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# Isolation of Antagonistic Fungi Associated with the Lichens Distributed in Southern Parts of Korea

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**Lichen-forming (LFF) or lichenicolous fungi (LCF) were isolated from the lichens collected at 'Backwoon' mountain area, 'Chiri' mountain area and 'Sorok' island in the southern regions of Korea and were screened for antagonistic efficacy against several phyto-pathogenic fungi. Symbiotic algae-free LFF and LCF were isolated by the following methods: I) discharged spores (ascospores), II) macerated thallus suspension and III) direct use of thallus fragments. Among 58 isolates obtained from 34 lichens, 8 isolates showed anti-fungal activity against *Rhizoctonia solani*. Antifungal activities of the strongest antagonistic isolate (LB9810) originated from the thallus of *Parmelia quercina* lichen were evaluated against 15 phyto-pathogenic fungi. When crude methanol extract of mycelia of the LB9810 isolate was employed at the rate of 0.5% (v/w), fungal growth of *Magnaporthe grisea* and *Rhizoctonia solani* was severely inhibited as much as approximately 60% compared to control. Growth of various food-borne bacterial pathogens was also severely inhibited by the same extract. The extract was successively partitioned with *n*-hexane, ethyl acetate and *n*-butanol. *n*-Hexane fraction displayed the strongest antifungal activities against *R. solani*. The LB9810 isolate was finally identified as *Fusarium equiseti* (Corda) Sacc., which has not been reported as LFF or LCF yet. Therefore, it is very likely that *F. equiseti* isolated in this study was originated from the contaminants associated with thallus fragments rather than from LFF or LCF.**

***Keywords*** **:** lichen, lichen-forming fungi, lichenicolous fungi, antifungal activity, *Parmelia quercina*, *Fusarium equiseti*.

Lichen is one of the most widely distributed eucaryotic organisms in the world. There are, to date, about 13,500 lichen species known, which accounts for approximately 20% of all the fungi described (Hwaksworth, 1988). All are the result of a symbiotic association between two unrelated organisms - a fungus and an alga (or cyanobacterium). When they are fully integrated, they form a new biological entity with very little resemblance to either one of their components. Lichen-forming fungi (LFF) have gained a notoriety for being difficult to isolate and cultivate in pure culture. Slow growth rates of the fungi, in particular, have presented a major obstacle to physiological investigations of axenic states. There are, in addition, about 1000 species of fungi described that occur obligately on lichens. These lichenicolous fungi (LCF) derive their nutrition from the lichen thallus either biotrophically, necrotrophically or saprotrophically (Hawksworth, 1982).

During the past 10 years, there has been a resurgence of interest in fungi and other microorganisms as sources of novel, pharmacologically active molecules (Monoghan and Tkacz, 1990; Porter and Fox, 1993). LFF produce a wide range of natural products among which approximately 350 secondary metabolites have been identified (Elix et al., 1984; Galun and Shomer-Ilan, 1988). Many of them are unique to lichens and appreciable numbers have been shown to have antimicrobial activity (Lawrey, 1986) or other biological activities of potential economic value (Nishitoba et al., 1987; Higuchi et al., 1993). The toxic properties of lichen products suggest that they might function in the chemical defence of lichen thalli against pathogens and grazing animals (Lawrey, 1986; 1989; Emmerich et al., 1993). LFF have been shown to retain in axenic cultures the capacity to biosynthesize secondary products found in the lichenized state (Leuckert et al., 1990; Culberson et al., 1992), although the metabolites produced in the greatest abundance might differ from those found in the lichen (Miyagawa et al., 1993; Hamada, 1993).

Although extensive researches performed on plants and microorganisms to screen very effective antimicrobial substances against plant pathogens in Korea for the past few years (Kim et al., 1996; Kim et al., 1998; Son et al., 1998), no attempt has been made on LFF and LCF. In this study, the fungal isolates obtained from the spore or thallus of various lichens distributed in southern parts of Korea were

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screened for antagonistic efficacy against several plant pathogenic fungi to select possible candidates for the development of less-harmful and safe protectants as agrochemicals.

## Materials and Methods

**Collection and storage of lichens.** Lichens were collected from various habitats in southern parts of Korea (Table 1). Specimens bearing reproductive structures were obtained whenever possible. Foliose and fruticose lichens were mainly collected owing to their relative easiness for collection and identification and the poor identification on crustose lichens in Korea. The samples were air-dried at the earliest opportunity and stored in Petri-dish at room temperature. Isolations were usually completed within 2 weeks after collection. Identifications were also carried out according to the classifications of Park (1990) and Yoshimura (1982) before the isolation to avoid multiple works of conspicuous common macrolichens.

**Isolation and culture of mycobionts.** LFF and LCF were isolated by the following method of Crittenden et al. (1995); (i) For

**Table 1.** Isolation of antagonistic fungi from the lichens distributed in southern parts of Korea

| Lichens | | | Type | Number of isolates | Location | Isolation method | Antifungal activity |
|---|---|---|---|---|---|---|---|
| Ascomycetes Lecanorales | Cladoniaceae | *Cladonia coniocrare* | fruticose | 1 | BW[a] | II[b] | -[c] |
| | | *Cladonia scarbriusula* | 〃 | 2 | BW, SR | II, III | ++ |
| | Collemataceae | *Leptogium cyanessens* | foliose | 1 | CR | II, III | - |
| | | *Leptogium moluccanum* | 〃 | 1 | SR | II, III | - |
| | | *Leptogium saturninum* | 〃 | 1 | SR | II, III | + |
| | Hypogymniaceae | *Menegazia terebrata* | 〃 | 2 | BW | II, III | - |
| | Lecanoraceae | *Lecanora allophana* | crustose | 3 | CR | I, III | ++ |
| | Parmeliaceae | *Cetrelia braunsiana* | foliose | 2 | BW, CR | II, III | - |
| | | *Canoparmelia aptata* | 〃 | 1 | BW | III | - |
| | | *Flavoparmelina sp.* | 〃 | 1 | BW | II, III | - |
| | | *Melanelia huei* | 〃 | 2 | CR | II, III | - |
| | | *Myelochroa leucotyliza* | 〃 | 1 | BW | I | - |
| | | *Parmelia marmariza* | 〃 | 1 | BW | I | - |
| | | *Parmelia quercina* | 〃 | 3 | BW | II, III | +++ |
| | | *Parmelia* sp. | 〃 | 3 | BW | II, III | + |
| | | *Parmotrema austrosinense* | 〃 | 4 | BW, CR, SR | II, III | - |
| | | *Parmotrema clavulifera* | 〃 | 1 | BW | II, III | - |
| | | *Parmotrema eciliatum* | 〃 | 1 | CR | II, III | - |
| | | *Parmotrema tinctorum* | 〃 | 2 | BW, CR, SR | III | - |
| | | *Punctelia rudecta* | 〃 | 2 | BW | II, III | + |
| | | *Xanthoparmelia mexicana* | 〃 | 1 | CR | III | - |
| | | *Xanthoparmelia subramigera* | 〃 | 1 | BW | I | - |
| | Peltigeraceae | *Peltigera collina* | 〃 | 1 | SR | III | - |
| | | *Peltigera* sp. | 〃 | 2 | CR | II, III | + |
| | Pertusariaceae | *Pertusaria* sp. | 〃 | 3 | CR | I, II | + |
| | Physciaceae | *Anaptychia parmulata* | 〃 | 1 | CR | II | - |
| | | *Heterodermia diademata* | 〃 | 1 | CR | III | - |
| | | *Heterodermia japonica* | 〃 | 2 | CR | II, III | - |
| | | *Heterodermia hypoleuca* | 〃 | 1 | BW | II | - |
| | | *Heterodermia leviformis* | 〃 | 2 | CR | II, III | - |
| | | *Heterodermia pseudospeciosa* | 〃 | 1 | BW | II | - |
| | | *Physcica* sp. | 〃 | 3 | SR | II, III | - |
| | Ramalinaceae | *Ramalina yasudae* | fruticose | 2 | BW, CR, SR | II, III | - |
| | Sterocaulaceae | *Sterocaulon nigrum* | 〃 | 2 | BW | II, III | - |

[a] Collection sites; BW: 'Backwoon' mountain area. CR: 'Chiri' mountain area, SR 'Sorok' island

[b] Details are described in Materials and Methods; simply, method I: use of discharged ascospores, method II: use of macerated thallus suspension, method III: direct use of thallus fragments.

[c] Degree of antifungal efficacy: -: not antagonistic. +: weakly antagonistic, ++: moderately antagonistic, +++: strongly antagonistic.

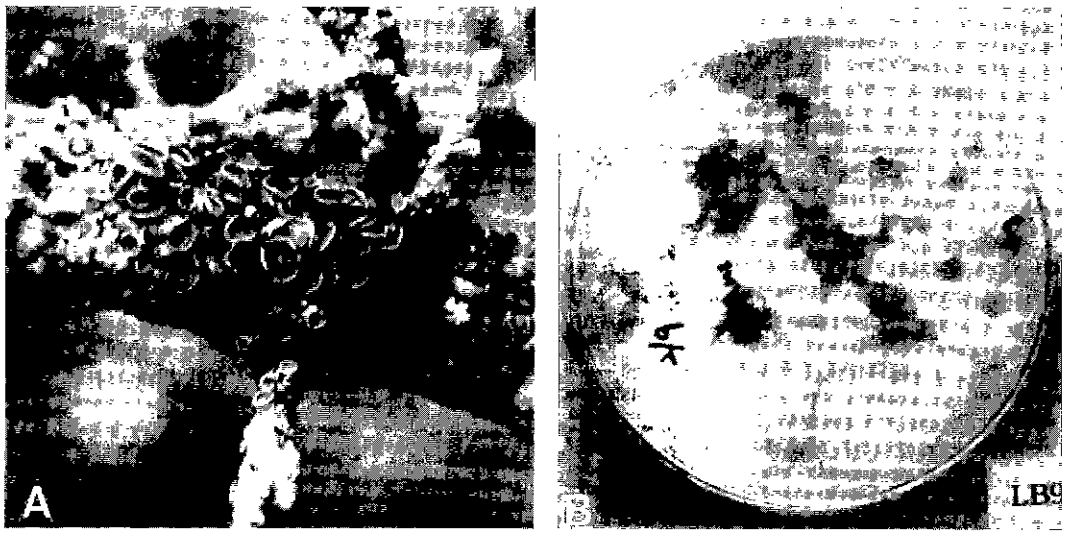


**Fig. 1.** Cup-shaped apothecia of foliose lichen, *Parmelia marmariza* (A) and several fungal isolates originated from the macerated thallus of *Parmotrema austrosinense* (B).

the majority of lichens bearing reproductive structures (fruiting bodies; Fig. 1(A)), the fungi were grown from discharged spores (ascospores). Thalli bearing fruiting bodies were washed for 1 h in a turbulent flow of tap water in order to remove as much surface contamination as possible. Either individual ascomata or fragments of thallus bearing many small ascomata were cut off and attached with petroleum jelly to the inside of Petri dish lids. Petri dishes containing agar were then inverted over the lids and the ascospores allowed to discharge upwards onto the agar medium. (ii) In the case of LCF, very small pieces of host bearing the reproductive structures of the fungus were washed as described above and then macerated in sterile water using a mortar and pestle. The resultant suspension was streaked onto agar medium. (iii) The fungal components of sterile lichens, or those in which isolation from spores had not proved successfully, were isolated from thallus fragments by the method of Yamamoto et al. (1985).

Cultures were incubated at 20°C in the dark and examined periodically during 20-day periods. Germinating single spore or groups of spores and thallus fragments that remained free of contamination were transferred to fresh medium. Mycobionts had produced a compact mycelium 5-10 mm in diameter 1 or 2 months after incubation and subcultured onto fresh medium for long-term storage. The culture medium of Crittenden et al. (1995) was routinely used for isolation and growth of LFF.

**Selection for antagonistic fungal isolates obtained from lichens.** *Rhizoctonia solani* (anastomosis group III) maintained at Department of Environmental Horticulture, the University of Seoul was used to select antagonistic isolates obtained from lichens. *R. solani* was grown on potato dextrose agar (Difco, USA) for about 5 days and then used to examine the antifungal activities of the isolates. When the fungal isolates obtained from lichens grew approximately 2 cm in diameter at 20°C on PDA, a mycelial disc (5 mm in diameter) of the isolates was removed from the margin of actively growing mycelial mat and transferred to the center of fresh PDA. The transferred isolates were incubated at 20°C until the diameter of mycelial mat reached to about 1.5 cm. Two 5-mm diameter agar plugs of the test fungus were placed on both sides of 3 cm apart from the center of the plate growing the isolate. Inhibition of mycelial growth of the test fungus was rated 5 days later. The antagonistic isolates were selected and stored at 4°C. Because the LB9810 isolate originated from the thallus of *Parmelia quercina* lichen displayed the strongest antifungal activity against the test fungus, they were selected for the further examination.

**Evaluation of antimicrobial activities of the LB9810 isolate.** A mycelial disc (1 cm in diameter) cut from the margin of actively growing cultures of the LB9810 isolate was inoculated into an 1-L Erlenmeyer flask containing 500 ml potato dextrose broth (Difco, USA). After 10-day incubation at 20°C and 150 rpm, liquid culture was filtered through Whatman No. 1 paper. Mycelial mats were harvested and then, dried at 60°C for 24 h in a dry oven. The dried mycelial mats were powdered with a mortar and pestle. About 0.5 g of the powdered sample was 3 times extracted with methanol (totalling 1000 ml) and the methanol extract was concentrated to 10 ml by a rotary evaporator. The concentrated methanol extract was used to evaluate the antifungal activities against 15 plant pathogenic fungal isolates such as *Collectotrichum gloeosporioides, Fusarium oxysporum, Magnaporthe grisea, Phomopsis soje, Pythium graminicola* and *Rhizoctonia solani*. Paper discs (8 mm in diameter) containing the concentrated extract (100 μl) were dried overnight and placed on both sides of 3 cm apart from the center of PDA plate. Agar plug (5 mm in diameter) cut from the margin of actively growing cultures of the 15 test fungi was placed at the center of the plate on which the papaer discs were placed. At the same time, a paper disc soaked with only 100 μl methanol as a control was also placed 3 cm apart from the center of the same plate. Inhibition of the fungal growth was examined by measuring the length of mycelial mat from the center of the plate 5 days after incubation at 25°C.

The concentrated methanol extract was also used to evaluate

the antimicrobial activities against 6 food-borne bacterial pathogens such as *Bacillus subtilis, Escherichia coli, E. Coli* O-157. *Salmonella anteroitidis, Streptococcus mutans* and *Pseudomonas aeruginosa.* To prepare seed inoculum, each strain of the bacteria was inoculated into a 250 ml Erlenmeyer flask containing 100 ml nutrient broth. After 1-day incubation at 30°C and 180 rpm, each bacterial suspension (100 μl) was smeared evenly on fresh nutrient agar and paper discs (8 mm in diameter) containing the concentrated methanol extract (100 μl) as prepared above were placed on the plate. Paper disc treated with only 100 μl methanol as a control was also placed on the same plate. Antimicrobial activity of the concentrated extract of LB9810 fungal isolate was evaluated by measuring the diameter of clear zone around the paper discs 2 days after incubation at 30°C.

**Partial purification of the crude methanol extract of the LB9810 isolate by solvent partition.** One liter of methanol extract as prepared above was concentrated to 250 ml, which was successively partitioned with equal volumes of *n*-hexane, ethyl acetate and *n*-butanol. Each solvent layer was concentrated to 10 ml with a rotary evaporator. Antifungal activity of the each concentrated solvent layer was evaluated against *R. solani* as described above.

## Results

**Isolation and antagonistic activity of fungal isolates from lichens.** To screen out novel antimicrobial agents from the lichens distributed in southern parts of Korea, 34 lichen species collected at mountain area of 'Backwoon' and 'Chiri' and coastal area of 'Sorok' island were investigated. Species of lichens attempted in this study are listed in Table 1 together with their geographical origin, the isolation method used and the antifungal activities of the isolates. Fifty eight fungal isolates free from symbiotic algae and contaminants such as bacteria and yeast were isolated by the three isolation methods described in materials and methods. All the tested lichen species produced fungal isolates associated with lichens (Fig. 1(B)). However, only 5 fungal isolates from total 34 lichens were obtained from discharged spores (method I) and the other isolates were obtained from macerated thallus suspension (method II) or direct use of thallus fragments (method III).

When 58 fungal isolates were evaluated their inhibitory effects on the mycelial growth of *R. solani,* 8 isolates were found to inhibit the fungal growth (Table 1). In particular, the isolate LB9810 originated from the thallus of *P. quercina* showed the strongest inhibitory activity to the mycelial growth of *R. solani.* Two antifungal isolates from *Lecanora allophane* or *Cladonia scarbriusula* also exhibited moderate inhibition effect on the fungal growth. In addition, five isolates showed no clear zone of inhibition but caused to loosely form mycelium of *R. solani* near the margin of mycelial mat of the isolates. Among the 8 antifungal isolates obtained, only 2 isolates from *L. allophane* and *Pertusaria sp.* were originated from discharged spores and the rest of them were obtained from thallus.

**Evaluation of antimicrobial activities of the LB9810 isolate.** The LB9810 isolate exhibiting the strongest antagonistic effect on the mycelial growth of *R. solani* was

**Table 2.** Antifungal activity of *Fusarium equiseti* LB9810 isolate originated from the thallus of *Parmelia quercina* lichen against various phyto-pathogenic fungi[a]

| Test fungus | Inhibition zone length (mm) of mycelial growth | Inhibition rate (%) compared to the control |
|---|---|---|
| *Bipolaris coicis* | 10.7 ± 1.5 | 44.3 ± 3.6 |
| *Botryosphaeria dothidea* | 3.7 ± 0.6 | 14.1 ± 1.8 |
| *Cercospora kikuchii* | 9.7 ± 2.3 | 38.3 ± 3.0 |
| *Colletotricum gloeosporioides* | 8.7 ± 1.5 | 35.9 ± 4.1 |
| *Fusarium graminearum* | 8.0 ± 0 3 | 33.4 ± 1.4 |
| *Fusarium oxysporum* | 9.7 ± 1.5 | 37.1 ± 4.5 |
| *Fusarium solani* | 8.3 ± 1.2 | 33.3 ± 3.6 |
| *Magnaporthe grisea* | 13.3 ± 2.1 | 59.5 ± 4.3 |
| *Phomopsis longieola* | 8.3 ± 0.6 | 35.8 ± 3.6 |
| *Phomopsis soje* | 14.3 ± 3.2 | 48.0 ± 7.2 |
| *Pythium aphanidermatum* | 4.7 ± 0.6 | 16.4 ± 1.5 |
| *Pythium graminicola* | 7.0 ± 2.0 | 25.0 ± 4.6 |
| *Pythium ultimum* var. *ultimum* | 11.7 ± 4.9 | 32.7 ± 12.1 |
| *Rhizoctonia solani* | 19.7 ± 0.6 | 60.3 ± 3.8 |
| *Sclerotinia sclerotiorum* | 3.7 ± 1.5 | 15.9 ± 7.1 |

[a]The antifungal activity was measured by placing the paper discs containing 100 μl methanol extract of LB9910 mycelia on both sides of PDA 3 cm apart from a mycelial disc of each fungus at the center of the plates. Inhibition of mycelial growth of each fungus was rated 5 days after inoculation of the test fungus. Each value represents a mean and standard deviation of three replicates.

selected and further examination on the antimicrobial spectra of several phyto-pathogenic fungi and food-borne bacterial pathogens was carried out. Inhibition of mycelial growth was evaluated against 15 phyto-pathogenic fungi listed in Table 2. Isolate LB9810 displayed the broad antifungal spectra against all of the fungi tested. However, inhibition rates widely ranged from 14.1% to 60.3% compared to the control. Especially, LB9810 severely inhibited mycelial growth of *R. solani* and *M. grisea*. Fungal growth of *Bipolaris coicis, Cercospora kikuchii, C. gloeosporioides, F. graminearum, F. oxysporum, F. solani, P. soje, Phomopsis longieola,* and *Pythium ultimum* was moderately suppressed by the isolate LB9810 and the inhibition rates were found to be more than 30%. On the other hands, *Botryosphaeria dothidea, Pythium aphanidermatum* and *Sclerotinia sclerotiorum* were weakly inhibited by the isolate LB9810 at the rates of less than 20%.

The LB9810 isolate also displayed antagonistic effect on several food-borne pathogenic bacteria such as *B. subtilis, E. coli, S. anteroitidis, S. mutans* and *P. aeruginosa* (Table 3). The diameter of clear zone formed by the isolate was not much variable in the test bacteria. Interestingly, the isolate had no effect on growth of *E. coli* O157 producing Shiga-like toxin, but the growth of a different serotype of *E. coli* was severely inhibited.

Crude methanol extract of LB9810 isolate was separated with different solvents such as *n*-hexane, ethyl acetate and *n*-butanol and the resulting fractions were examined to determine the antifungal activity against *R. solani*. Among the four fractions, *n*-hexane fraction exhibited the strongest antagonistic effect on the fungus at growth inhibition rate of 35% compared to the control. The fraction of ethyl acetate also showed inhibitory effect on the fungal growth of *R. solani* but antagonistic efficacy was less than that exhibited by *n*-hexane fraction. However, *n*-butanol or residual methanol fraction showed no clear antifungal activity against *R. solani*.

**Table 3.** Antimicrobial activity of *Fusarium equiseti* LB9810 isolate originated from the thallus of *Parmelia quercina* against various food-borne bacterial pathogens

| Test bacteria | Clear zone diameter (mm)[a] |
|---|---|
| *Bacillus subtilis* | 12.5 ± 0.71 |
| *Escherichia coli* | 13.5 ± 2.12 |
| *E. coli O-157* | no inhibition |
| *Salmonella anterotidis* | 13.0 ± 2.83 |
| *Streptococcus mutans* | 12.5 ± 0.71 |
| *Pseudomonas aeruginosa* | 12.0 ± 0.00 |

[a]The diameter of paper discs (8 mm) containing 100 μl methanol extract of LB9910 mycelia was included in the clear zone. The diameter was measured 2 days after treatment. Each value represents a mean and standard deviation of three replicates.

## Discussion

The number of isolates and the resulting isolates from each lichen species were largely affected by the isolation methods employed. Discharged spore method (method I) produced single isolate. However, macerated thallus suspension (method II) or direct use of thallus fragments (method III) usually produced more than two isolates from each lichen (Fig. (B)). The isolates originated from discharged spores (method I) were also detected with use of thallus fragments (method II or method III). This finding suggested that the isolates produced by the discharged spore method were likely to be LFF but those produced by macerated thallus suspension or thallus fragments may be the mixed components of LFF and LFC or contaminants associated with lichen thallus. Petrini et al. (1990) reported that surface sterilization was not an appropriate treatment for lichens because of the highly absorbent nature and their small cross-sectional dimensions. Therefore, it cannot be ruled out that the isolates obtained from the thallus (suspension or fragment) are the contaminants associated with thallus fragments on lichen surface or from within the inter-hyphal spaces of the thallus. Because isolation of antifungal fungi was mainly achieved by the method II or method III, most of antifungal isolates obtained from thallus fragments in this study were likely to be LCF or contaminants associated with lichen thallus rather than lichen-forming fungi.

Use of discharged ascospores has been considered as a very successful method to obtain LFF and the isolation success rates from discharged ascospores were reported to be about 30% (Crittenden et al., 1995). In this study, 22 ascolichen species were attempted by the discharged ascospore method but only 5 lichen species produced lichen-forming fungal isolates. The isolation success rate achieved in this study was lower than that of the previous results of Crittenden et al. (1995). Although the reasons for lower opportunity in the isolation of LFF from discharged spores are not certain, it is well known that ascospore viability is critically dependent on water availability and the physiological state of lichens at the time of collection (Hale, 1983). Therefore, failure in the isolation of LFF from discharged ascospores could be due to the following reasons: discharged ascorspores failed to germinate, ascospores were not discharged, ascospores germinated but growth was not sustained. According to the results of Crittenden et al. (1995), total isolation success rate of LFF from spore or thallus fragments was found to be 45% in *Lecanorales* order which all the lichens investigated in this study belong to. This result also indicates that certain numbers of the fungal isolates obtained from lichen thallus (suspension or fragments) in this study may belong to LCF or the contaminants associated with lichen thallus.

Eight antifungal isolates were obtained from the 34 lichens distributed in southern parts of Korea. The fungal isolates exhibiting antagonistic effect on the growth of *R. solani* were originated from various lichen species (genus) and thus the majority of lichen producing antifungal isolates was not distinguishable in this study. The isolate LB9810 originated from the thallus of *P. quercina* lichen exhibited strong antagonistic effects against several phytopathogenic fungi or food-borne bacterial pathogens. Although the antagonistic effects of LB9810 isolate were highly evaluated, origin of the isolate was not confirmed due to its isolation from thallus fragment. Identification of the LB9810 was performed by observing growth type, color development on PDA and morphology of conidia spore and chlamydospores. The LB9810 isolate was finally identified as *Fusarium equiseti* (Corda) Sacc. according to Nelson et al. (1983). It has been reported that the 13,250 species of LFF described constitute 46% of all known ascomycetes, about one fifth of all known fungi (Hawksworth, 1988). Unfortunately, there was no record on *F. equiseti* as a LFF or LCF. Therefore, it is very likely that *F. equiseti* LB9810 was originated from the contaminants associated with thallus fragments on lichen surface or from within the inter-hyphal spaces of the thallus. Two antifungal isolates originated from *L. allophane* or *C. scarbriusula* also displayed moderate antifungal activity against *R. solani*. Because the isolate of *C. scarbriusula* was originated from the lichen thallus, further identification of the fungal isolate should be performed and is now under investigation.

*F. equiseti* is one of the well known fungus producing several compounds of mycotoxins such as zearalenone, α-zearalenol and fusarochromanone (Xie et al., 1990; Jimenez et al., 1997). The isolate LB9810 identified as *F. equiseti* exhibited broad antifungal spectra against phytopathogenic fungi and also very strong antagonistic effects against food-borne pathogenic bacteria. This implies that the fungus can produce several antimicrobial metabolites effective in growth inhibition of both fungus and pathogenic bacteria. The result of strong antifungal activity in *n*-hexane fraction separated from the crude methanol extracts of the LB9810 mycelial mats would be helpful for the investigation.

Whatever origin of the antifungal isolates obtained in this study is, namely, LFF, LCF or the contaminants associated with lichen thallus, it is quite certain that all the isolates are closely associated with lichens. In addition, this study is the first attempt to isolate lichen-form fungi from the lichens distributed in southern parts of Korea and to screen the novel antimicrobial agents associated with the lichens which are effective in controlling pathogenic fungi or bacteria causing a serious damage to plants and animals. A great attention should be paid to the isolation of LFF and LCF due to either low success rates of isolation from discharged spore or high opportunity of contamination associated with lichen thallus.

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