CHAPTER I
INTRODUCTION

1.1 Significance and Background

The discovery and utilization of one of the first the first antibiotic, Salvarsan, in 1910 extended our past human lifespan by 23 years (Adedeji, 2016; Hutchings et al., 2019). The golden age of antibiotic discovery initiated in 1928, when Alexander Fleming found Penicillin and revolutionized our medical treatment of microorganism infections (Demain and Elander, 1999; Fleming, 1929). Besides the decline in antibiotics discovery and development rate (Katz and Baltz, 2016), misuse of antibiotics lead to a rapid rise in multidrug resistance (MDR) making the treatment of various infections tougher (Prescott, 2014). Nowadays, we still seek new antibiotics to treat infections caused by diverse MDR pathogens e.g., *Acinetobacter baumannii*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, etc. which happens to be one major threat to human life (Appelbaum, 2006). MDR has been a global health challenge as well as an expense that threatens our ability to control and treat life-threatening infections (Liu et al., 2021). Moreover, there have been reports that during the COVID-19 pandemic overall incident density (ID) of MDR infections increased by 23% mainly from methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* (Polly et al., 2022). Failure of the primary antimicrobial treatment results from multidrug-resistant bacteria increases the risk of secondary complications and death. Treatment is then forced to switch to second- or third-line drugs, which are much more expensive and more toxic as well (World Health Organization. Regional Office for South-East, 2011).

Bacteria in the genus *Streptomyces* have been reported to have a fascinating complex secondary metabolite (Wu et al., 2005) which is a major promising source of novel antibiotics (Challis and Hopwood, 2003) as the matter of fact that two-thirds of our currently used natured-based antibiotics came from this genus (Papagianni, 2012).

In order to trigger/increase antibiotic production, mimicking natural conditions where more than one bacterial species habits together could be done in the laboratory via co-cultivation method which involves repeating exposure of *Streptomyces* spp. to MDR pathogens (De Roy et al., 2014).

1.2 Research Objectives

This research aimed to investigate the potential of adaptive laboratory evolution (ALE) through co-cultivation with drug-resistant pathogens to enhance antibiotic production in *Streptomyces* sp. SSUT88A and to explore the underlying genetic changes associated with this adaptation.

1.2.1 Study the effect of adaptive laboratory evolution using co-cultivation method to trigger the production of antibiotics of *Streptomyces* sp. SSUT88A against drug-resistant bacteria.

1.2.2 Evaluate antimicrobial activities of crude compounds of wild-type and adapted strains of SSUT88A.

1.2.3 Investigate genetic background of adapted strains of SSUT88A compares to wild-type using whole genome sequencing and bioinformatics analysis.

1.3 Research Scope

This research will be using *Streptomyces* strain SSUT88A co-cultivate (both bi-culture and quadri-culture) with clinical isolate drug-resistant pathogens *Acinetobacter baumannii* SUTH-Isolate, methicillin-resistant *Staphylococcus aureus* (MRSA) DMST20651 and *Pseudomonas aeruginosa* SUTH-Isolate N90PS for 9 cycles, 10 days/cycle. Determination of adaptive laboratory evolution will be indicated via antimicrobial activities compared to wild-type strain (monoculture). The antimicrobial activity of crude extract will be tested with 16 human pathogens, listed in the material section, using agar well diffusion method. The genetic background of the most adapted strains based on antimicrobial activity improvement against MDR pathogens were analyzed compares to the wild-type strain using whole genome sequencing.

1.4 Research Hypothesis

Adaptive laboratory evolution using co-cultivation with drug-resistant bacteria can stimulate *Streptomyces* sp. SSUT88A to produce antibiotics with enhanced activity. This adaptation would be associated with genetic variations that contribute to increased antimicrobial production or stress response mechanisms.