. (de Goes, 1998) The spread of infection relies on rain or irrigation to the seeds and seedlings. The optimum temperature for germination of conidia is between 30-33 ° C at night and 22-25 C in the daytime. (Francisco Neto *et al*., 1994)

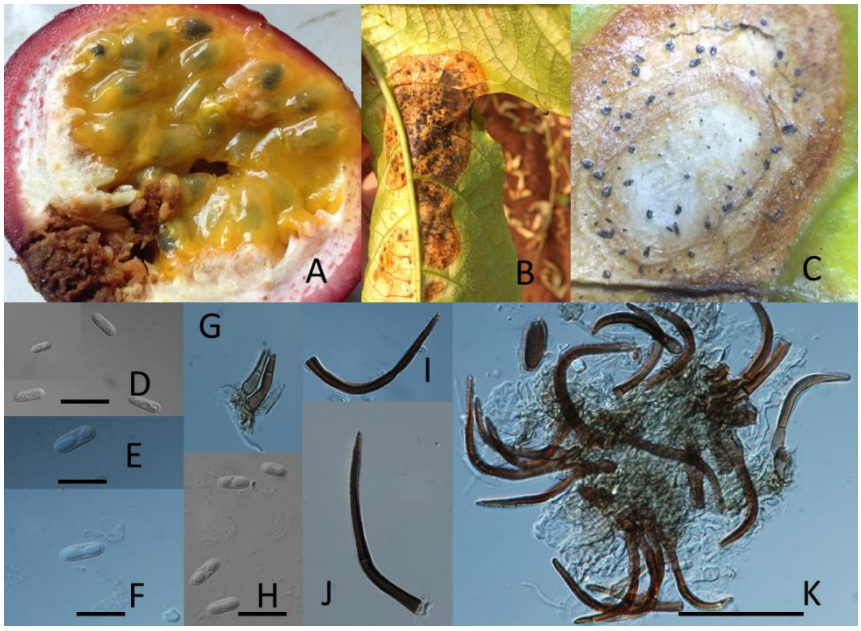

Figure 1. Anthracnose diseases on passion fruit (A- B), characteristic lesions on fruit(C), Group conidia on passion fruit (D, E, F, H: Scale bars = 5 µm), seta of the fungus 40X (G, I, J, K: Scale bars = 17 µm)

Ascomata perithecia, variable in shape but usually subglobose to pyriform, glabrous, medium brown, 100–300 × 100–200 µm, ostiolate, periphysate, neck hyaline to pale brown, to 100 µm in length, outer wall composed of flattened angular cells 4–15 µm diam. Interascal tissue composed of rather irregular thin-walled hyaline septate paraphyses. Asci in a basal fascicle, cylindric-clavate, 45–60 × 12.5–17 µm, 8-spored, with a ±

truncate apex and a small refractive apical ring. Ascospores initially hyaline and aseptate, becoming 1–3-septate, septation sometimes occurring inside the ascus, light to medium brown-pigmented, sometimes verruculose prior to the start of germination, allantoid, (12.5–)14–17(–18) × (4–)5–6(–6.5) μm, mean ± SD = 15.6 ± 1.4 × 5.4 ± 0.5 μm, L/W ratio = 2.9.

The ITS region of all five isolates (coll004, coll011 and coll013) was amplified using primers ITS5 and ITS4, Sequence analyses by BLAST indicated that the isolates were most similar to *C.boninense* isolate C24 and isolate 33-P1(GenBank Accession No.AB688389 and KJ865230) with sequence identity values of 100 % and the sequence obtained in this study were aligned using the Clustal-X program. Phylogenetic analysis based on ITS5, ITS4 and 5.8S rDNA sequences clearly confirmed that the isolated fungus was *C. boninense*. A phylogenetic tree was constructed with the MAGA7program, using the Neighbor-joining method with 1000 bootstrap replications (Figure 2). The MAGA7 program analysis confirmed that the fungus found on *Passiflora edulis* belongs to the genus *Colletotrichum* and can be grouped in the same clade as other taxa of the family *Glomerellaceae*, according to the new classification adopted by Martin *et al*. (2014). Morphological examination and MAGA7 program analysis revealed that the fungus found on *P. edulis* is *C. boninense*.

A phylogenetic tree was constructed using the MAGA7 program and phylogenetic distances were calculated using the neighbor-joining method. Bar = 0.005 genetic distance between samples.To our knowledge this is the first report of *C. gloeosporioides* causing anthracnose of passion fruit in Thailand.

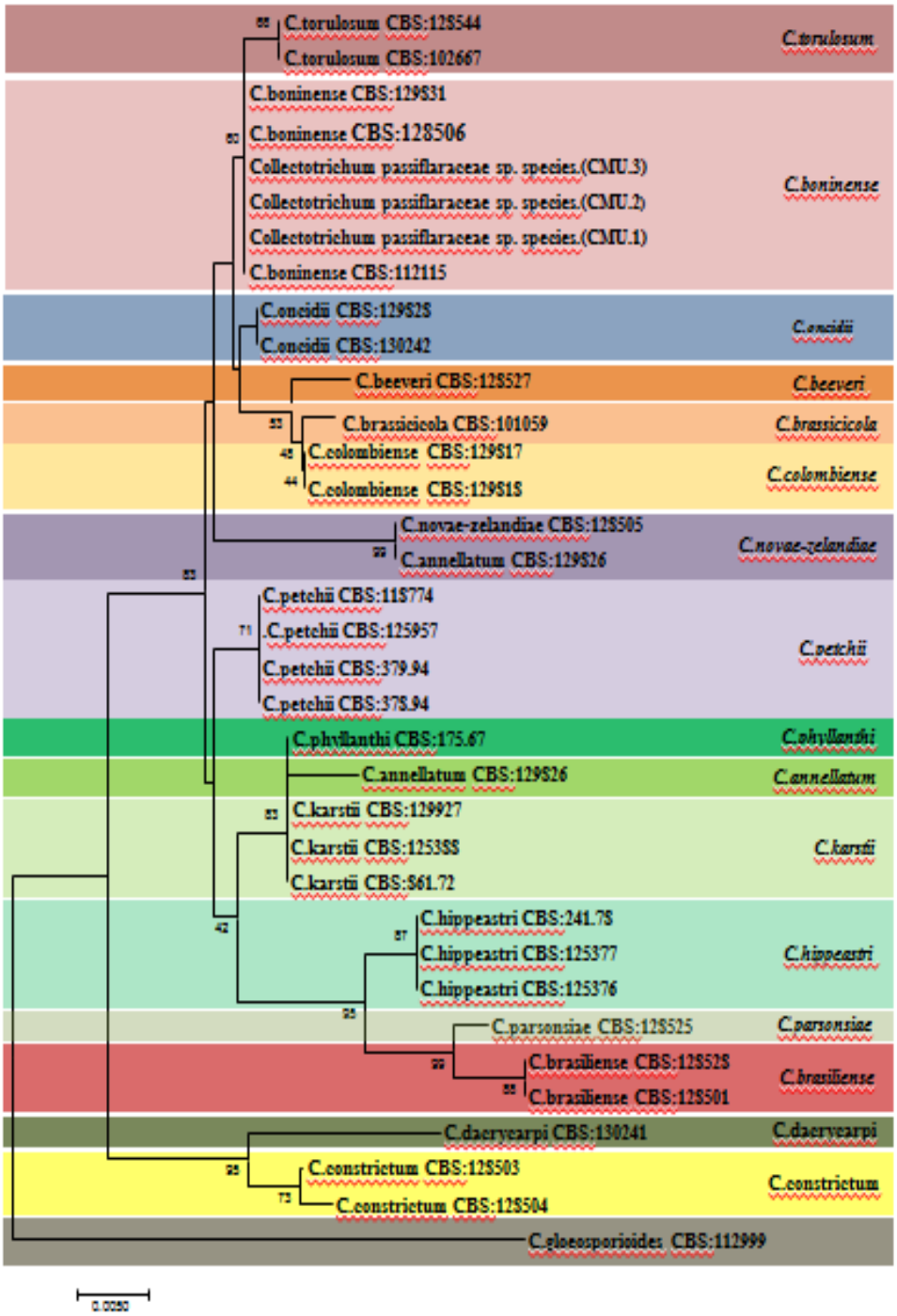

Figure 2. Phylogenetic relationships of *C. boninense*. based on internal transcribed spacer rDNA sequences. Numerical values on branches are the bootstrap values as percentage of bootstrap replication from 1,000 replicate analyses.

Acknowledgments

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