การยับยั้งราเขียวบนผลส้มโดยไอระเหยจากราเอนโดไฟท์ Muscodor albus CMU-Cib 462

Inhibition of green mold by volatile compounds from an endophytic fungus, *Muscodor albus* CMU-Cib 462

นครินทร์ สุวรรณราช[1,3], บุญสม บุษบรรณ์[1,2,3*], วิพรพรรณ์ เนื่องเม็ก[4], วาสนา พิทักษ์พล[4] และ สายสมร ลำยอง[1,2,3]

Nakarin Suwannarach[1,3], Boonsom Bussaban[1,2,3*], Wipornpan Nuangmek[4], Wasna Pithakpol[4] and Saisamorn Lumyong[1,2,3]

Abstract

Muscodor albus CMU-Cib 462 reside as an endophytic fungus within the leaf tissues of *Cinnamomum bejolghota* collected in Chiang Mai province. This fungus is able to produce active volatile organic compounds. Analysis of the volatile organic compounds by gas chromatography and mass spectrometry showed that *M. albus* CMU-Cib 462 primarily produced a number of esters, alcohols and small molecular weight acid compounds. These volatile compounds showed capability in control green mold (*Penicillium digitatum*) causal agent of fruit rot on tangerine fruit cv. Sai Nam Peung.

Keywords: Endophytic fungus, volatile compound, green mold

บทคัดย่อ

Muscodor albus CMU-Cib 462 เป็นราเอนโดไฟท์ที่แยกได้จากใบอบเชยในจังหวัดเชียงใหม่ ซึ่งราดังกล่าวสามารถผลิตสารอินทรีย์ระเหย จากการวิเคราะห์และบ่งบอกชนิดของสารอินทรีย์ระเหยที่ราเอนโดไฟท์ *M. albus* CMU-Cib 462 สร้างด้วยเทคนิค gas chromatography และ mass spectrometry พบว่าเป็นสารจำพวก เอสเทอร์ แอลกอฮอล์ และกรดอินทรีย์ที่มีน้ำหนักโมเลกุลขนาดเล็ก ซึ่งสารอินทรีย์ระเหยที่ผลิตจากราเอนโดไฟท์ชนิดนี้ มีศักยภาพในการยับยั้งราเขียว (*Penicillium digitatum*) ที่ก่อโรคผลเน่าในส้มพันธุ์สายน้ำผึ้งได้

คำสำคัญ: ราเอนโดไฟท์ สารอินทรีย์ระเหย ราเขียว

Introduction

Injuries sustained by citrus fruit during harvest allow the entry of wound pathogens, including *Penicillium digitatum* Sacc (green mold). These pathogens occur in almost all regions of the world where citrus is grown, and cause serious postharvest losses annually (Obawa and Korsten, 2003). Sodium *ortho*-phenylphenate (SOPP), thiabendazole (TBZ), and imazalil (IMZ) are fungicides commonly used in packing houses. These compound have different mode of action, they are used alone, combined in mixtures, or applied separately in sequence. Chemicals have been the primary method used to control citrus fruit decay during storage and marketing for more than 25 years (Ismail and Zhang, 2004). However, postharvest use of fungicides has been increasingly curtailed by the development of pathogen resistance to many key fungicides, lack of replacement fungicides, and public perception that pesticides are harmful to human health and the environment (Janisiewicz and Chen, 2002). An interesting candidate for biological control is *Muscodor* species, an endophytic fungus isolated from plants (Strobel *et al*., 2001). Therefore, the aim of current research is to use the fungus itself as a biological fumigant. Several reports have shown the potential of biofumigation with *Muscodor* spp. in controlling soil borne diseases (Mercier and Manker, 2005) and postharvest decay of fruit (Mercier and Jiménez, 2004). The purpose of this

[1] ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ 50200

[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

[2] สถาบันวิจัยเทคโนโลยีหลังการเก็บเกี่ยว มหาวิทยาลัยเชียงใหม่ 50200

[2] Postharvest Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand

[3] ศูนย์นวัตกรรมเทคโนโลยีหลังการเก็บเกี่ยว สำนักงานคณะกรรมการการอุดมศึกษา กทม. 10400

[3] Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand

[4] คณะเกษตรศาสตร์ และทรัพยากรธรรมชาติ มหาวิทยาลัยพะเยา 65000

[4] Faculty of Agriculture and Natural Resources, University of Phayao, Phayao 56000,Thailand

* Corresponding author: bussaban@hotmail.com

research is to investigate *in vitro* and *in vivo* effects of *M. albus* CMU-Cib 462 in control of green mold (*P. digitatum*).

Materials and Methods

1. Fungal strains

Muscodor albus CMU-Cib 462 was isolated from leaves of *Cinnamomum bejolghota*, collected Chiang Mai provinces during November 2010, using half plate method (Ezra *et al*., 2004). Pathogenic *P. digitatum* was isolated and stored in The Sustainable Development of Biological Resources Laboratory, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. The fungal cultures were preserved in potato dextrose agar (PDA) slants at 4°C and 15% glycerol solution at -20°C.

2. *In vitro* effect of volatiles compounds from *M. albus* CMU-Cib 462 on *P. digitatum*

Muscordor albus CMU-Cib 462 and *P. digitatum* were separately inoculated onto PDA and incubated at room temperature (25±2°C) for 12 and 5 days, respectively. A two-compartment plastic plate (92 × 16 mm) was loaded with PDA for dual culture volatile assay. All tested plates were sealed with Parafilm M® and incubated at room temperature for seven days. After incubation, plugs of *P. digitatum* were then transferred to fresh PDA and incubated for seven days to assess viability. The experiment was repeated two times with five replicates.

3. Qualitative analysis of *M. albus* CMU-Cib 462 volatiles

Muscordor albus CMU-Cib 462 was grown in 5 ml Aglelent® clear glass vials containing PDA for 10 days at room temperature (25±2°C). Volatile compounds produced by the fungus were analyzed using gas chromatographic and mass spectrometric techniques (Suwannarach *et al*., 2010).

4. *In vivo* effect of volatiles compounds from *M. albus* CMU-Cib 462 on *P. digitatum*

In vivo efficacy of volatile compounds was evaluated against *P. digitatum* on tangerine fruit. Early ripening tangerine fruit were obtained from local growers. The fruits were surface sterilized by 2% (v/v) sodium hypochlorite for 3 minutes and then washed three times with sterile distilled water. After air-dried, a uniform wound (6 points, 1 mm width and 2 mm depth) was made at an equator of each fruit using needles. An aliquot of 30 µl spore suspension of *P. digitatum* (10^6spore/ml) was injected into each wound. The inoculated fruits were arranged (6 fruits/box) in 4 liter plastic boxes. Twenty-one day-olds of rye culture *M. albus* CMU-Cib 462 were used for inocula. Six dosages of *M. albus* CMU-Cib 462 inocula (0, 10, 20, 30, 40 and 50 g) were applied separately into each box. The boxes were sealed by Parafilm M® and stored at 28°C for 24 h. The fungal inocula were then removed and the boxes were re-stored. After 9 days of storage, diameters of decay lesions were recorded. The experiment was repeated two times with five replicates.

Results and discussion

The inhibition effect of the volatile compounds produced from *M. albus* CMU-Cib 462 against the mycelial growth of *P. digitatum* was tested using a dual culture volatile test. The results showed that volatile compounds 100% inhibited the mycelial growth of *P. digitatum* (Figure 1). No growth of *P. digitatum* was observed when transferred mycelial plugs onto fresh PDA after one week of exposure to *M. albus* CMU-Cib 462 volatile compounds. According to the previous studies, endophytic fungi, genus *Muscordor* were capable of volatile antibiotics producing. Those volatile antibiotics have been reported that active against a wide range of plant pathogenic fungi, such as *Colletotrichum*, *Fusarium, Penicillium*, *Pythium, Rhizoctonia* and *Verticillium* (Strobel *et al*., 2001; Banerjee *et al*., 2010).

Muscodor albus (CMU-Cib 462) produced seven volatile compounds. These could be positively identified on the basis of a GC/MS comparison with authentic standards in Tabel 1.

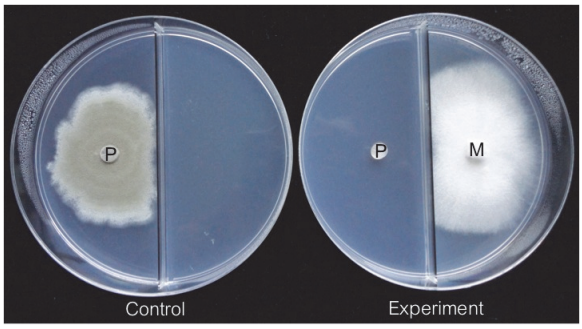

Figure 1 Dual culture volatile test showed 100% inhibition of fungal growth; M= *Muscodor albus* CMU-Cib 462 and P= *Penicillium digitatum*

Table 1 GC/MS analysis of the volatile compounds produced by *Muscodor albus* CMU-Cib 462 cultured in 5.0 ml clear glass vial Aglelent® for 12 days.

RT (min:s)	Total area (%)	Analysis compounds	M/z
3:32	4.84	Ethyl acetate	88
4:34	30.20	Propanoic acid,2-methyl,methyl ester	102
5:41	13.78	Cyclopentane	70
6:38	5.54	butanoic acid,2-methyl,methyl ester	116
11:12	8.34	1-butanol,3-methyl,acetate	130
27:42	1.78	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.α., 7.α., 8a. ß)]	204
30:90	6.40	Eudosma-4(14),11-diene	107

Note: Retention time (RT), Mass to charge ratio (M/z)

In the *in vivo* experiment, tangerine fruits inoculated with *P. digitatum* were fumigated with six different dosages of *M. albus* CMU-Cib 462 inocula. Treatment with *M. albus* CMU-Cib 462 at 30 g inocula showed the minimum dose that completely controlled *P. digitatum* (Tabel 2). This result indicated that *M. albus* CMU-Cib 462 had efficacy in control the causal agent of tangerine fruit rot disease. In addition, several reports have also shown that *M. albus* cultured in rye completely controlled decay of lemons (Mercier and Smilanick, 2005), apples, peaches (Mercier and Jiménez, 2004) and grapes (Gabler *et al*., 2006).

Table 2 The *in vivo* effect of volatiles compounds against green mold during 9 days.

Dosage of inocula (g)	Symptom diameter of infected fruit (mm)
0 (control)	43.0±1.8[a]
10	22.3±1.6[b]
20	9.2±0.9[c]
30	0
40	0
50	0

Means followed by different letter are significantly different (Fisher's LSD test, $P<0.05$)

Conclusion

Muscodor albus CMU-Cib 462 able to produce volatile compounds with 100% inhibited *in vitro* growth of *P. digitatum* and showed efficacy in complete controlling of *P. digitatum* causal agent of fruit rot disease on tangerine fruit cv. Sai Nam Peung.

Acknowledgements

This research was supported by the Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok, Thailand.

Literature cited

Banerjee, D., G. strobel, J. Seares, D. Ezra, O. Liarzi and J. Coombs. 2010. *Muscodor albus* strain GBA, an endophytic fungus of *Ginkgo biloba* from United States of America, produces volatile antimicrobials. Mycology 1:179-186.

Ezra, D., W.M. Hess and G.A. Strobel. 2004. New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. Microbiology 150: 4023-4031.

Gabler, F.M., R. Fassel, J. Mercier and J.L. Smilanick. 2006. Influence of temperature, inoculation interval, and dosage on biofumigation with *Muscodor albus* to control postharvest gray mold on grapes. Plants Disease 90:1019-1025.

Isamail, M. and J. Zhang. 2004. Post-harvest citrus diseases and their control. Outlooks on Pest Management 15:29-35.

Janisiewicz, Y. and C. Chen. 2002. Biological control of postharvest disease of fruits. Annual Review of Phytopathology 40:411-441.

Mercier, J. and D.C. Manker. 2005. Biocontrol of soil-borne diseases and plant growth enhancement in greenhouse soilless mix by the volatile producing fungus *Muscodor albus*. Crop Protection 24:355-362.

Mercier, J. and J.I. Jiménez. 2004. Control of fungal decay of apples and peaches by the biofumigant fungus *Muscodor albus*. Postharvest Biology and Technology 31:1-8.

Mercier, J. and J.L. Smilanick. 2005. Control of green mold and sour rot of stored lemon by biofumigation with *Muscodor albus*. BioControl 32:401-407.

Obagwa, J. and L. Korsten. 2003. Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biology and technology 28:187-194.

Strobel, G.A., E. Dirkse, J. Sears and C. Markworth. 2001. Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. Microbiology 147:2943-2950.

Suwannarach, N., B. Bussaban, K.D. Hyde and S. Lumyong. 2010. *Muscodor cinnamomi*, a new endophytic species from *Cinnamomum bejolghota*. Mycotaxon 114:15-23.