CHAPTER I

INTRODUCTION

Pseudomonas pseudomallei , a gram-negative rod, is the causative agent of melioidosis or glanders-like disease, was first recognized by Whitmore and Krishnaswami in 1912 (1). In the first report, the organism (isolated from an autopsy victim in Burma) was named *Bacillus pseudomallei* . *P. pseudomallei* is a free living bacterium and survives in soil and water (2,3). It is believed that the mode of transmission is via direct contact and respiratory tract (4).Its geographic distribution is located in tropical and subtropical zone in East Asia and Southeast Asia (5-7). Sporadic melioidosis cases can be found in Korea, Hong Kong, Iran, Turkey, England, France, Africa, Philippines, USSR and the United States (8-11).

Serological survey of melioidosis in Thailand was studied by Nigg in 1963 (12). It was reported that 29 percent (118/405 sera) of sera had antibodies to *P. pseudomallei*. Recently, 539 cases of melioidosis have been reported in the North and the South of Thailand (13). Clinical manifestation of melioidosis varies greatly, ranging from subclinical infection, chronic suppurative, subacute to acute fulminating bacteremia. *P. pseudomallei* can be isolated from lung, liver, spleen ,skin and cause pneumonitis, abscess and cellulitis. In chronic suppurative infection, almost all visceral organs are

involved and may develop to septicemia (10-11,14-16). Histopathological study in human melioidosis found that almost all visceral organs revealed necrotic lesions (14). These may be due to the action of the extracellular enzymes or toxins secreted by this organism (17). Due to the high mortality rate in septicemia, it is therefore gained a revival of interest in melioidosis.

The pathogenesis of melioidosis is not yet clearly defined, especially in regarding to the roles of virulence properties. The potential virulence factors such as endotoxin (18), intracellular macromolecule polypeptide extract (19,20), hemolysin (21), and exotoxin (17,22) may play roles in pathogenesis. Heckly and his colleague described 2 groups of exotoxins namely lethal toxin and necrotoxin (22). The lethal toxin has been the subject of extensive investigation and for serodiagnotic test (23). It has been reported that necrotoxin possessed proteolytic activity (24,25). At present,there has not been any report of separation of necrotoxin from proteolytic activity. Thus, it is interesting to isolate and to purify the proteolytic enzyme from this organism. If such protease could be purified, it could then be tested to find out whether it was associated with necrotoxic activity or not.

Proteases from many microbes such as *Pseudomonas aeruginosa*, *Serratia marcescens*, *Vibrio vulnificus* and *Legionella pneumophila* have been isolated and characterized (26-29). These proteases possess necrotizing factors, vascular enhancing factors and

cytotoxic factors (30-34,29) and seem to be important factors contributing to the virulence of the organisms. *Vibrio cholerae* protease possesses hemagglutinating activity and hydrolyzes fibronectin and mucin (35-37). It is believed that enteric *Bacteriodes.sp.* produces proteases which destroys maltase and sucrase, then causes disruption of brush borders (38).

The purpose of this study was to isolate and to purify extracellular protease from *P. pseudomallei* as an initial step in defining its role in pathogenesis. The specific aims were the followings :

1. to study the protease production of *P. pseudomallei* from clinical isolates.
2. to prepare purified protease from *P. pseudomallei*.
3. to characterize certain physicochemical properties of purified *P. pseudomallei* protease.
4. to assess the protease activity against some biological substrates.