

CHAPTER V

DISCUSSION

A total of 4 in 10 species of Thai medicinal plants collected in this study was found to harbor no endophytic fungus. This number was rather high in contrast to previously reported that endophytic fungi could be found in most of vascular plant species examined (35). The plant samples collected from these four species (*Abrus precatorium* Linn., *Cassia alata* Linn., *Datura metel* Linn., and *Punica granatum* Linn.) might be not colonized by endophytic fungi because the plant samples were re-collected for another two times to confirm the results. On the other hand, it might be resulted from the killing affect of sodium hypochlorite that might penetrate the tissue of these plant species. The finding that only 8.6% of endophytic fungus isolates were collected from leaf sample suggested that UV in sunlight might affect the colonization of endophytic fungi in leaves as previously proposed (36). During the spring and summer, sunleaves have much higher UV exposure relative to the inner shaded leaves. They were found to have lower endophytic infection densities than shade leaves. In contrary, it might imply that the condition used in surface sterilization (6% sodium hypochlorite for 5 min) were too strong for leaves. Deleterious effect of sterilizing condition on plant tissues, that were found to harbor no fungal endophyte, should be studied further in order that all endophytic fungi could be isolated.

More than 70% of endophytic fungus isolates were found to have antimicrobial activities. Most of them were active against filamentous fungi. This supported the proposal that endophytic fungi might confer benefit to host plant by enhancing the ability to resist pathogens (12). The high percentage of active endophytic fungus isolates is consistent with the previous idea that fungal endophytes are untapped source of bioactive compounds (3). In contrast to activity against gram-positive, number of endophytic fungi being active against gram-negative bacteria was very low. This phenomenon is common because gram-negative bacteria have an intrinsic resistance to antibacterial compounds. A small number of compound could pass gram-negative bacterial envelope (30). At present, efflux mechanisms are also

known to contribute significantly to intrinsic multidrug resistance in a number of gram-negative bacteria (37).

By dual-culture agar diffusion assay, the culture media was found to be the one of factor affecting expression of antimicrobial activity. Malt extract-containing media such as MCzA or MEA were effective in enhancing the expression of antimicrobial activity against test microbial strains except *P. aeruginosa* and *T. mentagrophytes*. YES was found to be the medium of choice for expression of anti-*P. aeruginosa* and *T. mentagrophytes* activities. This result was consistent with the previous report that these three media were useful for the production of secondary metabolites (34). Some endophytic fungus isolates expressed certain antimicrobial activity in culture medium-specific i.e. anti-*E. coli* and anti-*P. aeruginosa*. There is no single suitable culture medium that provides all of antimicrobial activities. Using numerous culture media probably will be increasing the opportunity to obtain more antimicrobial activities, but this has to be considered with the financial facility.

The instability of antimicrobial phenotype of endophytic fungus isolates is the common phenomenon found in fungi. The fungal mycelium should be considered as an unstable dynamic structure that is prone to change its developmental pattern in response to external or internal cues. Strain instability and degeneration is usually associated with laboratory subculture in industrial situations. It has been shown that the presence of individuals of *Fusarium compactum* in the Chihuahuan and Sonoran Deserts exhibiting a very degenerated morphology in culture with a restricted colony size was accompanied by the profuse aerial mycelium production and the poor pigment production. These isolates shew the ability to produce the metabolites which were not produced by the other isolates in the population (35).

By using gradient plate technique to select low susceptible *S. aureus* and *E. coli* strains, most of low susceptible strains, except *S. aureus* mutant strain with low susceptibility to tetracycline (Te^L), resisted to the respective antibiotic that was used in selection. Susceptibility of *S. aureus* to tetracycline is broad, MICs for the susceptible strains is $\leq 4 \mu\text{g/ml}$ (33). Therefore, the selected mutant, with MIC that was 16 times higher than that of wild type strain, was only the low susceptible mutant. Cross-resistance to the unrelated antibiotics was found in selected mutants. This might come from 2 possibilities. First, mutation in multiple chromosomal loci might occur in these strains and resulted in modification of several antibiotic targets. Target

modification is the primary mechanism of resistance to most antibiotics that is resulted from chromosomal mutation (38). Second, mutation might occur in some loci governing bacterial efflux mechanism (37,39). This might promote the efflux of unrelated drugs.

Using antibiotic resistant strains as test microorganism was one strategy in searching of new antifungal compound (9). By this approach, endophytic fungi producing the known compounds would be identified at early stage and thus eliminated from further consideration. Only 5 active isolates (17.2%) were found to be active against the resistant strains. The active endophytic fungi, isolate *Bore* 04, *Ccoc* 08, *Cfis* 01A, *Line* 13 and *Oind* 05A, having activities against the resistant strains, might be the potential sources of new compounds with anti-*S.aureus* activity.

In identification of endophytic fungus isolates with activity against the selected *S. aureus* mutants, 4 in 5 isolates were found to be mycelia sterilia. These isolates should be true endophytic fungi. One isolate identified as *Alternaria* sp. might be an epiphyte that penetrated into the host tissue. A number of fungi reported as endophytes are known to be epiphytes that are able to live endophytically within plant tissue (40). O'Donnell and Dickinson (41) demonstrate that some epiphytes including *Alternaria alternata* (Fr.) Keissler penetrate into living leaf tissues at the onset of the senescence process.

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