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# Studies on the Antibiotic Constituents of Korean Basidiomycetes (IV). Preliminary examination of the mycelial cultures of the 17 basidiomycetous strains

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**Abstract** □ To investigate the antibiotic constituents of Korean basidiomycetes the carpophores of the wood-rotting basidiomycetes were collected from several locations of Korea, and from them 17 mycelial strains were isolated on potato-dextrose-agar plates supplemented with tetracycline (20 $\mu$g/m*l*). The isolated strains were shake-cultured in glucose-peptone-yeast extract medium and then the antibacterial activities of the culture filtrates were assessed by disc-plate method. Among them, 12 strains (70.6%) were active, and basidiomycete strain LMCB-109 (*Daedalea quercina*) and LMCB-116 showed potent activities against all the six bacterial target organisms including *Serratia marcescens*.

**Keywords** □ Antibiotic constituents, basidiomycete, mycelial culture, disc-plate method, basidiomycete strain LMCB-109 (*Daedalea quercina*), basidiomycete strain LMCB-116.

In the late 1940s and early 1950s when screening for antibiotic activity was at its peak, many basidiomycetes were also surveyed mostly by Wilkins *et al.*[1-3], by Robbins *et al.*[4], and by many other investigators[5-7]. But none of the antibiotics from basidiomycetes is clinically used today. The growing prevailence of resistance to existing antibiotics, however, increases the utility of the previously rejected antibiotics, including some from basidiomycetes. Therefore, basidiomycetes have recently received attention as a new source of noble antibiotics mostly by Anke *et al.*[8].

In Korea, however, such kinds of investigations have been very scarce. In 1959, Yun[9] investigated some wild mushrooms for their antibiotic activity. It is commonly believed that Korea should have much more basidiomycetes than the already reported some 800 species, for more and more newly recognized species are being added to the list of Korean basidiomycetes each year. Therefore, considering the fact that Korea has a great variety of basidiomycetes most of which were not screened for antibiotic activity and that antibiotic production is subject to variations in strain or cultural conditions, it is supposable that noble and utilizable antibiotics could be developed from Korean basidiomycetes. To achieve this goal extensive screening test for and isolation and characterization of the active constituents of Korean basidiomycetes are needed. In 1985, Chung, one of the authors, and his collaborators launched a research program of screening for antibiotic components of Korean basidiomycetes. The previous three reports[10-12] contained the results of screening for antibiotic activity of the carpophores, but this fourth report is the result of the antibiotic screening of the cultured mycelia.

## EXPERIMENTAL METHODS

### *Bacterial Strains*

The following six bacterial strins were used at target organisms: *Sarcina lutea* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538p, *Escherichia coli* ATCC 25922, *Proteus mirabilis* H8-4 and *Serratia marcescens* 0101 RI MD996002. These were from the stock culture of our laboratories.

### *Media*

Potato-dextrose-agar medium (Bacto PDA medium), peptone (Bacto Peptone) and nutrient broth (Bacto Nutrient Broth) were purchased from Difco Laboratories, Detroit, Michigan U.S.A. Yeast extract (Gibco Yeast Extract) was from Gibco Diagnostics, Madison, Wisconsin, U.S.A. Glucose (anhydrouse) was from OSKA Chemical Co. Ltd., Ja-

pan, and glucose-peptone-yeast extract medium (GPY medium: glucose 50g, peptone 10g, yeast extract 10g per one liter) was prepared in our laboratory.

### *Basidiomycetes Samples*

The carpophores of the wood-rotting Basidiomycetes and the wood samples showing the mycelial growth were collected from several locations of Korea including Gong-ju, Gyeryong Mt., the campus of Chung-nam National University at Yu-seong, Taejon City, and Ga-pyong during the period of July to October, 1987. Identification was undertaken by comparing the observed macroscopic and microscopic characteristics with those described by Imazeki *et al.*[12,13], Philips[14] and Lincoff[15]. The voucher specimens are kept at the Laboratory of Microbial Chemistry, College of Pharmacy, Chungnam National University.

### *Shake-culture of Basidiomycetes Strains*

The mycelia together with a small agar block was separated from the stock culture and homogenized with a few milliliters of GPY medium in a glass homogenizer. The homogenate was transferred into 50 m*l* of GPY medium in a 250 m*l* Elenmyer flask and then shake-cultured for seven to 10 days on a rotary shaker at 27°, 180rpm. The mycelial culture was filtered and the culture filtrate was used as a sample for antimicrobial test.

### *Isolation of Basidiomycetes Strains*

Small tissue fragments (about 3×3 mm) were aseptically separated from the uncontaminated parts of the carpophores or the wood samples and then layed on a PDA plate supplemented with tetracycline (20 $\mu$g/m*l*). After incubation at 27° for three to five days, small agar blocks were separated from the edges of hyphal growth and transferred onto fresh PDA plates. The pure isolates were obtained from the edges of new hyphal growth and implanted on PDA slants in screw-capped tubes. After incubation the isolated strains were kept at 4° as stock cultures.

### *Antimicrobial Test*

Disc-plate method was used to assess the antibiotic activity of the samples. The bacterial stock

**Table I. Characteristics of the isolated basidiomycete strains**

| | | Characteristics[a] | | | |
|---|---|---|---|---|---|
| Strain | Scientific Name | Growth | Color of mycelia | Soluble pigment in the medium | Clamp connection |
| LMCB-001 | *Coriolus versicolor* | cottony | white | yellow | yes |
| LMCB-101 | N.I. | flat | brown | -[b] | yes |
| LMCB-102 | *Podoscypha multizonata* | fluffy | white | - | yes |
| LMCB-103 | N.I. | cottony | white | - | yes |
| LMCB-104 | *Lenzites betulina* | cottony | white | - | N.O.[c] |
| LMCB-106 | N.I. | fluffy | white | - | N.O. |
| LMCB-107 | *Ganoderma lucidum* | cottony | white | - | yes |
| LMCB-109 | *Daedalea quericina* | cottony | brown | brown | yes |
| LMCB-110 | *Ganoderma lucidum* | flat | brown | brown | N.O. |
| LMCB-112 | *Schizophyllum commune* | fluffy | white | - | yes |
| LMCB-113 | *Schizophyllum commune* | fluffy | white | - | yes |
| LMCB-116 | N.I. | cottony | white | - | yes |
| LMCB-123 | N.I. | cottony | pale pink | brown | N.O. |
| LMCB-127 | N.I. | fluffy | white | - | yes |
| LMCB-129 | N.I. | flat | brown | - | N.O. |
| LMCB-130 | N.I. | flat to cottony | brown | brown | N.O. |
| LMCB-135 | *Merulius tremellosus* | flat | white | - | N.O. |

[a] The characteristics are those of the mycelial strains on PDA slants.
[b] No soluble pigment was formed. [c] Not observed. N.I.: not identified.

culture was inoculated into nutrient broth and incubated overnight at 37°. One milliliter of the overnight culture was inoculated into 100 m*l* of molten nutrient agar and then 5 m*l* of it was spread over the basal layer in a Petri dish (9cm in diameter). The paper disc (6mm in diameter) was loaded with 60 $\mu l$ of the sample and applied on a seeded agar plate. After overnight incubation at 37° the diameters of the inhibition zones were measured.

## RESULTS AND DISCUSSION

### *Basidiomycetes Isolates*

From the collected basidiomycetes which belong to the Family Polyporaceae, 17 strains were successfully isolated (Table I). Eight of them were identified on the basis of the morphological characteristics of their carpophores, but nine strains await further studies to be identified. Some of the macroscopic and microscopic characteristics of the isolated strains are shown in Table I.

### *Antibiotic Activity*

Of the 17 basidiomycetes, 12 strains (70.6%) were antibiotically active (Table II). And it is noteworthy that *B. subtilis* was inhibited by all these active strains. This result gives credibility to the sugestion that *B. subtilis* should be included among the test organisms to assess the antibiotic activities of the basidiomycetes. Among the active strains nine were active against both gram positive and negative bacteria including *S. marcescens*. When considering the fact that *S. marcescens* is insensitive to majority of the existing antibiotics the reason of this phenomenon is not easily explained.

These nine strains (75%) of the 12 active strains could exert antibiotic activities against this gram negative bacterium. Three basidiomycetes strains showed activity against only gram positive bacteria, but none exhibited any activity against only gram negative bacteria. Strains LMCB-109 (*Daedalea quercina*), LMCB-116, LMCB-110, and LMCB-135 (*Merulius tremellosus*) showed antibiotic activities against all the test bacteria. Among these, the strain LMCB-116 showed the most potent antibiotic activity and its potent activity against all the bacterial strains provides a good reason for a further study to isolate and characterize the active constituents. The

**Table II. Antibiotic activities of the mycelial culture filtrates of some wood-rotting basidiomycetes against six bacterial strains tested by disc-plate method**

| Basidiomycete strains | Antibiotic activity[a] against | | | | | |
|---|---|---|---|---|---|---|
| | S.l. | S.a. | B.s. | E.c. | P.m. | S.m. |
| LMCB-001 | + + | + + + | + + | − | − | − |
| LMCB-101 | − | − | − | − | − | − |
| LMCB-102 | + + | + | + | − | + | + |
| LMCB-103 | + + | + | + | − | − | + |
| LMCB-104 | − | − | − | N.D. | − | − |
| LMCB-106 | − | − | − | − | − | − |
| LMCB-107 | − | − | + | − | − | − |
| LMCB-109 | + + + | + + | + + + | + + | + + | + + |
| LMCB-110 | + + + | + + + | + + | + + | + | + |
| LMCB-112 | − | − | − | − | − | − |
| LMCB-113 | − | − | − | − | − | − |
| LMCB-116 | + + + + | + + + + | + + + + | + + + | + + + | + + + + |
| LMCB-123 | + + | + | + | − | − | − |
| LMCB-127 | + | − | + | − | − | + |
| LMCB-129 | + + | + | + + | − | − | + |
| LMCB-130 | + + | + | + | − | + | + |
| LMCB-135 | + + + | + | + + | + | + | + + |

[a] The paper disc (Toyo No. 25, 6mm in diameter) was soaked with 60 $\mu l$ of the culture filtrate with intermittant drying. −: no inhibition zone; + : < 10.0mm; + + : ≧ 10.0mm, < 15.0mm; + + + : ≧ 15.0mm, < 20.mm; + + + + : ≧ 20.0 mm. N.D.: not done, S.l., Sarcina lutea; S.a., Staphylococcus aureus; B.s., Bacillus subtilis; E.c., Esherichia coli; P.m., Proteus mirabilis; S.m., Serratia marcescens.

strain LMCB-109, which was tentatively identified as *Daedalea quercina,* is also thought to deserve a further study to illucidate its active constituents. The strains LMCB-109 (*Daedalea quercina*), and LMCB-102 (*Podoscypha multizonata*) were newly recognized as antibiotic producers by this study, though the latter showed only moderate to weak activity against five bacterial strains.

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