CHAPTER I

INTRODUCTION

Pseudomonas pseudomallei is a gram-negative rod , found as a free-living saprophyte of soil and water, which causes the disease melioidosis in animals and humans (1,2). This etiologic agent was recognized by Whitmore and Krishnaswami in 1912. It was presently transferred to the newly proposed genus *Burkholderia* in 1992 (3). In this work it is still mentioned in former *P. pseudomallei*. Melioidosis has a major endemic focus in Southeast Asia and Northern Australia, although sporadic cases have been reported worldwide (4,5). Serological survey of melioidosis in Thailand was studied by Nigg in 1963 (6) . It was reported that 29% (118/405 sera) of sera had antibodies to *P. pseudomallei*. In humans,meioidosis is acquired mainly by contamination of pre-existing skin abrasions (or ulcers) with soil and water or by inhalation of dust particles (1,5). According to a broad spectrum of clinical manifestations, melioidosis is often called " a great immitator ". The disease varies greatly in its clinical presentation (1,4-7), ranging from the subclinical infection being common in endemic area manifested only by the presence specific antibodies; localized infection in form of either acute suppurative or chronic granulomatous lesions; subacute or acute fulminating bacteremia. Furthermore, *P. pseudomallei* may remain dormant in asymptomatic individual only to recrudesce, if conditions are favorable and in many years after initial exposure,as an acute exacerbation.

Hispathological study in human melioidosis found that almost all visceral organs reveals necrotic lesions (8). These may be due to the action of the extracellular enzymes or toxins such as endotoxin (9), exotoxin (10,11), hemolysin (12), protease (11,13) and malleobactin (14,15) secreted by this organism. Nevertheless, the pathogenesis of melioidosis is not yet clearly defined, especially in regarding to bacterial pathogenicity.

Hemolysin is an extracellular substance which lyses erythrocyte and differentiated eukaryotic cell (16). Bacterial hemolysins seem to play an important role in virulence of the organisms. For example, *Escherichia coli* strain causing extraintestinal infections produced hemolysin (17,18). *Proteus mirabilis* hemolysin was involved in the invasion (19). *Listeria monocytogenes* possessed hemolytic activity associated with the ability to grow within macrophage cytoplasm to escape from host immune system (20). Heat-labile hemolysin (phospholipase C, PLC) of *Pseudomonas aeruginosa* was contributed to virulence and pathogenesis (21,22), nevertheless, its mechanism is still unknown.

At present, there is no clear report about the mechanism, and role of *P. pseudomallei* hemolysin in the pathogensis of melioidosis. Genetic methods including recombinant DNA technique, have accelerated the detailed studies of hemolysin of *P. pseudomallei* for elucidation. Kongcharoensuntorn (23) had cloned the genes from *P. pseudomallei* strain K1/88 that expressed hemolytic activity in *E. coli*. This was an initial step to study hemolysin from *P. pseudomallei* and define its

role in pathogenesis. Therefore, the hemolysin-expressing *E. coli* was used in this study in order to characterize the cloned gene for better understanding of the regulation of hemolysin gene expression and further study of the function of hemolysin in pathogenesis of melioidosis.

The purpose of this work was the followings:

1. To sequence *P. pseudomallei* gene in hemolysin-expressing *E. coli*.

2. To characterize the gene product produced by this cloned gene.