CHAPTER 1 INTRODUCTION

1.1 Background

Nowadays, agrochemical usages for protecting plants pose negative effects on environment and affecting animal and human health. The natural products that are highly effective, possess low toxicity, and have a minor environmental impact are sought (Strobel and Daisy, 2003). Microorganisms are rich sources of bioactive substances. Among various natural sources of bioactive compounds, endophytic fungi are one of the interesting groups of organism that are high potential producers of bioactive compounds. Many endophytic fungi produce antimicrobial compounds which strongly exhibit antifungal and/or antibacterial activity against other microorganisms including plant-pathogens. The endophytic fungi can be considered as a source of novel bioactive metabolites for controlling phytopathogenic diseases.

Boonmakard *et al*. (2007) and Ratnaranthorn *et al*. (2009) isolated endophytic fungi from leaves and roots of *Stemona colinsae, S. burkillii, S. tuberosa* and *S. kerii* and found that they could inhibit many fungal plant pathogens (i.e. *Alternaria brassicola, Penicillum* sp., *Fusarium solani* and *F. oxysporum*) and bacterial plant pathogens (i.e. *Erwinia caratovora*, *Pseudomonas solanacearum* and *Xantromonas citrii*). Among endophytic fungal isolate, NHL-L 6/6 isolated from *S. burkillii* leave showed the highest activity against all test fungi and bacteria. Therefore, it was interesting to use this fungus for production of anti-phytopathogenic compound. However, as NHL-L 6/6 fail to sporulate in culture medium, the morphological characteristics of mycelium, spore formation and shape of spores cannot, therefore, be applied for identifying the fungal isolate (Lodge *et al*., 1996; Sette *et al*., 2006). To overcome such difficulty, molecular techniques are employed for fungal identification in the present study.

For the production of anti-phytopathogenic compounds, which are secondary metabolites, the common practice is the development of medium to obtain maximum metabolic product yield. Hence, screening of medium component is an essential part of successful fermentation, as there is no recommended medium for production of these unknown compounds. The production of fungal secondary metabolites is directly affected by the components of the culture medium and culture conditions. The

constituents of culture medium play a major role on growth and production of secondary metabolites (Betina, 1994). Medium screening and optimization strategies often involve the one-factor-at-a-time technique. This method is, however, time consuming and does not guarantee the determination of optimal conditions. The present study showed that the application of statistical experimental design techniques could result in improved product yield compared with conventional practice of single factor optimization. Response surface methodology can be used to evaluate the relative significance of several factors even in the presence of interactions. Pairoj *et al*. (2011) have investigated the effects of medium components on the antibiotic activity of NHL-L 6/6 by one-factor-at-a-time approach; nonetheless no information exists on their interactive effects has been reported. In this study, response surface methodology (RSM) was employed to optimize the medium components for the anti-phytopathogenic compound production by NHL-L 6/6.

1.2 Objectives

1) To identify NHL-L 6/6, a selected endophytic fungus isolated from *Stemona burkillii* leave, which showed powerful activity against several plant pathogens.
2) To study the effects of different sources of nitrogen and carbon on the anti-phytopathogenic compound production.
3) To evaluate the significance of factors greatly influencing anti-phytopathogenic compound production by fractional factorial design (FFD).
4) To optimize the medium components for anti-phytopathogenic compound production by a response surface methodology.
5) To explore the antimicrobial activity spectrum of the compounds against several plant pathogens.

1.3 Scopes

1) The sterile endophytic fungus, NHL-L 6/6, isolated from *Stemona burkillii* was identified using nucleotide sequence analysis of the internal transcribed spacer (ITS) region of the ribosomal RNA genes.
2) Primary screening of different sources of nitrogen (i.e. yeast extract, whey, ammonium sulfate and urea) and carbon (i.e. sucrose, glucose, cassava starch and molasses) for the anti-phytopathogenic compound production was studied by one-variable-at-a-time technique. The indicator pathogen adopted in this study was *Erwinia caratovora*.
3) A quick identification of the best carbon and nitrogen sources were achieved by a screening design (i.e. fractional factorial design, FFD), followed by steepest ascent design to identify the direction towards response maximization, and the response surface methodology (RSM) using central composite design (CCD) for further optimization.
4) The ethyl acetate extract of anti-phytopathogenic compound from optimized medium was tested for its antimicrobial activity spectrum against several plant pathogens, i.e. *Pseudomonas solanacearum*, *Xanthomonas citrii*, *Alternaria brassicicola, A. porri, Penicillium* sp., *Colletotrichum* sp., *Fusarium solani* and *F. oxysporum*.

1.4 Expected outputs

1) The unknown endophytic fungus, NHL-L 6/6, can be identified.
2) The optimum medium for antimicrobial compound production in submerged fermentation will be established.