CHAPTER I

INTRODUCTION

1.1 BACKGROUND

The rapid emergence of antibiotic resistant pathogenic bacteria has increased medical concerns in the past decades. Since the first finding of antibiotic, penicillin, by Alexander Fleming in 1928, the golden age of antibiotic has improved human health. Despite so, this revolution also led to drug resistant bacteria in almost all developed antibiotic (Liu & Pop, 2009; Ventola, 2015; Walsh & Wencewicz, 2014). Under the appearance of bacteria resistance phenomena, medical world has been brought into a race in drug innovation and bacterial resistant where resistance can appear in less than 50 years (Walsh & Wencewicz, 2014). The resistance emerged due to misuse of antibiotic (overuse, inappropriate prescription, and uncontrolled agricultural used) in a wide community. The inappropriate antibiotic usage is also supported with lack of new drugs development due to economic difficulties, long processes, and regulatory barriers (Ventola, 2015; Walsh & Wencewicz, 2014). Furthermore, repetition of drug mechanism which targets translation, DNA replication, cell wall synthesis, and folate biosynthesis have led bacteria to develop mutation and resistant genes (K. Blount, Puskarz, Penchovsky, & Breaker, 2006). According to Antibiotic Resistance Gene Database (ARDB), there are more than 13.000 resistant genes to more than 200 antibiotics in 2008 which pushes concern and importance of new antibiotics (Liu & Pop, 2009).

There are many unexplored targets in antibiotic development. One of the most promising target is ribonucleic acid (RNA)-targeting antibiotics. RNA has long known to be the carrier of genetic information for protein translation. However, recent reports have shown interesting properties of RNA that was found to be able to fold into 3D structure, bind to ligand, and undergo structural changes upon binding. These properties showed versatile ability of RNA in gene regulation. One example of RNA gene regulation can be found in riboswitches, a regulator in mRNA level in bacteria

(Deigan & Ferré-D'Amaré, 2011; Kirchner, Schorpp, Hadian, & Schneider, 2017; Winkler, Nahvi, Roth, Collins, & Breaker, 2004).

Riboswitches are regulatory elements located in 5' untranslated region (UTR) of their regulated gene. In general, riboswitches have two domains: aptamer and expression domain. The aptamer domain enables RNA to recognize small metabolites such as uncharged tRNA, secondary messenger, amino acid, and metal (Collins, Irnov, Baker, & Winkler, 2007; Kirchner & Schneider, 2017; Machtel, Bąkowska-Żywicka, & Żywicki, 2016). This aptamer domain is unique to each riboswitch class and a highly conserved region (McCown, Roth, & Breaker, 2011). Upon binding to its ligand, riboswitch regulates transcription, translation, or mRNA splicing through conformational change. In transcription level, conformational change led to rho-independent terminator formation, while in translational level riboswitch regulates gene by modifying ribosome binding site or start codon availability (Kirchner & Schneider, 2017; McCown et al., 2011). To date, there are 17 classes of riboswitch in 36 human pathogenic bacteria. Among these classes, *glmS* riboswitch is recently identified in 5' UTR of *glmS* gene in *Bacillus subtilis* which showed different mechanism to other riboswitches (Collins et al., 2007; Winkler et al., 2004).

The *glmS* riboswitch controls *glmS* gene, which encodes for glucosamine-6-phosphate synthase. This enzyme produces glucosamine-6-phosphate (GlcN6P) and glutamate from fructose-6-phosphate and glutamine. GlcN6P plays an important role in cell wall synthesis pathway as uridine 5'-diphospho-*N*-acetyl-D-glucosamine (UDP-GlcNAc). GlcN6P also functions as *glmS* riboswitch activator. Upon binding, *glmS* riboswitch induces self-clevage resulting in 2',3'-cyclic phosphate and 5' hydroxyl fragments. The 5' hydroxyl fragment which linked to *glmS* gene is then degraded by RNaseJ1 (K. Blount et al., 2006; Collins et al., 2007; Kalamorz, Reichenbach, März, Rak, & Görke, 2007; Machtel et al., 2016; Winkler et al., 2004). Due to its important role in cell wall biosynthesis, *glmS* riboswitch is shown to be a potential drug target. However, there is no developed *in vivo* screening system for *glmS* riboswitch drug discovery. Current screening systems are mostly based on fluorescence which is high in cost and low in detection sensitivity (Zhao et al., 2014). By utilizing *in vivo* screening system, it

can reduce cost, while increasing sensitivity detection as *in vivo* system is able to mimic biological events (Kirchner et al., 2017; Zang, Li, Tang, Wang, & Yang, 2012).

1.2 OBJECTIVE

The main project is currently carried out at Protein-ligand Engineering and Molecular Biology (MPMB) laboratory, BIOTEC, Thailand. The objective of the project is to establish *Escherichia coli* screening system to identify compounds that can activate *glmS* riboswitch activity. This thesis is a part of the ongoing research project and scopes of work in this thesis include:

1. To optimize production of RNaseJ1 in *E. coli*
2. To investigate RNaseJ1 function
3. To validate *glmS* riboswitch activity in the established system