CHAPTER I

INTRODUCTION

1.1 Background / Problem

Melioidosis is a serious infectious disease with high mortality rate caused by *Burkholderia pseudomallei*, an environmental aerobic Gram-negative bacillus. The endemic area of melioidosis is the most common in Northern Australia, Southeast Asia, especially in Northeast Thailand. The infection in humans typically results from contact with contaminated environmental sources (soils and surface waters) by inoculation, ingestion, or inhalation (Wiersinga et al., 2006). Rice paddy workers are one of the highest risk occupational groups for contracting melioidosis. The clinical presentation following *B. pseudomallei* infection is broad and includes nonspecific symptoms range from skin and soft tissue abscesses to acute pneumonia and septicemia with fatal outcomes. The acute septicemic can occur following an incubation period of a few days and its clinical presentations are similar to other conditions such as malaria, enteric fever, typhus, leptospirosis making them difficult to differentiate. The chronic type may occur newly or follow the subacute type, either directly or as a recurrence after treatment. The illness can progress slowly and lasts over months to years, and is characterised by chronic abscesses, closely mimicking tuberculosis (Karunanayake, 2022) and chronic lung disease can also occur and can be difficult to distinguish from pulmonary tuberculosis. Hence, early diagnosis of *B. pseudomallei* infection can lead to timely treatment and the administration of appropriate medication, ultimately contributing to saving lives. Identification of *B. pseudomallei* from clinical specimens in a hospital laboratory is typically done through laboratory tests followed by biochemical identification. Although this technique is specific and relatively inexpensive, definitive identification of *B. pseudomallei* requires expertise and can be time-consuming (5 to 7 days). The culture method is not a perfect gold standard for detecting *B. pseudomallei* in clinical samples due to its low sensitivity and the presence of unculturable forms of the organism that have been associated

with previous antibiotic treatment in some patients (Selvam et al., 2021). To achieve early diagnosis of melioidosis, other techniques have been developed for detecting *B. pseudomallei* in clinical specimens. For instance, immunological tests such as immunofluorescent assays (IFAs) and molecular methods such as real-time PCR and loop-mediated amplification (LAMP) have been used. However, these methods require expensive instruments and professional operation, which limits their application in the field.

Recently, nucleic acid detection technology based on clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) has been developed. This technology has the advantages of being rapid, simple, and low cost. The detection relies on the target-activated nonspecific endonuclease activity of Cas12a after binding to a specific target DNA via programmable guide RNAs (Jolany Vangah et al., 2020). By combining the programmable specificity of Cas12a with a reporter molecule that is activated upon target recognition, these enzymes result in specific and sensitive indications of the presence or quantity of nucleic acid. CRISPR/Cas-based diagnostic technology has been successfully applied to detect a variety of highly pathogenic viruses, such as Zika virus (ZIKV), Dengue virus (DENV), human papillomavirus (HPV), and Avian Influenza A Virus (H7N9) (Wang et al., 2020). In this study, we will develop a rapid diagnosis tool based on a molecular detection system for *B. pseudomallei* using CRISPR-CAS12a technology. In this study we selected *orf2* and *orf11* from T3SS-1 gene clusters of T3SSs. The T3SS-1 gene cluster is present only in *B. pseudomallei* and not in avirulent *B. thailandensis*.

Furthermore, *orf2* was found to be present in *B. pseudomallei* and not in the related *B. mallei* or *B. thailandensis* while *orf11* is a specific marker for *B. pseudomallei* (Thibault et al., 2004)

1.2 Research objectives

1.2.1 To design a specific guided RNA for genes encoding components of the type three secretion systems (T3SS) of *B. pseudomallei.*

1.2.2 To validate an appropriate condition for *B. pseudomallei* DNA sample preparation.

1.2.3 To determine the specificity of the designed guided RNA using *in vitro* digestion of DNA of *B. pseudomallei* with CRISPR-Cas12a.

1.3 Research hypothesis

CRISPR-Cas12a technology incorporating the endonuclease CAS12a, and a specific guided RNA could be used for the development of a rapid diagnosis method for *B. pseudomallei* infection.

1.4 Scope and limitation of the study

In this study, we used *orf2* and *orf11* located in the T3SS of *B. pseudomallei*, the specific genes were used to design a guided RNA (gRNA) complementary to the target site as well as a 5' TTTV protospacer adjacent motif (PAM) on the DNA strand opposite the target sequence. DNA sample of *B. pseudomallei* were prepared using a modifying boiling procedure method that involved thermal lysis, and DNA precipitation and isolation. After that, the specificity of the guided RNA was determined by comparing the endonuclease CAS12a digestion reaction of *B. pseudomallei* genomic DNA with another Gram-negative bacilli (*Burkholderia thailandensis*) DNA. Finally, the sensitivity of the guided RNA was determined by varying the concentration of *B. pseudomallei* DNA.

1.5 Expected results

CRISPR-Cas12a technology could be applied for development of a diagnosis test for *B. pseudomallei* infection and provide a high sensitivity and specificity for the bacterial detection.