CHAPTER I

INTRODUCTION

1.1 Background/Problem

The research of nanotechnology is one of the most important developing fields of modern study, with applications in technology and science to manufacture new materials at the nanoscale level (Albrecht et al., 2006). Metallic nanoparticle sizes are ranged from 1 to 100 nm (Alanazi et al., 2010). Among all metallic nanoparticles, gold nanoparticles (AuNPs) have stable nature, unique surface morphologies, large surface area, controlled geometry, and high properties of dispersion, catalytic, optical, magnetic, electrical, biocompatibility, and non-toxicity (Yee et al., 2015). Gold nanoparticles have been used in various applications, including optical devices, food, synthetic biology, cellular transportation, and health care (Mohanpuria et al., 2008), biosensor, optical bio-imaging, immune-analysis, detection, drug delivery, and photo-thermolysis of cancer cells and microorganisms (Dykman and Khlebtsov, 2011) and they are applied for treatments, diagnosis, and detection of several diseases (Alvarez et al., 2016; Khan et al., 2014). Besides, the antimicrobial activity of gold nanoparticles has been investigated (Geethalakshmi and Sarada, 2013).

The antibacterial properties of metallic nanoparticles mostly attach to the surface of the microorganism's cell. Thus, the cell has structural changes across the plasma membrane, resulting in distorted membranes (Li et al., 2010). Moreover, metallic nanoparticles also help to enhance in redox processes of the gene expression, depressing the activity of respiratory chain enzymes leading to cell death (Sharma et al., 2009). The major antibacterial activity mechanisms of nanomaterial are described as followed: disruption of energy transduction, inhibition of cell membrane/wall synthesis, production of toxic reactive oxygen species (ROS), enzyme inhibition, photocatalysis, and reduced DNA production (Figure 1.1) (Singh et al., 2014;

Weir et al., 2008). Gold nanoparticles could probably have the antibacterial mechanism, such as accumulation at cell surfaces, heavy electrostatic attraction, and interaction with the cell membrane (Chamundeeswari et al., 2010; Johnston et al., 2010).

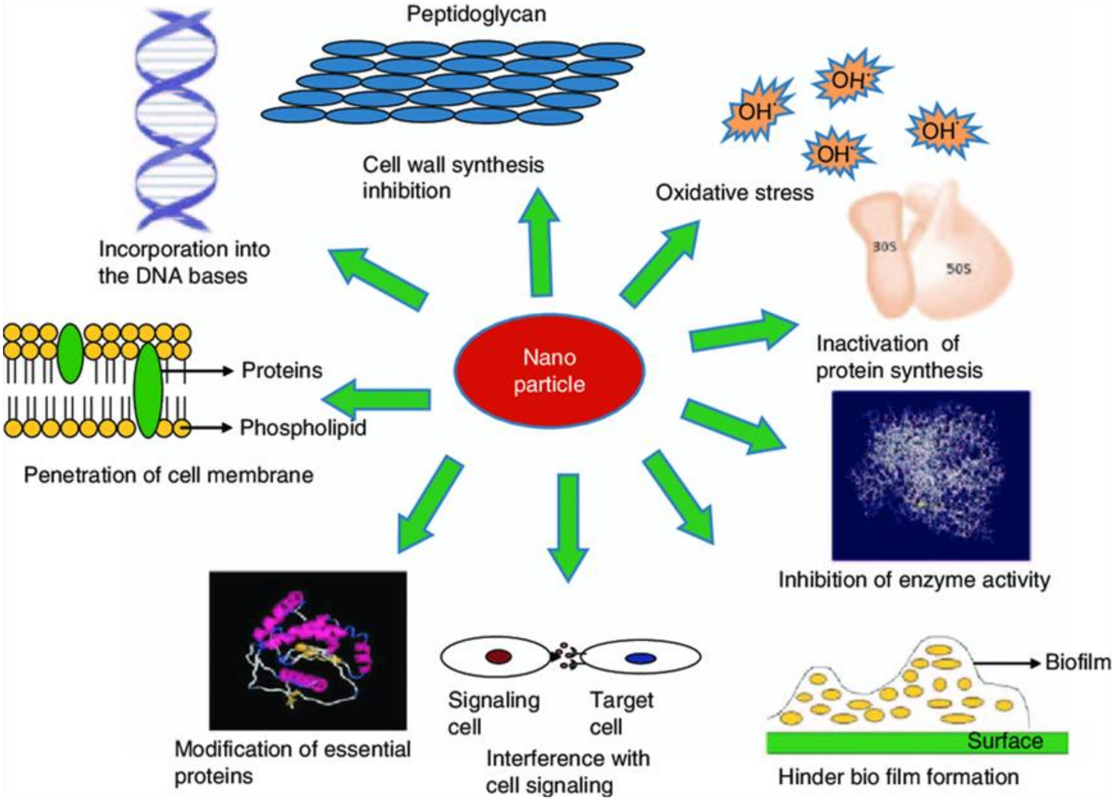

Figure 1.1 Mechanisms for antibacterial activity of nanoparticles (Singh et al., 2014).

Antibacterial activities of gold nanoparticles have been reported against both Gram-positive and Gram-negative bacteria. The most common antibacterial activity against antibiotic-resistant bacteria of gold nanoparticles is *Acinetobacter baumannii*, *Enterrococcus faecium*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (Rice, 2010). Several gold nanoparticles are synthesized, and their antibacterial activity have been evaluated (Monic et al., 2014). The gold nanomaterial is recognized as a multifunctional material useful for a wide variety of applications. Because of its shape and size, it has a high biological, chemical, and physical value (Dreaden et al., 2012). The development of new materials or new methodologies for controlling the synthesis of gold nanoparticles is important for several research applications (Grubbs, 2007; Tang et al., 2002; Wong et al., 1998).

Several methods have been used to precisely synthesize nanoparticles in terms of shape, size, and properties such as physical (laser ablation, sonication, and radiation), chemical (sol-gel method, condensation, and lactic acid method), and biosynthesis methods (plant, bacteria, and fungi). However, the synthesis processes in the chemical or physical methods involve the use of stringent synthetic conditions, toxic solvents and chemicals, and non-eco-friendly protocol which was raised toxic chemicals in nature for human consumption and toxic to the environment. In contrast, the biological method of nanoparticles is clean, safe, non-toxic, dynamic and energy-efficient, bio-compatible, economical, and ecofriendly acceptable procedures concerning microorganisms (Khadivi et al., 2012). Moreover, microorganisms can be grown in a low-cost medium, maintaining the safety level by reducing the metal ion into nanoparticles by an enzyme produced by metallic processes (Kumar et al., 2014).

Actinobacteria are Gram-positive, aerobic, non-motile, filamentous bacteria that are commercially interested in their unrivaled ability to produce a wide range of bioactive secondary metabolites and extracellular enzymes with interesting biological activities (Kumar et al., 2014; Zotchev, 2012). Among the Actinobacteria, the *Streptomyces* group is considered the most economically important, because more than 50–55% of bioactive secondary metabolites producing antibiotics of the order Actinomycetales are produced by *Streptomyces* (Chater, 1993; Manivasagan et al., 2014). Only a few *Streptomyces* species have been reported for biosynthesis of AuNPs. The *Streptomyces* isolated from different ecosystems has been accepted as the potential biosynthesis of metal nanoparticles, such as *Streptomyces hygroscopicus* (Sadhasivam et al., 2012), *Streptomyces viridogens* (HM10) (Balagurunathan et al., 2011), *Streptomyces naganishii* (MA7) (Shanmugasundaram et al., 2013), *Streptomyces* spp. (Karthik et al., 2013b) and *Streptomyces avidinii* (Park et al., 2006).

This study was focused on the biosynthesis of the gold nanoparticle using *Streptomyces* sp. The biosynthesized gold nanoparticles were used to investigate antimicrobial activity against Gram-positive and Gram-negative bacteria using the agar well diffusion method. The biosynthesis of gold nanoparticles was characterized using UV–visible spectroscopy, X-ray diffraction (XRD) spectroscopy, Energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM) Fourier transform infrared (FTIR) spectroscopy, and X-ray absorption near edge structure (XANES) spectroscopy.

1.2 Research objectives

a) To isolate and identify *Streptomyces* from terrestrial soil
b) To synthesize AuNPs by a green synthesis method using fermented broth of *Streptomyces* sp. MSK03 and MSK05
c) To characterize AuNPs and evaluate their antimicrobial activity against pathogenic microorganisms

1.3 Research hypothesis

The soil-isolated *Streptomyces* sp. MSK03 and MSK05 have the ability to synthesize AuNPs and the biosynthesized AuNPs exhibit antimicrobial activity against tested pathogens.

1.4 Scope and limitation of the study

This work involved the biosynthesis of AuNPs using *Streptomyces* spp. isolated from terrestrial soil in Nakhon Ratchasima province, Thailand. *Streptomyces* sp. (MSK03 and MSK05) was identified based on cultural characteristics and 16S rRNA gene analysis. AuNPs were tested for antimicrobial activity against tested pathogens such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Serratia marcescense*, *Klebsiella pneumonia*, *Proteus mirabilis*, *salmonella typhi, Enterobacter aerogenes, Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus* *epidermidis* (MRSE). The biosynthesized AuNPs was characterized by using UV–visible, XRD, EDX, TEM, FTIR, and XANES spectroscopy.