CHAPTER I

INTRODUCTION

Background and rational

Melioidosis is an infectious disease caused by the gram-negative bacterium *Burkholderia pseudomallei*. It is known to be endemic to Southeast Asia and Northern Australia, although melioidosis cases are reported from nearly every part of the world. Melioidosis also has other names in the literature such as Vietnam lung disease, time bomb disease and the great imitator[1,2]. In Thailand, melioidosis can be found in all ages, but is mostly found in the 40-50 year-old age group, and within that group, predominantly in male rice farmers[2]. Nearly every organ except the gastrointestinal tract has been reported as being infected with *B. pseudomallei*, and the signs and symptoms of a particular case depend on the type of infected organ[3,4]. The clinical symptoms can be classified into 5 groups - disseminated septicemic melioidosis, non-dissemi-nated septicemic melioidosis, localized melioidosis, transient bacteremia and subclinical melioidosis[5]. The acute form of the first group has a high mortality rate even with appropriate antibiotic treatment. The subclinical form may comprise the largest melioidosis group according to melioidosis antibodies in the serum. The range of clinical presentations and the potential for asymptomatic infection presumably reflect differences in the route of inoculation, inoculum size, virulence of the infecting strain, immune competence and genetic predisposition of the host[6]. Epidemiological data suggests that melioidosis infection occurs via ingestion, inhalation or subcutaneous inoculation of contaminated soil and surface water[7,8].

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Currently, little is known about melioidosis pathogenesis. From experimental study, *B. pseudomallei* is known to have a broad range of virulent determinants that likely influence both pathogenesis and clinical presentation. These include various exotoxins, rhamnolipids, endotoxins, protease, malleobactin *(B. pseudomallei* siderophore), flagella, capsules, and fimbriae (pili)[6]. Polysaccharides and flagella are reported to be conjugated vaccine candidate. The specific role of these virulent determinants in the pathogenesis of melioidosis remains unclear. In modern genetics and molecular biology, new approaches to the study of bacterial pathogenesis focus on genes and gene products that facilitate survival, virulence and multiplication of bacterial pathogens in the host [9,10]. Knowledge of *in vivo* expressed genes may help to elucidate bacterial pathogenesis, treatment, vaccine development and diagnosis. Some of the *in vivo* expressed gene approaches have been reported in bacterial pathogenesis studies, including *in vivo* expression technology (IVET),[11] signature tagged mutagenesis (STM)[12] and differential fluorescence induction (DFI)[13]. IVET is a promoter trap approach that uses animals as a selective medium to reveal *in vivo* induced bacterial genes. In STM, the bacterial genes are mutated by the insertion of a transposon carrying a uniquely tagged DNA sequence. The animal models are inoculated with the pooled mutant strains and then the mutant strains that are absent from the output pools can be identified by comparative hybridization. DFI has the same principle as IVET but differs in the type of reporter genes. DFI employs a green fluorescence protein (gfp) as a reporter in the plasmid. The *in vivo* expressed genes can be monitored by using a fluorescence activated cell sorter (FACS)[13]. Although new insights in bacterial pathogenesis are demonstrated by those technique, there are some limitations[14].

Host immune response has long been studied for vaccine development. There are only a few works which have used antibodies to probe *in vivo* expressed gene by immunoscreening.The serum from actively infected and heat killed *Borrelia burgdorferi* immunized mice are used as probes in immunoscreening of the expressed genomic library. One gene (p21) encoding a 20.7 kDa antigen is recovered by this approach[15]. Recently, an *in vivo* induced antigen technology(IVIAT) was developed to use pooled serum which is extensively absorbed with *in vitro* grown bacterial cells and bacterial cell lysate [16]. Then the absorbed serum is used as a probe in immunoscreening of expressed genomic library. The gene profiles recovered by this approach may reflect direct interaction between the bacteria and host.

Hypothesis

According to the general understanding of bacterial pathogenesis, after *B. pseudomallei* enters a host, it is expected that it should express some groups of genes that are essential for each step in the infection process. Some of the proteins encoded by the expressed genes would stimulate the host immune response system to produce specific antibodies. The induced antibodies in the patient's serum may then be used as a probe to detect *in vivo* expressed genes by immunoscreening of the expressed genomic library. These *in vivo* expressed genes might be detected by a combination of immunoscreening, bioinformatics and molecular biology. The genes that are expressed only in the patient and not in normal people in endemic areas may play an important role in the pathogenesis of melioidosis .

Objective

1. To clone the genes of *B. pseudomallei* that are expressed in bacteremic melioidosis by using antibodies as probes for screening
2. To characterize the genes and antigens encoded by these genes

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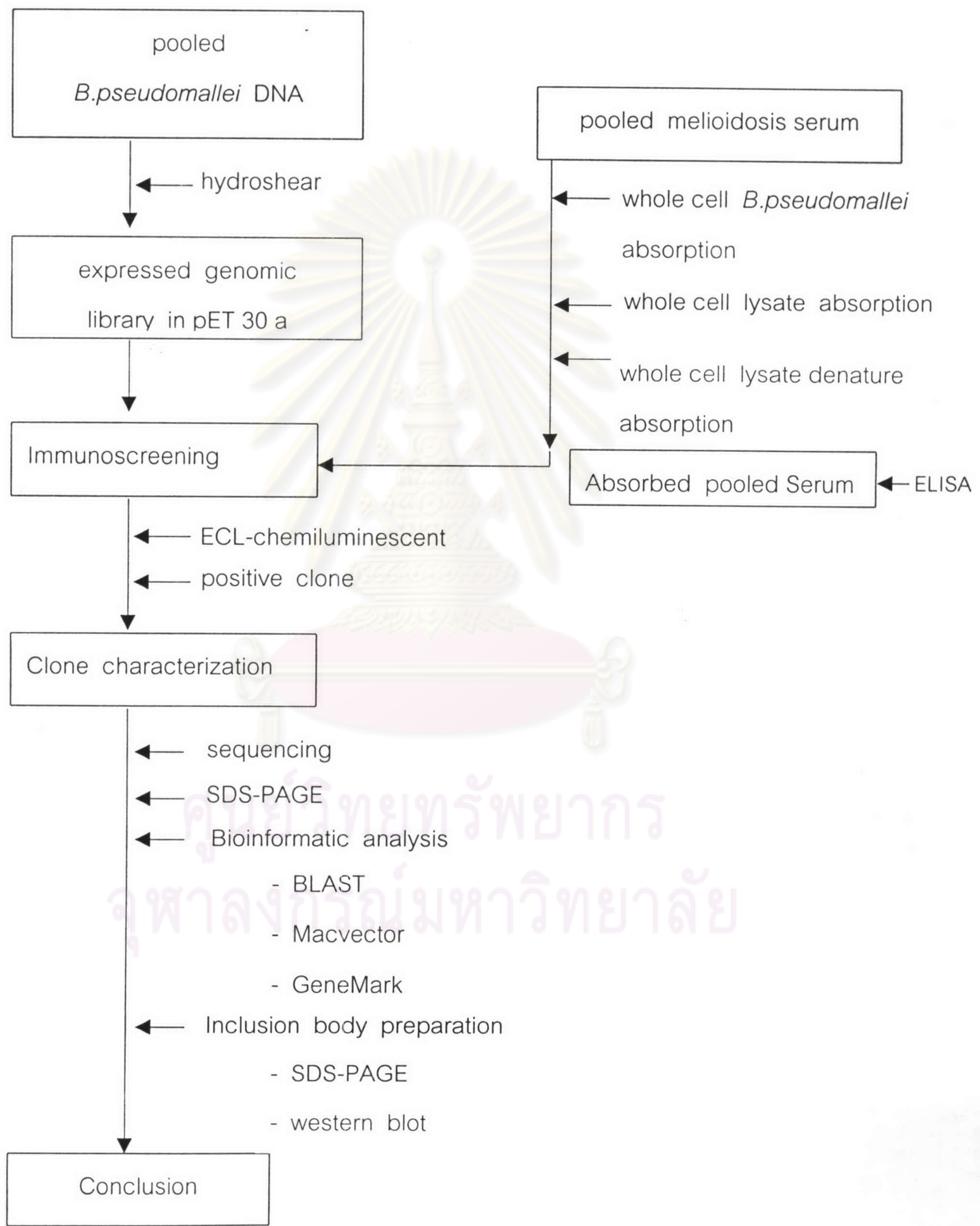
Flow chart of experimental design I

An approach to isolating *in vivo* expressed genes of *Burkholderia pseudomallei* using *In vivo* induced antigen technology(IVIAT)



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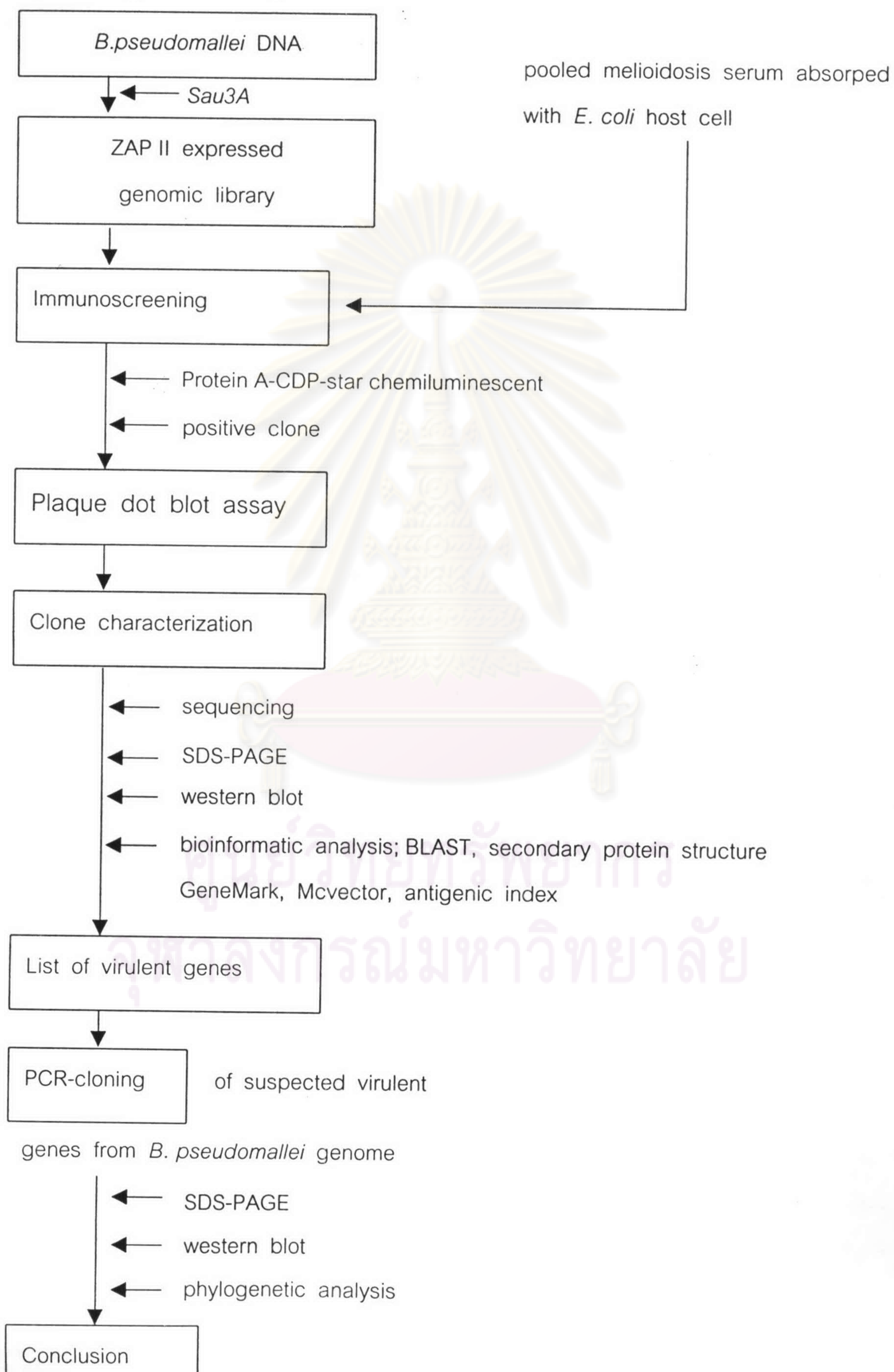
Flow chart of experimental design II

Searching for virulent *B.pseudomallei* genes by immunoscreening the ZAP II expressed genomic library



Expected outcomes and significance

1. Some *in vivo* expressed genes of *B. pseudomallei* would be recovered by this novel approach.
2. The type of expressed genes may provide some basic and significant new knowledge of melioidosis.
3. Some of the isolated genes may have high potential to be used as serological markers for melioidosis.
4. This approach may have application for other bacterial pathogen studies.

Research methodology

1. Sample collection

Melioidosis patient serum samples :16 sera were collected from the bacteremic patients who admitted at the hospitals in northeastern, central and southern parts of Thailand.

Normal control sera : 20 normal healthy sera from blood bank donors.

B.pseudomallei collection : 20 strains were collected from the bacteremic patients.

2. Process of study

- blood sample collection
- pooled melioidosis serum absorption
- expressed genomic library construction in pET30a
- expressed genomic library construction in ZAP II
- immunoscreening using ECL chemiluminescence
- immunoscreening using protein A-CDPstar
- clone characterization
- PCR-cloning of suspected *in vivo* expressed gene from *B.pseudomallei* genome

3. Data analysis

Phylogenetic analysis was performed by Mcvector version 7.0

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