CHAPTER 1

INTRODUCTION

1.1 Principle, Rationale and Hypotheses.

Actinobacillus pleuropneumoniae (*A. pleuropneumoniae*) is the major etiologic agent of swine pleuropneumonia in pigs of all ages. Swine pleuropneumonia, a severe and contagious pulmonary disease of pigs, causes significant economic losses in the industrialized swine production countries (Frey, 1995, Taylor, 1999). *A. pleuropneumoniae* is a gram-negative bacteria of the *Pasteurellaceae* family. *A. pleuropneumoniae* can be categorized into 2 groups on the basis of nicotinamide adenine dinucleotide (NAD) or so called "*V* factor" requirement for their growth (Frey, 1995, Møller and Kilian, 1990, Taylor, 1999). Biovar 1 is the NAD dependent strains and biovar 2 is the NAD independent strains. By using the capsular polysaccharides (CPS) and the lipopolysaccharides (LPS), Biovar 1 can be categorized into 12 serotypes including serotype 5a and 5b. Biovar 2 shares the antigenic characterization with serotype 2, 4, 7 and 9 of biovar 1 (Frey, 1999, Taylor, 1999). There are significant differences in virulence among 12 serotypes of biovar 1. Recently, serotype 13, 14 and 15 have been reported (Schaller et al., 2001, Blackall et al., 2002). Serotype 1, 5, 9 and 11 of biovar 1 are involved in the severe outbreaks with high mortality and morbidity rate. The other serotypes are generally less virulent

and less mortality. The virulence of *A. pleuropneumoniae* depends on many factors, such as capsular polysaccharides, lipopolysaccharides, adhesion factors, membrane proteins and exotoxins (Bosse' et al., 2002, Frey, 1995, Gerlach et al., 1992, Haesebrouck et al, 1997). The exotoxin producing by *A. pleuropneumoniae* are ApxI, ApxII, ApxIII, and ApxIVA toxin, respectively (Beck et al., 1994, Cho and Chae, 2001a, 2001b, Frey, 1995, Jansen et al., 1993a, 1993b, Schaller et al., 1999, 2000, 2001). *A. pleuropneumoniae* can be found in tonsils of infected pigs (Gram et al., 1996, 1998). In pig's lung tissue, *A. pleuropneumoniae* are phagocytized by alveolar macrophage and polymorphonuclear leukocytes (Cruijsen et al., 1992, Jacobsen et al., 1996, Lohansson et al., 2003, van de Kerkhof et al., 1996, van Overbek et al., 2002). However, *A. pleuropneumoniae* can produce their belonging exotoxins to destroy tissue surrounding and cause toxemia by absorption of toxin into the circulation (Frey, 1995, Kamp et al., 1997, Taylor, 1999). The clinical signs of this disease, depending on pathogenic serotype and its exotoxin, can be either peracute and acute disease characterized by extensive and fibrinohemorrhagic lung or chronic disease characterized by localized, necrotizing and associated with pleuritis (Jacobsen et al., 1996, Taylor, 1999).

The subclinical infected pigs are the particular reservoir host in the swineherds (Taylor, 1999). These pigs can not be identified by bacterial cultivation neither nor serological tests but can be possibly detected by polymerase chain reaction assay (Gram et al., 1996, 1998, Taylor, 1999). Several conserved genes were used for identifying *A. pleuropneumoniae* by multiplex PCR based on the *apx* toxin gene and the outer membrane lipoprotein (*omlA*) gene (Gram et al., 2000a). The *apx* toxin genes can be detected in *A. pleuropneumoniae* consisted of *apxI*, *apxII*, *apxIII* and

apxIVA genes, respectively (Beck et al., 1994, Frey, 1995). Epidemiological data of *A. pleuropneumoniae* outbreaks and observed serotypes in Thailand indicated that the serotype 1, 2, 3, 5, and 7, were the commonly found serotypes (Sakpuaram, 1990). During 1997-1998 outbreaks serotypes in Thailand were consisted of serotype 1, 2, 5 and 7, respectively (Sakpuaram, unpublished data).

The aims of this study was using the *apxI, apxII, apxIII* and *apxIVA* gene of *A. pleuropneumoniae* to design a specific primer set for directly detection and serotype identification from fresh swine pleuropneumonic lung samples by using the polymerase chain reaction.

Objectives of this study were:

1. to develop PCR assay for the detection of reference strains pure cultures of *A. pleuropneumoniae*
2. to develop PCR assay for the serotype identification of *A. pleuropneumoniae*
3. to apply both PCR assays for the detection and serotype identification to swine pleuropneumonic lung samples
4. to apply PCR assay for the serotype identification to field isolates
5. to determine the detectability level and the accuracy

Hypotheses:

1. The new PCR assay yield similar results to bacterial culture and the rapid slide agglutination test (SAT)
2. The new PCR assay can identify *A. pleuropneumoniae* in various samples such as pure culture of *A. pleuropneumoniae* 13 reference strains, swine pleuropneumonic lung samples and field isolates.

1.2 Purposes of the Study

The aims of this study was to develop the PCR assay for the detection and the serotyping of *A. pleuropneumoniae* using a specific set of primers designated for the *apxI, apxII, apxIII* and *apxIVA* genes of *A. pleuropneumoniae*. The assay was directly performed using pure culture and the fresh swine pleuropneumonic lungs. The detectability level and the accuracy of this assay were also determined.

1.3 Educational Advantages

Our developed PCR may provide an alternative tool for the routine detection and the serotype identification of *A. pleuropneumoniae* with the nested PCR and the multiplex PCR, respectively. This PCR method may be performed in a routine diagnosis laboratory work using both pure single colony and fresh swine pleuropneumonic lung samples.