Antimicrobial activity of some saprobic fungi isolated from *Magnolia liliifera* and *Cinnamomum iners* leaves

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(Received 22 February 2013; final version received 19 April 2013)

Six saprobic fungal isolates were assayed for the *in vitro* antimicrobial activity against nine pathogenic microorganisms. The results showed that four fungal isolates including **Clonostachys rosea** MFLU10-0261, **Fusicoccum aesculi** MFLU10-0260, MFLU10-0266, and **Ophioceras cf. leptosporum** MFLU10-0281 were effective inhibitors against some test microorganisms. Of these, **Ophioceras cf. leptosporum** MFLU 10-0281 and **Fusicoccum aesculi** MFLU10-0260 exhibited a maximum inhibition zone of 15 ± 1 mm and 11 ± 1.6 mm against **Staphylococcus aureus** TISTR 1466 and **Candida albicans** TISTR 5779, respectively. These fungi also act as strong decomposers on leaves of *Magnolia liliifera* during the decay process from previous study. The results suggest that saprobic fungi could be a promising resource of useful bioactive compounds.

**Keywords:** bioactive compounds; dead leaves; decomposer fungi

**Introduction**

In the past several years, the emergence and overgrowth of important infections caused by multi-drug resistant organisms have been more serious and become untreatable (Aksoy & Unal 2008). Therefore, there is an urgent need for searching new and effective antimicrobial agents. Fungi serve as a significant source of bioactive compounds as they have diverse chemical structures and activities (Zhong & Xiao 2009). Moreover, most secondary metabolites from fungi represent the drug-like structures leading to a new research program in drug discovery (Strobel 2003; König et al. 2006). Recently, the screening of secondary metabolites produced by microorganisms which are isolated from unusual or unexplored habitats has been a challenge and become an attractive research topic (Gunatilaka 2006). The investigation of fungi isolated from plant debris or dead plant materials is also an interesting alternative for finding novel bioactive compounds because these fungi can degrade organic materials in decaying process and might secrete secondary metabolites for promoting of their growth (Duong et al. 2004). There have been a few studies about screening bioactive compounds from saprobic fungi (Wang et al. 2008). Some examples of these bioactive compounds which are derived from freshwater fungi are two new eudesmane sesquiterpenes (rhombidiol and rhombitriol) isolated from **Beltrania rhombica** (Rukachaisirikul et al. 2005) and ophiocerins A-D, ophioceric acid, an africane sesquiterpenoid isolated from **Ophioceras venezuelence** (Reategui et al. 2005). Another interesting research related to screening antimicrobial substances from saprobic fungi is plectasin (small cysteine-rich peptides) isolated from **Pseudoplectania nigrella** showed active against **Streptococcus pneumoniae**, in which the strains of this pathogen are resistant to conventional antibiotics (Mygind et al. 2005). The discovery of saprobic fungi as a new source of antimicrobial protein has been considered for the development of a human therapeutic (Mygind et al. 2005).

During the study of diversity of saprobic fungi from *Magnolia liliifera* and *Cinnamomum iners* leaves in northeastern Thailand, we describe many strains of saprobic fungi (Monkai et al. 2013). These fungal isolates were selected based on the criteria that (1) they belonged to the genera known to produce the active compounds with biological activities; and (2) they were possibly new species. This study was therefore undertaken with an aim to investigate if these saprobic fungi exhibit antimicrobial activity.

**Materials and methods**

**Fungal strains and test microorganisms**

Six saprobic fungal strains were isolated from dead leaves of *Magnolia liliifera* collected from Doi Suthep-Pui forest, Chiang Mai, Thailand (Monkai et al. 2013). Five pathogenic bacteria and four pathogenic fungi were used as test microorganisms. *Bacillus cereus* TISTR
687, Escherichia coli TISTR 780, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium TISTR 292, Staphylococcus aureus TISTR 1466, and Candida albicans TISTR 5779 were obtained from Biology and Biotechnology Laboratory of the Scientific and Technological Instruments Center, Mae Fah Luang University, Chiang Rai. Colletotrichum fructicola MFLUCC10-0202, Fusarium sp. MFU11-0219, and Alternaria sp. MFU11-0213 were obtained from Mae Fah Luang University Culture Collection (MFUCC), Mae Fah Luang University, Chiang Rai.

Fungal extracts preparation

Initially, the fungal strains were cultivated on sabouraud dextrose agar (SDA) for 30 days at 28°C. The fungal extracts were then prepared using a modified solid phase extraction method (Chamyuang 2010). For this procedure, the fungal cultures were macerated with 30 ml ethyl acetate (EtOAc) using a sterile stainless blender (Waring 800BU). The agar slurry was incubated for 24 h at room temperature to separate the solid and solvent phase. The solvent phase was then collected and the agar solid was re-extracted twice with ethyl acetate. All these EtOAc extracts were combined and transferred to a pre-weighed vial and air dried yielding the crude extracts. The crude extracts were then stored at 4°C in airtight bottles until use.

Antimicrobial activity assay

A modified paper disk method was used for antimicrobial activities assay (Wang et al. 2008; Gu 2009). Bacteria were grown in nutrient broth at 37°C for 18 h and yeast was grown in potato dextrose broth at 30°C for 18 h. After that, they were adjusted to approximately 10⁸ colony-forming units per milliliter (CFU/ml). Agar plates were swabbed uniformly by test bacteria on nutrient agar (NA) and yeast on potato dextrose agar (PDA). Whereas, other fungi were grown in potato dextrose broth at 30°C for 18 h. After that, they were adjusted to approximately 10⁸ colony-forming units per milliliter (CFU/ml). Agar plates were swabbed uniformly by test bacteria on nutrient agar (NA) and yeast on potato dextrose agar (PDA). Whereas, other fungi were grown in PDA at 28°C for 2–3 days until radius of colony are reached to approximately 20 mm. Sterile paper disks (6 mm diameter) each containing 20 µl of sample solution (0.1 g/ml) were dried thoroughly and placed on the surface of medium. The test plates were then incubated at 37°C for 24 h for bacteria and 28°C for 1–4 days for fungi. Methanol was used as negative controls. Streptomycin sulphate (10 µg/ml) was used for bacteria as a positive control. For each test, five replicates were performed. The diameter (mm) of the growth inhibition was examined and measured.

Results and discussion

The antimicrobial assay of six fungal metabolites was processed in vitro by modified disk diffusion methods against nine pathogenic microorganisms. The result of microbial growth inhibition testing by fungal extracts is shown in Table 1. It was found that four fungal strains including Clonostachys rosea MFLU10-0261, Fusicoccum aesculi MFLU10-0260, MFLU10-0266, and Ophioceras cf. leptosporum MFLU 10-0281 inhibited the growth of at least one test bacteria. Among them, Ophioceras cf. leptosporum MFLU 10-0281 showed the highest antibacterial activity against S. aureus. Fusicoccum aesculi MFLU10-0266 also exhibited antimicrobial activity against Ps. aeruginosa and C. albicans.

These results are supported by many previous studies on screening for biologically active fungal metabolites. The aquatic fungi, Ophioceras dolichostomum YMF1.00988 was found to exhibit strong antifungal activity against several plant pathogens. Their antifungal compounds were isolated composed of novel active compound as ophiocerol, as well as two known compounds, isoamericanic acid A and caffeine acid. Reategui et al. (2005) reported that Ophioceras venezuelense produced antibacterial and antifungal compounds named as ophiocerins A-D. Fusicoccum species have been reported to produce xanthofusin and fusicoccin for antifungal and phytoxic activity, respectively (Ballio et al. 1964; Breinholt et al. 1993). Furthermore, Clonostachys rosea is known as mycoparasites of several pathogens including other fungi, bacteria, and insects and produces a wide range of volatile organic compounds (Yu and Sutton 1997; Toledo et al. 2006).

Table 1. Antimicrobial activities of fungal isolates against some pathogenic microorganisms (Mean ± SD).

| Fungal isolates                  | BC | EC | PA | ST | SA | CA | CF | F | A |
|---------------------------------|----|----|----|----|----|----|----|---|---|
| Beltrania rhombica MFLU10-0293  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 |
| Clonostachys rosea MFLU10-0261  | 8 ±| 0  | 0  | 0  | 0  | 0  | 8 ±| 0 | 0 |
| Fusicoccum aesculi MFLU10-0260  | 0  | 0  | 9 ±| 0.7| 0  | 11 ±| 1.6| 0 | 0 |
| Fusicoccum aesculi MFLU10-0266  | 0  | 0  | 0  | 0  | 0  | 10 ±| 1  | 0 | 0 |
| Ophioceras cf. leptosporum MFLU 10-0281 | 8 ±| 0.3| 0  | 0  | 0  | 15 ±| 1  | 0 | 0 |
| Stachybotrys parvispora MFLU10-0292 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0 |
| Methanol                        | 20 | 20 | 15 | 20 | 14 | 0  | 0  | 0 | 0 |

Note: BC = Bacillus cereus, EC = Escherichia coli, PA = Pseudomonas aeruginosa, ST = Salmonella typhimurium, SA = Staphylococcus aureus, CA = Candida albicans, CF = Colletotrichum fructicola, F = Fusarium sp. and A = Alternaria sp.
The study of saprobic fungi on dead leaves of *M. liliifera* found that *Ophioceras cf. leptosporum* MFLU 10-0281 and *Fusicoccum aesculi* MFLU10-0260, MFLU10-0266 were observed on young and mostly decaying leaves (Monkai et al. 2013). The results showed that these fungal isolates are strong decomposers because they grow on the leaves over the decay process. During this process, they might produce many enzymes for decaying chemical composition of leaves and also secrete secondary metabolites such as antimicrobial compounds to compete other organisms. Therefore, saprobic fungi are potential to produce significant bioactive compounds. The potential fungi should be further studied for investigation of other bioactivities such as antioxidant, antitumor, and cytotoxicity and characterization of chemical compounds. They may produce novel bioactive compounds for agricultural and pharmaceutical industry.

**Acknowledgments**

This research was funded by the Mae Fah Luang University (No. 56101020033) and the Thailand Research Fund (BRG5280002). A. Jatuwong, A. Khuanpet, and P. Noireung are thanked for providing test microorganisms. The authors also acknowledge the Scientific and Technological Instruments Center, Mae Fah Luang University for providing facilities.

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