2. Antifungal Activity and Structure Elucidation of Antibiotics from Microbial Secondary Metabolites.

Beom Seok Kim[1], Surk Sik Moon[2] and Byung Kook Hwang[1]. [1]Department of Agricultural Biology, Korea University, Seoul 136-701, Korea. [2]Department of Chemistry, Kongju National University, Kongju 314-701, Korea

Microbial secondary metabolites, represented by antibiotics, have been important sources of antifungal compounds for the development of fungicides. Antibiotics of microbial origin have promising characteristics as fungicides for control of plant diseases. The antibiotics from microorganisms would be likely to degrade rapidly and easily in biosphere, thus leading to low residue levels. Additionally, their versatility in biological activity and chemical structure provides highly specific modes of action.

During the screening procedure for potent antifungal metabolites as a plant chemotherapeutic agent, we isolated a number of actinomycete strains active against some plant pathogenic fungi from sea-mud soils in Korea. Among the thirty-seven antifungal-active actinomycetes, including *Streptomyces* spp., *Micromonospora* spp. and Nocardioform isolates, the strains As1, Ao58 and Ao108 showed substantial antifungal activities against some plant pathogenic fungi.

The actinomycete strain As1 was identified as *Streptomyces libani* using the Taxon program, based on the morphological and cytochemical characteristics. The antibiotic As1A was isolated from the broth culture of *S. libani* strain As1 using various chromatographic procedures. The molecular formula of the antibiotic As1A was deduced to be $C_{45}H_{74}O_{11}$ (M+H, m/z 791.5307) by HR-FAB mass spectroscopy. The analysis of various NMR spectral data revealed that the antibiotic As1A is a macrolide antibiotic having a 26-membered, α,β-unsaturated macrolactone ring with a conjugated diene fused to a bicyclic spiroketal. Based on NMR data and other chemical properties, the antibiotic As1A turned out to have the same structure as oligomycin A. The actinomycete strain Ao58 was identified as *Micromonospora coerulea* based on morphological and physiological characteristics. The molecular formula of the antibiotic Ao58A purified from the culture of *M. coerulea* strain Ao58 was deduced to be $C_{16}H_{23}NO_4$ (M+H, m/z 294.1707) by HR-FAB mass spectroscopy. Analyses of various spectral data revealed that the antibiotic Ao58A is a glutarimide antibiotic streptimidone, 4-(2-hydroxy-5,7-dimethyl-4-oxo-6, 8-nonadienyl)-2,6-piperidinedione. The actinomycete strain Ao108 that produced antifungal metabolites active against some plant pathogenic fungi was identified as *Actinomadura roseola* by analyzing of morphological and physiological characteristics. The antibiotic Da2B was isolated from the culture of *A. roseola* strain Ao108 using various chromatographic procedures. On the basis of various NMR data, the antibiotic Da2B was confirmed to have the structure of an anthracycline antibiotic, daunomycin.

The antibiotic As1A, Ao58A, and Da2B showed not only potent *in vitro* antifungal activity but also substantial control efficacy against the development of some plant diseases under greenhouse condition. A high inhibitory activity of the antibiotic As1A against *Botrytis cinerea, Cladosporium cucumerinum, Colletotrichum orbiculare, Magnaporthe grisea,* and *Phytophthora capsici* was observed in a range from 3 to 5 μg mL^{-1} of minimum inhibitory concentrations (MICs). Developments of the Phytophthora disease, anthracnose and leaf blast under greenhouse condition were markedly inhibited on pepper, cucumber, and rice plants by treatments with the antibiotic As1A, respectively. The antibiotic Ao58A was very effective in inhibiting mycelial growth of *P. capsici*, *Didymella bryoniae*, *M. grisea* and *B. cinerea* in a range of 3-10 μg mL^{-1} of MICs. The *in vivo* evaluation of the antibiotic Ao58A showed strong control efficacies against

the development of *P. capsici*, *B. cinerea* and *M. grisea* on pepper, cucumber and rice plants, respectively. *In vitro* antimicrobial spectrum tests showed that the antibiotic Da2B had substantial antifungal activity (10 μg mL^{-1} of MICs) against *P. capsici* and *Rhizoctonia solani*. The antibiotic also showed antiyeast activity against *Saccharomyces cerevisiae* but the growth of *Candida albicans* was not affected. Antibacterial activity was found only against Gram-positive bacteria.

Pseudomonas aeruginosa strain B5 isolated from rhizosphere of pepper plants produced antibiotic substance B5 which showed substantial antifungal activity against *P. capsici*. The antibiotic substance was identified as a glycolipid antibiotic, rhamnolipid B, that caused cessation of motility and lysis of the entire zoospores at a concentration of 10 μg mL^{-1}. The mechanism for zoospore lysis is that upon exposure to rhamnolipid B, rhamnolipid intercalates into and disrupts the zoospore plasma membrane. However, rhamnolipid B also inhibited the mycelial growths of *P. capsici* and *C. orbiculare*, that implied other mechanism might be implicated in its antifungal activity against various plant pathogenic fungi.