CHAPTER 5

DISCUSSION

In this experiment, total 191 fungal endophytes were isolated from 5 healthy living part of Thai dwarf fishtail palm fronds and the overall colonization rate is 71.48% (Table 4). Fröhlich (1997) and Petrini *et al*. (2000) reported that the colonization rate of *Licuala ramsayi* and *Licuala* sp. from Australia and Brunei were 81%-89% but Rodrigues (1994) found that colonization rate of Amazonian palm *Euterpe oleracea* samples were 21-30%. Futhermore, the colonization rate on PDA (74.81%) was higher than PDA containing rose bengal (65.92%). Also the number of isolation by using PDA (101 isolates) was greater than PDA containing with rose bengal (90 isolates) especially in leaflet, using PDA yielded only 36 isolates but PDA containing rose bengal yielded 26 isolates. The percentage of isolate from each part of frond; leaflet, primary rachis and secondary rachis were 32.46, 33.56 and 32.98%, respectively. This result was accordance with many other species abundance between plant tissues such as between midrib and laminar tissues (Rodrigues, 1994). Previous studies have indicated the species specificity (Rodrigues, 1994; Clay and Holah, 1999) of endophyte, the different tissue types might be a capacity for utilizing the substrate along with factors like tissues physiology and chemistry (Petrini and Carroll, 1981; Pothita *et al*., 2001)

Statistic analysis showed that using different medium and part of frond were not affected to the colonization rate (Table 5). Thecha (2002) reported that the overall colonization rates of endophytic fungi isolated from *Wallichia caryotoides* were not

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different from different part palm. This study found that secondary rachis produced highest colonization rate and within the leaflet; central leaflet yielded a higher colonization rate than distal and basal part (Table 5). Another related research was conduced by Canon and Simmons (2002) reported that the colonization rate in midrib was greater than laminar and tip laminar was greater than base of the leaf.

All endophytic fungi in this study could be identified and classified into 10 genera, 30 morphotypes and 1 unidentified coelomycetes thus many plant species could be associated with tens to hundreds of endophyte species (Saikkonen *et al*., 2000; Stone *et al.*, 2004). The dominant genus was *Colletotrichum* spp. (21.9 %) which they were the common fungal endophyte associated with tropical plant (Canon and simmons, 2002; Thecha, 2002). *Collectotrichum* spp., morphotype 9 and morphotype 10 were found in every frond part and several genera or morphotypes were isolated only from specific part (Table 6) such as *Chaetomium globosum* which was isolated from the rachis. *Diplodia* sp. was isolated only from the secondary rachis. Morphotype 20 was isolated from central leaflet. *Phaeoisaria* sp., *Trichobotrys* sp., morphotype 15, 23, 24, 25, 29 and 30 were isolated from primary rachis only. *Phoma* sp., *Phomopsis* sp., morphotype 2, 3, 14 and 27 were found only in secondary rachis. In total number of genera or morphotypes from rachis were higher than leaflet. Fungal communities overlapping between leaflet tissues (37.16%) was higher than rachis (16.66%)

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In this research 89 isolates which failed to produce conidia were induced to produce fruiting body using filter paper technique which successful to induced the sporulation of *Pestalotiopsis microspora*; fungal endophyte from Himalayan yew

(Matz *et al*., 2000). In total only 19 isolates were successful to produce fruiting bodies which 5 isolates were produced young fruiting bodies.

Antagonistic activity test resulted 3 isolates; P59 (*Phaeoisaria* sp.), R12 (Morpho type18) and R14 (*Colletotrichum* spp.) (Appendix B). presented very high potential and broad-spectrum antifungal activity to suppressed all of 3 tested plant pathogenic fungi. Types of interaction were presented in this research; deadlock and replacement (Reaves and Crawford 1994; Badalyan *et al*., 2002; Badalyan *et al*., 2004; Campanile *et al*., 2007). Each fungal endophyte was expressed with each pathogen in different type of interaction. Interaction between endophytic fungi and *Sclerotium* sp. mostly were replacement of endophytic by *Sclerotium* sp (95.81%) (Table 8) and interaction with *Phythopthora* sp. were mostly deadlock (95.29%) (Table 9). Interactions between endophytic fungi and *Curvularia senegalensis* were deadlock for 77.47% and replacement type was present only replacement of pathogen by fungal endophyte (Table 10).

The most important parameter to determine antagonistic activity was the speeds of colony growth rate (Campanile *et al*., 2007) beside that interaction between endophytic fungi and tested pathogens also had to considered (Badalyan *et al*., 2002; Badalyan *et al*., 2004; Campanile *et al*., 2007). To choose the suitable isolate for further study both colony growth and interaction with tested pathogens. Table 11 presented interesting candidates for further study which had high to very high potential with subtype CA2 and CB2 (partial and complete replacement after initial deadlock at a distance, respectively) (Reaves and Crawford 1994; Badalyan *et al*., 2002; Campanile *et al*., 2007)

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Identification and selection of effective antagonistic was the first and foremost step in biological control (Kamalakannan *et al*., 2004). In this work were attempting to identify unknown fungal endophyte by using filter paper, however, only 17.6% were successfully induced to sporulated. Therefore, molecular technique is probably the most useful technique to identify endophytic fungi. Fungal isolates which showed the potential to control tested pathogens in preliminary test by simple method need further test such as field level. Analysis of active compound for biotechnological and agricultural purpose is also important.

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