Potential of the Volatile-Producing Fungus *Nodulisporium* sp. CF016 for the Control of Postharvest Diseases of Apple

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***In vitro* and *in vivo* mycofumigation effects of the volatile-producing fungus *Nodulisporium* sp. CF016 isolated from stem of *Cinnamomum loureirii* and the role of its volatile compounds were investigated against phytopathogenic fungi. The volatile compounds produced by *Nodulisporium* sp. CF016 inhibited and killed a wide range of plant and storage pathogens including to *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora capsici*, *Sclerotinia sclerotiorum*, *Colletotrichum coccodes*, *Magnaporthe oryzae*, *Alternaria panax*, *Botrytis cinerea* and *Penicillium expansum*. Mycofumigation with wheat bran-rice hull cultures of *Nodulisporium* sp. CF016 showed *in vivo* antifungal activity against gray mold caused by *B. cinerea* and blue mold caused by *P. expansum* of apple. The most abundant volatile compound produced by *Nodulisporium* sp. CF016 was β -elemene followed by 1-methyl-1,4-cyclohexadiene, β -selinene and α -selinene. *Nodulisporium* sp. CF016 could be an attractive mycofumigant in controlling postharvest diseases of various fruits including apple.**

Keywords : biological control agent, endophytic fungus, mycofumigation, *Nodulisporium* sp., postharvest disease

Postharvest losses can exceed 50% of the harvested fruits in developing countries (Eckert and Ogawa, 1988). Postharvest losses during storage of apples are mainly caused by fungal pathogens. More than 90 species of fungi have been reported to cause decay of apples during storage (Jones and Aldwinckle, 1991). Two of the important postharvest diseases of apple worldwide are blue mold and gray mold, caused by *Penicillium expansum* and *Botrytis cinerea*, respectively.

Control of postharvest diseases still relies on the synthetic fungicides. However, several fungicides are no longer used for postharvest diseases due to toxicological risks and the appearance of fungicide resistant isolates. These reasons have increased the need for alternative control strategies.

Biological control with microorganisms has emerged as a promising alternative with reduced environmental impact (Wilson and Wisniewski, 1994). The use of yeast or bacteria to control postharvest diseases of fruits has been reported (Janisiewicz and Korsten, 2002). The several commercial products are currently registered for the control of postharvest diseases in Europe and USA. The biofungicide such as Bio-Save 101p (Jet Harvest Solutions, Longwood, FL, USA) which contain *Pseudomonas syringae* as the active ingredient, respectively, have been registered in USA for the control of postharvest diseases on apples (Zhou et al., 2001). A commercial formulation of *Candida sake* was recently developed and registered for use on pome fruit in Spain under the name "Candifruit" (Sipcam. Inagra, Spain) (Droby et al., 2009).

Several fungi producing volatile antibiotics have been previously reported (Lee et al., 2009; Strobel et al., 2001; Stinson et al., 2003a and 2003b). *Muscodor albus* isolated from limbs of *Cinnamomum zeylanicum* is a mycofumigant that inhibits and kills fungi and bacteria in enclosed areas by producing a mixture of volatile compounds (Strobel et al., 2001). The natural mycofumigant (AgraQuest, Inc., Davis, CA, USA) by *M. albus* strain QST 20799 has been registered in USA for the control of plant pathogens. In addition, a mixture of volatile compounds of *M. albus* and *M. roseus* effectively inhibited soilborne diseases caused by *Pythium ultimum*, *Aphanomyces cochlioides*, *Rhizoctonia solani* and *Verticillium dahlia* (Stinson et al., 2003a). *Gliocladium* sp. effectively killed *P. ultimum* and *V. dahlia* by producing a mixture of volatile compounds (Stinson et al., 2003b). The most abundant antifungal volatiles of *M. albus* and *Gliocladium* sp. were 1-butanol and 3-methylacetate, and annulene, respectively (Strobel et al., 2001; Stinson et al., 2003b). *Oxyporus latemarginatus* EF069 isolated from healthy tissue of red pepper showed fumigation activity against postharvest apple decay and *Rhizoctonia* root rot on moth orchid caused by *B. cinerea* and *R. solani*, respectively, through production of 5-Pentyl-2-furaldehyde (Lee et al., 2009). The antifungal volatile-producing fungi are expected to be useful as potential sources of new bioactive compounds and as biocontrol agents by mycofumigation against postharvest diseases.

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In this study, the antifungal volatile-producing endophytic fungus *Nodulisporium* sp. CF016 was selected as a potential biological agent from various fungi isolated from leaf and stem tissues of Lauraceae trees. The mycofumigation effects of *Nodulisporium* sp. CF016 were examined against plant pathogens *in vitro* and *in vivo*. The volatile compounds were identified by gas chromatograph-mass spectrometer (GC-MS).

Materials and Methods

Isolation of endophytic fungi. Samples of leaf and stem tissues from Lauraceae trees were collected from regions of Jeju Island, Korea. The samples were washed in running tap water and cut into small pieces of approximately 4 mm². Tissue pieces were washed in 0.1% Tween 20 for 10 s, surface-sterilized in 2% sodium hypochlorite for 10 s, and then washed in sterile distilled water. The surface-sterilized tissue pieces were placed on cornmeal malt extract agar medium [17.0 g cornmeal agar (Becton, Dickinson and Company, MA, USA), 20.0 g malt extract agar (Becton, Dickinson and Company), and 2.0 g yeast extract (Becton, Dickinson and Company) per liter, to which 50 mg/l chloramphenicol was added (Sigma Chemical Co., St. Louis, USA)] (Suto et al., 2002) and incubated at 25 °C. The fungi growing out of the tissue pieces were transferred to potato dextrose agar (PDA; Becton, Dickinson and Company). A total of 38 endophytic fungi were isolated from Lauraceae trees (Table 1). These isolates were stored in sterile distilled water at 4 °C and 20% glycerol at –80 °C.

Screening for fumigation activity of endophytic fungi.

All of the endophytic fungi isolated from Lauraceae trees were tested by mycofumigation assay (Strobel et al., 2001; Lee et al., 2009) to select potential biological control agents for postharvest diseases. For fumigation assay for postharvest apple decay caused by *B. cinerea*, PDA medium was poured into half-plate separated Petri plates (90×15 mm; SPL Life Science, Korea). Each endophytic fungus was inoculated on one side of the plate and incubated for 3 days at 25 °C. Subsequently, an agar plug of *B. cinerea* was placed on other side of plate and the plates were sealed with Parafilm. Control experiments were conducted in which *B. cinerea* was subjected to the same procedures minus endophytic fungi on the test side of the petri plates. The fumigation assay plates were incubated for 3 days at 25 °C. Then the radii of fungal mycelia were measured and compared with that of control. Each experiment was performed in three replications.

Identification of CF016. Isolate CF016 obtained from stem tissue of *Cinnamomum loureirii* Ness was identified

Table 1. *In vitro* fumigation activity of endophytic fungi isolated from Lauraceae trees against *Botrytis cinerea*

Fungus isolate	Plant origin	Part of plant	Fumigation activity ^a
CF001	<i>Cinnamomum loureirii</i>	Leaf	–
CF002	<i>Litsea japonica</i>	Stem	–
CF003	<i>Litsea japonica</i>	Stem	–
CF004	<i>Machilus thunbergii</i>	Stem	–
CF005	<i>Machilus thunbergii</i>	Stem	–
CF006	<i>Neolitsea sericea</i>	Stem	–
CF007	<i>Neolitsea sericea</i>	Leaf	–
CF008	<i>Neolitsea sericea</i>	Stem	–
CF009	<i>Neolitsea sericea</i>	Stem	–
CF010	<i>Cinnamomum japonicum</i>	Stem	–
CF011	<i>Cinnamomum japonicum</i>	Stem	–
CF012	<i>Cinnamomum japonicum</i>	Stem	–
CF013	<i>Neolitsea aciculata</i>	Leaf	–
CF014	<i>Cinnamomum loureirii</i>	Leaf	–
CF015	<i>Litsea japonica</i>	Stem	–
CF016	<i>Cinnamomum loureirii</i>	Stem	+
CF017	<i>Cinnamomum camphora</i>	Stem	–
CF018	<i>Neolitsea sericea</i>	Stem	–
CF019	<i>Cinnamomum japonicum</i>	Leaf	–
CF020	<i>Cinnamomum japonicum</i>	Leaf	–
CF021	<i>Cinnamomum camphora</i>	Stem	–
CF022	<i>Cinnamomum camphora</i>	Stem	–
CF023	<i>Neolitsea aciculata</i>	Leaf	–
CF024	<i>Cinnamomum japonicum</i>	Leaf	–
CF025	<i>Cinnamomum camphora</i>	Stem	–
CF026	<i>Litsea japonica</i>	Leaf	–
CF027	<i>Neolitsea aciculata</i>	Stem	–
CF028	<i>Machilus thunbergii</i>	Leaf	–
CF029	<i>Neolitsea sericea</i>	Stem	–
CF030	<i>Neolitsea aciculata</i>	Stem	–
CF031	<i>Neolitsea sericea</i>	Leaf	–
CF032	<i>Litsea japonica</i>	Stem	–
CF033	<i>Cinnamomum loureirii</i>	Leaf	–
CF034	<i>Neolitsea aciculata</i>	Stem	–
CF035	<i>Litsea japonica</i>	Stem	–
CF036	<i>Cinnamomum camphora</i>	Leaf	–
CF037	<i>Cinnamomum camphora</i>	Leaf	–
CF038	<i>Cinnamomum camphora</i>	Leaf	–

^a Fumigation activity was determined by inhibition of mycelial growth of *B. cinerea*; +, inhibition activity; –, no inhibition

based on morphological characteristics and sequence analyses of internal transcribed spacer (ITS) regions of rRNA genes. Colony and morphological characteristics were examined on culture grown in darkness at 25 °C for 5 days on PDA.

The isolation of genomic DNA and PCR amplification of

the ITS regions of rRNA gene were performed using previously described methods (Park et al., 2005). The PCR fragments were purified using Wizard PCR prep Kits (Promega, WI, USA) and directly sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems, CA, USA), according to the manufacturer's instruction. The same primer sets for the PCR amplification were used to sequence both DNA strands. The gel electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

The sequences were compared with ITS regions of rRNA gene sequences available in the Genbank database by the BLAST search. Sequences generated from materials in this study and retrieved from GenBank were initially aligned using the program CLUSTAL X (Thompson et al., 1997), and then the alignment was refined manually using the PHYDIT program version 3.0 (Chun 1995; available at <http://plaza.snu.ac.kr/jchun/phydit>). Ambiguously aligned regions were excluded from the subsequent analyses. A neighbor-joining tree was reconstructed with Kimura's 2-parameter distance model (Kimura, 1980) using the PHYLIP 3.57c package (Felsenstein, 1985). The bootstrap analysis using 1,000 replications was performed to assess the relative stability of the branches.

In vitro assay for fumigation activity of CF016. For fumigation assay for plant pathogen, PDA medium was poured into half-plate separated Petri plates. A total of 10 plant pathogens were used in this study; *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora capsici*, *Sclerotinia sclerotiorum*, *Colletotrichum coccodes*, *Magnaporthe oryzae*, *Alternaria panax*, *Botrytis cinerea* and *Penicillium expansum*. These plant pathogens were isolated from infected plant materials in our laboratory, except *P. expansum*, which was kindly provided by Dr. S.H. Yu, Chungnam National University. CF016 was inoculated on one side of half-plate separated Petri plates and incubated for 5 days at 25°C. An agar plug of a plant pathogen was placed on the other side of the plate and the plates were sealed with Parafilm. Control experiments were conducted in which *B. cinerea* was subjected to the same procedures minus endophytic fungi on the test side of the petri plates. The fumigation assay plates were incubated for 3 days at 25°C and then the radii of fungal mycelia were measured and compared with that of control. Each experiment was performed in three replications. Agar plugs of a plant pathogen that did not grow were transferred to fresh PDA to assess their viability. The experiments were repeated two times.

In vivo assay for fumigation activity of CF016. Each fruit was wounded with 8 mm diameter cork borer, and then 8

mm diameter mycelial plugs of *P. expansum* and *B. cinerea* were inoculated. Four-inoculated fruits were arranged in a moistened 6.5 l container as a replicate of each treatment. To produce wheat bran-rice hull culture of *Nodulisporium* sp. CF016, 70g of wheat bran and 10g of rice hull were placed in Erlenmeyer flasks (1 l) with 100 ml of distilled water and autoclaved twice for 20 min at 121 °C twice with a 24 h interval. The solid medium was inoculated with mycelia plugs from 5-day-old PDA plate of the fungus. One week-old culture of *Nodulisporium* sp. CF016 on wheat bran-rice hull was used in mycofumigation assay. Wheat bran-rice hull culture of *Nodulisporium* sp. CF016 was applied at 12.5 g, 25.0 g and 50.0 g in a plastic cup for each container immediately after inoculation and the containers were closed. The containers were sealed and stored at 20°C (*B. cinerea*) and 25°C (*P. expansum*) in dark. After 7 days, the radii of rotted symptoms were measured from edge of the agar inoculum plug on the fruit surface. The experiment was conducted twice with two replicates per treatment and the four values for each treatment and control percentages were calculated.

Analysis of volatile compounds produced by CF016.

The gas in the air space above cultures of CF016 grown on 20 ml of PDA in 100 ml culture glass bottle (61×77 mm; KisanBiotech, Seoul, Korea) was analyzed by GC-MS (Shimadzu GC-MS QP5050, Shimadzu co., Kyoto, Japan). A solid phase micro-extraction syringe (Supelco Inc., PA, USA) was used to trap fungal volatiles. The syringe tip was inserted through a hole in the screw-caps of CF016 culture bottles and exposed to the gas phase for 1 h. The syringe was then inserted into the splitless injection port of the GC-MS. A capillary column SPB-5 (30 m×0.25 mm i.d., 0.25 µm film thickness; Supelco Inc.) was used. The initial temperature of the column was held at 30°C for 2 min, programmed at 5°C min⁻¹ to 220°C. The injection port and interface were set at 240°C and 200°C, respectively. Helium was the carrier gas at a flow rate of 2.2 ml min⁻¹.

Statistical analysis. Analysis of variance was performed on the PROC GLM procedure (SAS institute, Cary, NC). If $P > F$ was less than 0.01, means were separated with Duncan's multiple range test at the $P = 0.05$ level.

Results

Isolation and identification of endophytic fungi producing antifungal volatile compounds. Among the 38 endophytic fungi isolated from Lauraceae trees, one fungus, designated CF016, which was isolated from stem of *Cinnamomum loureirii* showed antifungal activity against *B. cinerea* by fumigation (Table 1). The colony of isolate

Fig. 1. Neighbor-joining tree based on ITS sequences showing relationships among endophytic *Nodulisporium* sp. CF016 and other xylariaceous fungi. The values above each branch indicate the percentage levels of bootstrap support (> 50%) for the branch point based on 1000 resamplings. The bar represents 0.1 substitutions per nucleotide position.

CF016 rapidly grew on PDA and was pale smoke gray. The reverse was pale brown at first, turning to blackish brown with age. Conidia were smooth, rounded at the apex, and ellipsoidal to obovoid.

Based on the sequence analyses of ITS regions of rRNA gene, *Nodulisporium* species were divided into two groups; one group produces nodulisporic acid and the other group does not. Isolate CF016 formed a monophyletic group with isolates of *Nodulisporium* sp. which does not produce nodulisporic acid (Fig. 1). Isolate CF016 shared a 99% sequence similarity with *Nodulisporium* sp. JP3665 isolated from woody plant tissue. Thus, based on these results, isolate CF016 was identified as *Nodulisporium* sp. CF016.

In vitro assay for fumigation activity of *Nodulisporium* sp. CF016. *Nodulisporium* sp. CF016 inhibited the growth of all of the plant pathogenic fungi tested in this study (Table 2). *R. solani*, *S. sclerotiorum*, *C. coccodes*, and *B. cinerea* were completely dead 3 days after exposure to *Nodulisporium* sp. CF016. Mycelial growth of *A. panax* and *P. expansum* was inhibited by 87% and 80% compared with the untreated control, respectively. *P. ultimum*, *F. oxy-*

Table 2. Inhibition of mycelial growth of various phytopathogenic fungi by volatile compounds produced by *Nodulisporium* sp. CF016.

Phytopathogen	Inhibition rate ($\pm$ SD) (%) ^a	Viability ^b
<i>Pythium ultimum</i>	32 ($\pm$ 7.1)	Fungistatic
<i>Rhizoctonia solani</i>	100 ($\pm$ 0.0)	Fungicidal
<i>Fusarium oxysporum</i>	46 ($\pm$ 10)	Fungistatic
<i>Phytophthora capsici</i>	54 ($\pm$ 11)	Fungistatic
<i>Sclerotinia sclerotiorum</i>	100 ($\pm$ 0.0)	Fungicidal
<i>Colletotrichum coccodes</i>	100 ($\pm$ 0.0)	Fungicidal
<i>Magnaporthe oryzae</i>	43 ($\pm$ 9.6)	Fungistatic
<i>Alternaria panax</i>	87 ($\pm$ 12)	Fungistatic
<i>Botrytis cinerea</i>	100 ($\pm$ 0.0)	Fungicidal
<i>Penicillium expansum</i>	80 ($\pm$ 2,9)	Fungistatic

^a The inhibition rate was calculated as percentage growth inhibition 3 days after exposure to volatile compounds of *Nodulisporium* sp. CF016 as compared to an untreated control. Each value represents the mean of two times with three replications ($\pm$ the standard deviation).

^b Viability of the test phytopathogenic fungi was determined 3 days after exposure to volatile compounds of *Nodulisporium* sp. CF016 and transfer to a fresh PDA plate.

sporum, *P. capsici*, and *M. oryzae* were relatively resistant to the volatile compounds produced by *Nodulisporium* sp. CF016.

In vivo assay for fumigation activity of *Nodulisporium* sp. CF016. The apple fruits inoculated with *B. cinerea* and *P. expansum* were fumigated with wheat bran-rice hull culture of *Nodulisporium* sp. CF016. The results clearly displayed that the volatile compounds produced by *Nodulisporium* sp. CF016 suppressed lesion development of gray mold and blue mold on apple fruits. The effect on controlling blue mold and gray mold on apple fruits depended on fumigant dose. The effect was dose-dependent; treatment with 50.0 g of the solid culture of *Nodulisporium* sp. CF016 gave the best control. Fumigation with CF016 suppressed gray mold lesion development by 35%, 68% and 88% at 12.5, 25.0 and 50.0 g for 6.5 container, respectively (Fig. 2). For blue mold, the control efficacies were 38%, 58% and 76% at 12.5, 25.0 and 50.0 g for 6.5 l container, respectively (Fig. 3).

Analysis of volatile compounds of CF016. *Nodulisporium* sp. CF016 was subjected to analysis of the head-space volatiles in a 5-day-old culture using GC-MS. The identification of volatile organic compounds has only been tentatively identified on the basis of the The National Institute of Standards and Technology (NIST) database information. The headspace analysis of *Nodulisporium* sp.

Fig. 2. *In vivo* antifungal activity of mycofumigation with *Nodulisporium* sp. CF016 against gray mold caused by *Botrytis cinerea*. Gray mold development (above) and control value (below) on apple as applied at dosages of 12.5 g, 25.0 g, 50.0 g, and 0 g of solid culture of *Nodulisporium* sp. 7 days after incubation at 20°C in dark. Control value (%) = $100 \times \{\text{rotted symptom radius of untreated fruit} - \text{rotted symptom radius of treated fruit}\} \div \text{rotted symptom radius of untreated fruit}$. The lower-case letters indicate values that are not significantly different ($P = 0.05$) to other values with the same letter in column, according to Duncan's multiple range test.

Fig. 3. *In vivo* antifungal activity of mycofumigation with *Nodulisporium* sp. CF016 against blue mold caused by *Penicillium expansum*. Blue mold development (above) and control value (below) on apple as applied at three dosages of 12.5 g, 25.0 g, 50.0 g, and 0 g of solid culture of *Nodulisporium* sp. 7 days after incubation at 25°C in dark. Control value (%) = $100 \times \{\text{rotted symptom radius of untreated fruits} - \text{rotted symptom radius of treated fruits}\} \div \text{rotted symptom radius of untreated fruits}$. The lower-case letters indicate values that are not significantly different ($P = 0.05$) to other values with the same letter in column, according to Duncan's multiple range test.

CF016 culture displayed four major volatile compounds and three minor compounds. The most abundant compound, based on the total area of the GC-MS analysis, was β -elemene (33.0%) followed by 1-methyl-1,4-cyclohexadiene (32.2%), β -selinene (14.5%) and α -selinene (9.6%) (Table 3). The four major components accounted for 89.3% of the total volatile compounds produced. 5,5-dimethyl-1,3-cyclopentadiene, 2-methyl-5,6-dihydro-2H-pyran, and cyclohexane were detected as minor compounds.

Discussion

The antifungal volatile-producing isolate CF016 obtained from stems of *C. loureiroi* was selected as potential biological control agent for postharvest disease of apple fruit. Based on the sequence analysis of ITS regions of rRNA

gene, CF016 was grouped with *Nodulisporium* sp. not producing nodulisporic acid. The sequence of ITS regions of rRNA gene of CF016 was 99% similar to *Nodulisporium* sp. JP3665 isolated from woody plant tissue. Thus, it is reasonable to identify the isolate CF016 as *Nodulisporium* sp.

The genus *Nodulisporium* Preuss was reported as an anamorph of at least 10 genera (e.g., *Hypoxylon* Bull, *Xylaria* Hill: Schrank, *Camillea* Fr., and *Biscogniauxia* Kuntze) of Xylariaceae (Whalley 1996). These teleomorph genera are distributed worldwide and known to be plant pathogen, wood decay or endophytic fungi. Endophytic *Nodulisporium* sp. was found to produce nodulisporic acid, an indole terpene, which exhibited insecticidal activity for first instars of *Lucilia sericata* (Ostlind et al., 1997). Recently, seven new compounds and ten known compounds

Table 3. GC-MS analysis of the volatile compounds produced by *Nodulisporium* sp. CF016.

Retention time (min)	Total area (%)	Possible compound
4.5	1.8	5,5-Dimethyl-1,3-cyclopentadiene
5.9	32.2	1-Methyl-1,4-cyclohexadiene
14.2	1.1	2-Methyl-5,6-dihydro-2H-pyran
22.6	2.7	Cyclohexane
22.9	33.0	β -Elemene
24.6	14.5	β -Selinene
24.8	9.6	α -Selinene

from a culture extract of endophytic *Nodulisporium* sp. isolated from *Juniperus cedre* exhibited herbicidal, anti-fungal and/or antibacterial activity (Dai et al., 2006). In particularly, 1-(2,6-dihydroxyphenyl) butan-1-one strongly inhibited *Bacillus megaterium*, *Septoria tritici* and *Chlorella fusca* in an agar diffusion test.

While endophytic fungi have been known to produce volatile compounds, the production of volatile antibiotics produced by endophytic fungi has been little investigated. *Muscodor albus* is known to make volatile compounds having the power to inhibit and kill other fungi and bacteria (Strobel et al., 2001). The most effective class of antibiotic compounds was esters, of which isoamyl acetate was the most biologically active. *M. albus* has been developed by AgraQuest, Inc. (Davis, CA) for control of plant pathogens, insects and nematodes. Recently, AgraQuest, Inc. received Environmental Protection Agency (EPA) approval of natural fumigant based on *M. albus* strain QST 20799. In addition, *Gliocladium* sp. and *O. latemarginatus* EF069 were found to volatile antibiotics (Stinson et al., 2003; Lee et al., 2009). The primary volatile compound produced by *Gliocladium* sp. is annulene, a compound previously not discovered as natural product. *Gliocladium* sp. effectively killed *P. ultimum* and *V. dahlia* by producing a mixture of volatile compounds (Stinson et al., 2003). *O. latemarginatus* EF069 effectively inhibited *in vitro* and *in vivo* the growth of fungal plant pathogens by producing 5-pentyl-2-furaldehyde (Lee et al., 2009).

In vitro assay for fumigation activity, inhibitory activity of the volatile compounds produced by *Nodulisporium* sp. CF016 was different according to plant pathogen. The volatile compounds killed *S. sclerotiorum*, *C. coccodes*, and *B. cinerea*, while other plant pathogens tested in this study were inhibited. Mycofumigation with *Nodulisporium* sp. CF016 was effective in controlling blue mold and gray mold of apples caused by *P. expansum* and *B. cinerea*, respectively.

Nodulisporium sp. CF016 growing on PDA was found to

produce a number of volatile compounds. *Nodulisporium* sp. CF016 growing on PDA produced more than seven volatile compounds including β -elemene, 1-methyl-1,4-cyclohexadiene, β -selinene and α -selinene in the headspace. β -Elemene is a volatile terpene found in botanicals such as celery, mint, and in many herbs used in traditional medicine. The extract containing β -elemene isolated from *Rhizoma zedoariae*, a type of ginger plant, has been reported to relieve pain, decrease the side effects of chemotherapy, and increase the quality of life in cancer patients. *In vitro* studies show possible anti-proliferative effects through cell-cycle arrest and induction of apoptosis (Li et al., 2005; Wang et al., 2005; Yao et al., 2008). Malele et al. (2003) reported that essential oil of *Hyptis suaveolens* containing β -elemene (10.4%) exhibited significant antimicrobial activity against *Mucor* sp. (Malele et al., 2003). β -Selinene was detected as a minor component in the essential oils from needles of *Pinus koraiensis*, aerial parts of *Artemisia absinthium* and aerial parts of *Hypericum heterophyllum* showing anti-fungal activities against various plant pathogens (Cakir et al., 2003; Hong et al., 2004; Kordali et al., 2005). α -Selinene was also found as a minor component in essential oils from aerial parts of *A. santonicum* (Kordali et al., 2005). 1-Methyl-1,4-cyclohexadiene as a volatile compound is structurally similar to benzene and toluene (Raines et al., 2004), but biological activity of 1-methyl-1,4-cyclohexadiene previously was not reported. The volatile compounds of *Nodulisporium* sp. CF016 are totally different from those of *M. albus* and *Gliocladium* sp. To our knowledge, this is the first report of a *Nodulisporium* isolate producing antifungal volatiles. All of the four major compounds produced by CF016 were first found in fungi.

In this study, *Nodulisporium* sp. CF016 killed or inhibited mycelial growth of various fungal plant pathogens and effectively suppressed the development of postharvest diseases of apple without direct contact with the apples to be treated. *Nodulisporium* sp. CF016 producing volatile antibiotics may be more useful for controlling postharvest disease during storage and shipment systems than current biological or chemical fungicides requiring spraying or drenching as application methods. *Nodulisporium* sp. CF016 has the potential to replace the use of chemical fungicides. Potential applications of CF016 against soil borne plant pathogen *in vivo* and in the field are currently being investigated.

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