Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 3 (5): 368-374, 2008
ISSN 1816-4935

Endophytic Fungi from Wild Banana (*Musa acuminata* Colla) Works Against Anthracnose Disease Caused by *Colletotrichum musae*

[1]W. Nuangmek, [2]E.H.C. McKenzie and [3]S. Lumyong
[1]School of Agriculture and Natural Resources,
Naresuan University Phayao, Phayao 56000, Thailand
[2]Landcare Research, Private Bag 92170, Auckland, New Zealand
[3]Department of Biology, Faculty of Science, Chiang Mai University,
Chiang Mai 50200, Thailand

Abstract: Screening of endophytic fungi which antagonize *Colletotrichum musae*, the cause of anthracnose disease, was carried out. The *in vitro* screening was studied by dual culture method. The inhibition due to fast competitive growth of *Cordana* sp. (KPP-3) and the antibiotic producing endophyte; *Nodulisporium* sp., reached a high percentage (90% *C. musae* inhibition). Spore germination assay showed 91% *C. musae* germination, while only 0.63 and 1.88% germinated in the conidial suspension of *Cordana* sp. and *Nodulisporium* sp., respectively. There was a significant difference between the Disease Severity Index (DSI) of banana fruits treated with *Cordana* sp. and those treated with *Nodulisporium* sp. The results presented in this research highlight the possibility of using endophytic fungi as biological control agents for anthracnose disease of banana.

Key words: Anthracnose, biocontrol, *Colletotrichum musae*, endophytic fungi, *Musa acuminate*

INTRODUCTION

Banana (*Musa* spp.) is found throughout most tropical and warm temperate regions, especially those with high precipitation. Banana species are important for the daily life of Thai people and are utilized for food, drugs, utensils and are used for various traditions and culture. In addition, chemical extracts from green banana skin contains antibiotic compounds that inhibit pathogenic fungi (Anonymous, 1998). On the other hand, there are several banana diseases whose control is problematic.

The genus *Colletotrichum* includes about 50 species that cause serious worldwide plant diseases, usually known as anthracnose (Sutton, 1992). *Colletotrichum* species cause major damage to crops in tropical, subtropical and temperate regions. Cereals, vegetables, legumes, ornamentals and fruit trees may be seriously affected by this pathogen (Freeman, 2000). *Colletotrichum musae* causes anthracnose in banana fruit and is one of the few *Colletotrichum* diseases that is confined to mature fruits (Waller, 1992). This disease results in scarring from the infection lesion and is the main quality defect of ripe fruit causing consumer rejection. Fungicides have been used to reduce postharvest disease in banana together with hot water dips and modified atmosphere packaging (Wade *et al*., 1993; Costa and Erabadupitiya, 2005). Because the infection is latent and does not manifest itself until the fruit ripens, it is difficult to control the disease. There is a need to develop new control methods, including biological control using antagonistic fungi and these could then become a component of an integrated control program (Jones, 2000).

Corresponding Author: W. Nuangmek, School of Agriculture and Natural Resources,
Naresuan University Phayao, Phayao 56000, Thailand
Tel: 66 8 6728 9571 Fax: 66 5446 6663

In the last decade, the interest in endophytic fungi as potential producers of novel, biologically active products has increased (Petrini *et al*., 1992; Monaghan *et al*., 1995). Endophytes are a potential sources of novel chemistry and biology to assist in solving human and animal health problems (Strobel, 2002). The objective of this study was to screen potential antagonistic endophytes and to study their efficacy in inhibiting growth of *Colletotrichum musae*.

MATERIALS AND METHODS

Isolation

Colletotrichum musae was isolated from anthracnose lesions of banana fruits. The diseased areas were superficially disinfected, cut into small pieces (3-5 mm in diameter) and each piece was sterilized in 10% Clorox for 3-5 min and washed in 3 series in sterile water. The washed tissues were then placed separately on Potato Dextrose Agar (PDA) plates and incubated at room temperature (25-30°C). After incubation, hyphal tip isolation technique was used to transfer mycelium to fresh PDA plates. Identification of the fungal isolate was carried out under microscopic observation according to appropriate taxonomic key and description (Sutton, 1980).

Antagonistic Test

Seven hundred and twenty three isolates of endophytic fungi were isolated from healthy leaves, petioles and pseudostems of wild banana (*Musa acuminata*) in Thailand from July 2004 to April 2005 using the method developed by Photita *et al*. (2001). Screening of the endophyte strains for their antagonistic effects was carried out by inoculating each endophyte in dual cultures with *Colletotrichum musae* in 9 cm Petri dishes containing PDA. Each agar plate was inoculated with a 5 mm in diameter agar disk of the actively growing endophyte; this was positioned at the opposite side of a 5 mm diameter agar disk of the actively growing pathogen. The distance between discs was approximately 5 cm. Five replications were run for this experiment for each pairing. Mycelium plugs of the endophytic fungi were taken from the margins of young colonies grown on PDA.

Plates inoculated with *C. musae* alone were used as controls. All plates were then incubated at room temperature (25-30°C) and the two colonies were allowed to grow towards each other. After 7 days, the diameters of the *C. musae* colony in the control and in each paired culture were recorded. The percentage of inhibition was calculated by using the equation:

$$\text{Percentage of inhibition} = \frac{A - B}{A} \times 100$$

Where:

A = Radius of pathogen in control plate
B = Radius of pathogen in dual culture plate

The endophytes showing highest percentage inhibition were selected as candidates for *in vitro* germination and *in vivo* studies.

In vitro Germination Assay

Cordana sp. and *Nodulisporium* sp., the two endophytes significantly reduced the growth of *C. musae*, were selected for an *in vitro* germination assay. Fungal suspensions of *C. musae* were prepared from 9 to 13 day old cultures. PDA plates with growing fungi were flooded with sterilized water and surface gently rubbed with a sterile grass rod. The concentration of conidia mL^{-1} was determined using a haemacytometer and adjusted to 1×10^{6} mL^{-1}. Fungal suspensions of two endophytic strains were also carry out in the same method and same concentration of test pathogen.

Five replicates of 1 mL of conidial suspension of each endophytes and pathogen were incubated at room temperature on microscope slides in humidified Petri dishes for up to 12 h. The percentage of spore germination was determined hourly. Conidial suspensions of *C. musae* incubated in distilled water were served as a control.

***In vivo* Screening of Antagonists**

The selected endophytes were chosen as candidates for the *in vivo* screening test. They were incubated on PDA for one week at room temperature. Conidia of *Colletotrichum musae* were gently scraped from the surface of one PDA plate into 25 mL sterile distilled water to prepare a conidial suspension. The suspension was adjusted to 10^6 conidia per milliliter. Ten fruits per replicate per treatment were tested. Banana fruits were soaked in the endophyte suspensions at room temperature for 30 min. The control treatment was soaked in distilled water. Banana fruits were sprayed with 5 mL of the tested fungal suspension. The treatments were maintained in plastic boxes (10×12×10 cm) with regularly moistened tissue papers and incubated at room temperature. The treated banana fruits were incubated for 1-2 weeks at room temperature. The effectiveness of endophytes was determined as percentage of disease reduction using the disease index (Ohata *et al*., 1995) with slight modifications. Disease severity was based on the percentage of fruit surface covered by lesion.

RESULTS AND DISCUSSION

Antagonistic Test

Out of 723 isolates of endophytic fungi isolated from wild banana (*Musa acuminata*), *Cordana* sp. (strain KPP4-3) and *Nodulisporium* sp. (JWL1-8) were the most effective fungi in reducing the radial mycelium growth of *Colletotrichum musae*. *Cordana* sp. (KPP4-3) and *Nodulisporium* sp. (JWL1-8) were paired in culture. The inhibition produced by *Cordana* sp. occurs through its fast competitive growth, while the inhibition due to *Nodulisporium* sp. is produced by antibiotic production. The inhibition reached a high percentage (90% of *C. musae* inhibition) (Fig. 1).

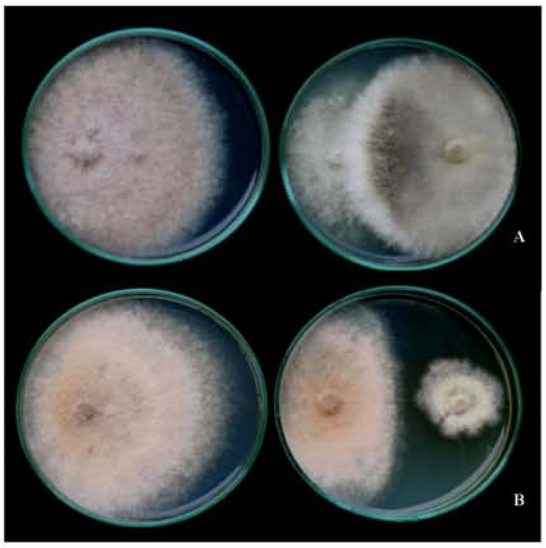

Fig. 1: Endophytic strains showing inhibition zone against growth of *Colletotrichum musae* on PDA; A = *Cordana* sp. (strain KPP4-3) and B = *Nodulisporium* sp. (JWL1-8). Control plates on left. *C. musae* inoculated on left-hand side of plates, endopohyte test strain on right-hand side

The dual culture method indicates the ability of certain endophytes to inhibit the growth of pathogens and has been shown previously (Dennis and Webster, 1971; Lee and Hanlin, 1999; Krauss *et al*., 2001; Georgakopoulos *et al*., 2002; Larena *et al*., 2002; Park *et al*., 2002).

In vitro Germinating Assay

Despite the normal spore germination of *Colletotrichum musae* was 91%, the reduction in its germination was only 0.63 and 1.88% when germinated in conidial suspension of *Cordana* sp. and *Nodulisporium* sp., respectively (Table 1). The germ tubes of *C. musae* were short and coiled when incubated in conidial suspension of endophytes (Fig. 2). Dennis and Webster (1971) observed that the aerial mycelium of pathogens were both shorter and smaller when inhibited by other fungi. The morphological changes included changes in form, size and structure of the hyphae and also changes in the direction of growth and even cessation of growth, the segments of hyphae became shorter.

In vivo Screening for Antifungal Activity

This assay showed that disease severity was significantly reduced in banana fruits treated with endophytes compared to the untreated ones (Table 2). This research highlights the possibility of using endophytic fungi as biological control agents for anthracnose disease of banana. It was reported that

Table 1: Germination percentage of *Colletotrichum musae* conidia

Treatments	Spore germination[1]	Germination[2] (%)
Colletotrichum musae (Control)	182	91.00
C. musae × *Cordana* sp. (KPP4-3)	2	0.63*
C. musae × *Nodulisporium* sp. (JWL1-8)	6	1.88*

[1]: at 24 h [2]: The one-way classification, ?

Table 2: Severity of anthracnose disease caused by *Colletotrichum musae* on banana

Treatments	Disease severity (%) 7th day
Colletotrichum musae (Control)	84[a]
C. musae × *Cordana* sp. (KPP4-3)	53[b]
C. musae × *Nodulisporium* sp. (JWL1-8)	33[c]

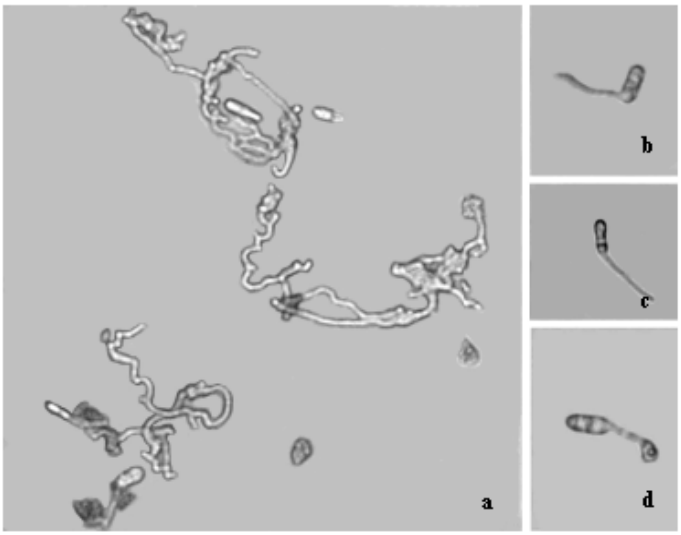

Fig. 2: Germ tube of *Colletotrichum musae* after 24 h incubation in conidial suspension of the endophytes; *Cordana* sp. and *Nodulisporium* sp. a = Control; b-d = conidia treated with endophytes

Cordana musae can cause large pale brown leaf spots on banana (Jeger *et al*., 1995; Photita *et al*., 2004). *Nodulisporium* sp. an endophyte from *Bontia daphnoides* can produce nodulisporic acids, that exhibit potent anti-insect properties against the larvae of the blowfly (Demain, 2000), endophytes from *Juniperus cedre* can produce compounds exhibit herbicidal antifungal and/or antibacterial activities (Dai *et al*., 2006). *Nodulisporium* sp. (JWL1-8) is good endophyte for further antifungal producing strain. There are reports showing that endophytes can produce bioactive compounds e.g., *Acremonium* sp., an endophyte of European yew produces a leucinostatin (Strobel *et al*., 1997), endophytes of *Spondias nimbin* can produce secondary metabolites for biological control (Rodrigues *et al*., 2000) and *Peslalotiopsis* sp. and *Monochaetia* sp. isolated from rain forest plants can produces ambuic acid (Li *et al*., 2001). Moreover, endophytes may be beneficial to the host because of their competition with, or chemical inhibition of pathogens or by activating host defense mechanisms (Brown *et al*., 2003). It has been proposed that fungal endophyes could be potential biological control agents, particularly for control of latent pathogens (Petrini, 1993). They are also thought to induce resistance against disease through their ability to alter the alleochemical defenses of a plant (Clay, 1991). Antagonists produce toxic metabolites near the infection site and thereby inhibit growth of the pathogens by antibiosis (Singh *et al*., 2003). Endophyte cultures have been tested for antagonism against pathogenic fungi in leaf and stem *in vitro* assays with significant inhibition recorded for many isolates (Brown *et al*., 2003). Two isolates of *Phialocephala fortinii*, endophytes from root of eggplant and Chinese cabbage almost completely suppressed the effects of post-inoculated virulent strains of *Verticillium dahliae* and *V. longisporum* causing Verticillium yellows in Chinese cabbage (Narisawa *et al*., 2003).

The above example illustrates the potential for using endophytes in the biological control of certain plant pathogens. Further research is needed to determine which fractions of the extract are responsible for the inhibition of pathogen growth caused by the endophytic strains. The structures of the metabolites then needed to be elucidate, as they may represent novel therapeutic agents.

ACKNOWLEDGMENTS

This study was supported by The Thailand Research Fund (MRG47800005). We are thanked Dr. Boonsom Bussaban and Assoc. Prof. Dr. Manus Tityavan for good advice in writing the manuscript. Miss Pranorm Khrueawan is thanked for photographic technique. Department of Agricultural Extension is thanked for providing tissue culture banana plant.

REFERENCES

Anonymous, 1998. Seminar and Exhibition of Banana: 15-17 January 1998 at Museum and Culture of Agriculture, Kasetsart University.

Brown, K.B., G.I. Johnson and D.I. Guest, 2003. Interactions between the endophytic fungi of durian (*Durio zibethinus*) and *Phytophthora palmivora*. In: Proceedings of the 8th International Congress of Plant Pathology, Christchurch, New Zealand, pp: 41.

Clay, K., 1991. Endophytes as Antagonists of Plant Pests. In: Microbial Ecology of Leaves, Andrews, J.H. and S.S. Hirano (Eds.). Berlin, Springer-Verlag, pp: 331-357.

Costa, D.M. and H.R.U.T. Erabadupitiya, 2005. An integrated method to control postharvest diseases of banana using a member of the *Burkholderia cepacia* complex. Posthar. Biol. Tec., 36: 31-39.

Dai, J., K. Krohn, U. Flörke, S. Draeger, B. Schulz, A.K. Szikszai, S. Antus, T. Kurtán and T. Ree, 2006. Metabolites from the endophytuc fungus *Nodulisporium* sp. from *Juniperus cedre*. Eur. J. Org. Chem., 15: 3498-3506.

Demain, A.L., 2000. Microbial Natural Products: A Part with a Future. In: Biodiversity: New Leads for Pharmaceutical and Agrochemical Industrics, Wringley, S.K., M.A., Hayes, R., Thomas, E.J.T. Chrystal and N. Nicholson (Eds.). The Royal Society of Chemistry, Cambridge, United Kingdom, pp: 3-6.

Dennis, C. and J. Webster, 1971. Antagonistic properties of species-groups of *Trichoderma* I. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc., 57: 25-32.

Freeman, S., 2000. Genetic Diversity and Host Specificity of *Colletotrichum* Species on Various Fruits. In: *Colletotrichum*: Host Specificity, Pathology and Host-Pathogen Interaction, Prusky, D., S. Freeman and M.B. Dickman (Eds.). APS Press, Minnesota, USA., pp: 1-20.

Georgakopoulos, D.E., P. Fiddaman, C. Leifert and W.E. Malathrakis, 2002. Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimum* with bacterial and fungal antagonists. J. Applied Microbiol., 92: 1078-1086.

Jeger, M.J., S. Eden-Green, A. Johanson, J.M. Waller and A.E. Brown, 1995. Banana Diseases. In: Banana and Plantains, Gowen, S. (Ed.). Chapman and Hall, London, UK., pp: 317-381.

Jones, D.R., 2000. Diseases of Banana, ABACÁ and Enset. Commonwealth Mycological Institute, Kew, UK.

Krauss, U., P. Matthews, R. Bidwell, M. Hocart and F. Anthony, 2001. Strain discrimination by fungal antagonists of *Colletotrichum musae*: Implications for biocontrol of crown rot of banana. Mycol. Res., 105: 67-76.

Larena, I., P. Melgarejo and A. DeCal, 2002. Production, survival and evaluation of Solid-substrate inocula of *Penicillium oxalicum*, a biocontrol agent against Fusarium wilt of tomato. Phytopathology, 92: 863-869.

Lee, S. and R.T. Hanlin, 1999. Phylogenetic relationships of *Chaetomiun* and similar genera based on ribosomal DNA sequences. Mycologia, 91: 434-442.

Li, J.Y., J.K. Harper, D.M. Grant, B.O. Tombe, B. Bashyal, W.M. Hess and G.A. Strobel, 2001. Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* sp. and *Monochaetia* sp. Phytochemistry, 56: 463-468.

Monaghan, R.L., J.D. Polishook, V.J. Pecore, G.F. Bills, M. Nallin-Omstead and S.L. Streicher, 1995. Discovery of novel secondary metabolites from fungi-is it really a random walk through a random forest? Can. J. Bot., 73 (Suppl.): S925-S931.

Narisawa, K., R.S. Currah and T. Hashiba, 2003. The root endophytic fungus *Phialocephala fortinii* suppresses *Verticillium* yellows in Chinese cabbage. Proceedings of the 8th International Congress of Plant Pathology, Christchurch, New Zealand, pp: 39.

Ohata, K., T. Araki, A. Kiso and H. Takahashi, 1995. Methods for isolation, cultivation, inoculation of plant pathogens. Japan Plant Protection Association, Tokyo (In Japanese).

Park, J.Y., G. Okada, M. Takahashi and H. Oyaizu, 2002. Screening of fungal antagonists against yellows of cabbage caused by *Fusarium oxysporum* f. sp. *conglutinans*. Mycoscience, 43: 447-451.

Petrini, O., T.N. Sieber, L. Toti and O. Vivet, 1992. Ecology, metabolite production and substrate utilisation in endophytic fungi. Nat. Toxins, 1: 185-196.

Petrini, O., 1993. Endophytes of *Pteridium* sp. some considerations for biological control. Sydowia, 45: 330-338.

Photita, W., S. Lumyong, P. Lumyong and K.D. Hyde, 2001. Endophytic fungi of wild banana (*Musa acuninata*) at Doi Suthep Pui national Park. Mycol. Res., 105: 1508-1513.

Photita, W., S. Lumyong, P. Lumyong, E.H.C. McKenzie and K.D. Hyde, 2004. Are some fungi isolated as endophytes of *Musa acuminata* latent pathogens? Fungal Divers., 16: 131-140.

Rodrigues, K.F., M. Hesse and C. Werner, 2000. Antimicrobial activities of secondary metabolites produced by endophytic fungi from *Spondias mombin*. J. Basic Microbiol., 40: 261-267.

Singh, D.V., R. Aggarwal and K.D. Srivastava, 2003. The antagonism by *Chaetomium globosum* against spot blotch of wheat caused by *Drechshlera sorokiniana*. In: Proceedings of the 8th International Congress of Plant Pathology, Christchurch, New Zealand, pp: 32.

Strobel, G.A., R. Torczynski and A. Bollon, 1997. *Acremonium* sp. a leucinostatin A producing endophyte of European yew (*Taxus baccata*). Plant Sci., 128: 97-108.

Strobel, G.A., 2002. Gifts from the rainforest. Can. J. Phytopathol., 24: 14-20.

Sutton, B.C., 1980. The Coelomycetes. Commonwealth Mycological Institute, Kew, UK.

Sutton, B.C., 1992. The Genus *Glomerella* and Its Anamorph *Colletotrichum*. In: *Colletotrichum*: Biology, Pathology and Control, Bailey, J.A. and M.J. Jeger (Eds.). CAB International, Wallingford, UK., pp: 1-26.

Wade, N.C., E.E. Kavanagh and M. Sepiah, 1993. Effects of modified atmosphere storage on banana postharvest diseases and the control of bunch main-stalk rot. Post. Biol. Tech., 3: 143-154.

Waller, J.M., 1992. *Colletotrichum* Disease of Perennial and Other Cash Crops. In: *Colletotrichum*: Biology, Pathology and Control, Bailey, J.A. and M.J. Jeger (Eds.). CAB International, Wallingford, UK., pp: 167-186.