CHAPTER II

LITERATURE REVIEW

2.1 Antibiotics

2.1.1 Definition and Classification

The term antibiotics was first used in 1942 by Selman Waksman and his collaborators to describe any substance produced by a microorganism that opposes/inhibits the growth of other microorganisms in high dilution (Waksman, 1947).

This definition is then redefined as - any medications which kill or inhibit growth of microorganisms - to include synthetic antibiotics.

Three types of antibiotics were classified based on production: (1) natural products which are the antibiotics produced solely from microorganisms themselves, for instance, penicillin, cephalosporins; (2) semi-synthetic products which are modified natural substances, like carbapenems; and (3) fully synthetic products which are medications that are 100% synthesized from commercially available precursors e.g., the sulfonamides, the quinolones, and the oxazolidinones (von Nussbaum et al., 2006).

The other way to classify antibiotics is based on their mechanism of action which could categorize them into

- Cell wall synthesis inhibitors/disruptors: primarily prevent peptidoglycan synthesis consequently led to bacterium lysis e.g., Cycloserine, Vancomycin, Bacitracin, Penicillin, Cephalosporins, Monobactams, Carbapenems, etc. (Kapoor et al., 2017)
- Cell membrane disruption: binding or integrating into the lipid moiety of the plasma membrane e.g., Polymyxins, Daptomycins, etc. (Etebu and Arikekpar, 2016)

- Nucleic acid synthesis inhibition: suppress DNA or RNA synthesis e.g., Nalidixic acid, Ciprofloxacin, Novobiocin, Actinomycin, Rifampin, Streptovaricins, etc.
- Protein synthesis inhibitors: targeting small and large subunit of ribosome by various approach accordingly blocking protein synthesis e.g., Erythromycin, Chloramphinicol, Clindamycin, Lincomycin, Tetracyclines, Streptomycins, Gentamycin, Kanamycin, Puromycin, Amikacins, Nitrofurans, etc.
- Metabolic pathway inhibitors: by mimicking or bonding with substrate within metabolic pathway and ultimately suppress the production of essential molecules e.g., Sulfonamides, Trimethoprim, Izoniacid, etc.

  (Etebu and Arikekpar, 2016)

2.1.2 Brief History of Antibiotics

In 1910 Paul Ehrlich developed the synthetic arsenic-based drugs salvarsan (salvation arsenic) and neo-salvarsan circa for treating syphilis caused by *Treponema pallidum* (Gelpi et al., 2015). Salvarsan had been widely used as a broad-spectrum antibiotic until the discovery of penicillin by Alexander Fleming who observed blue-green mold contaminated on *Staphylococcus aureus* petri dish in 1928 (Fleming, 1929). Since Gramicidin was discovered from soil bacterium, *Bacillus brevis*, soil bacteria have gained interest as a potential approach to finding novel antibiotics (Andersen, 1984).

The introduction of antibiotics into clinical use was conceivably the substantial medical improvement of the 20th century. From the 1930s to the 1960s, the discovery of antibiotics accelerated rapidly, leading to what is known as the "Golden Age of Antibiotics." During this period, numerous groundbreaking compounds such as penicillin and streptomycin were introduced, transforming the treatment of bacterial infections. However, this era also marked the first observations of antibiotic-

resistant strains, signaling the beginning of the ongoing challenge posed by antimicrobial resistance (AMR) (Figure 2.1).

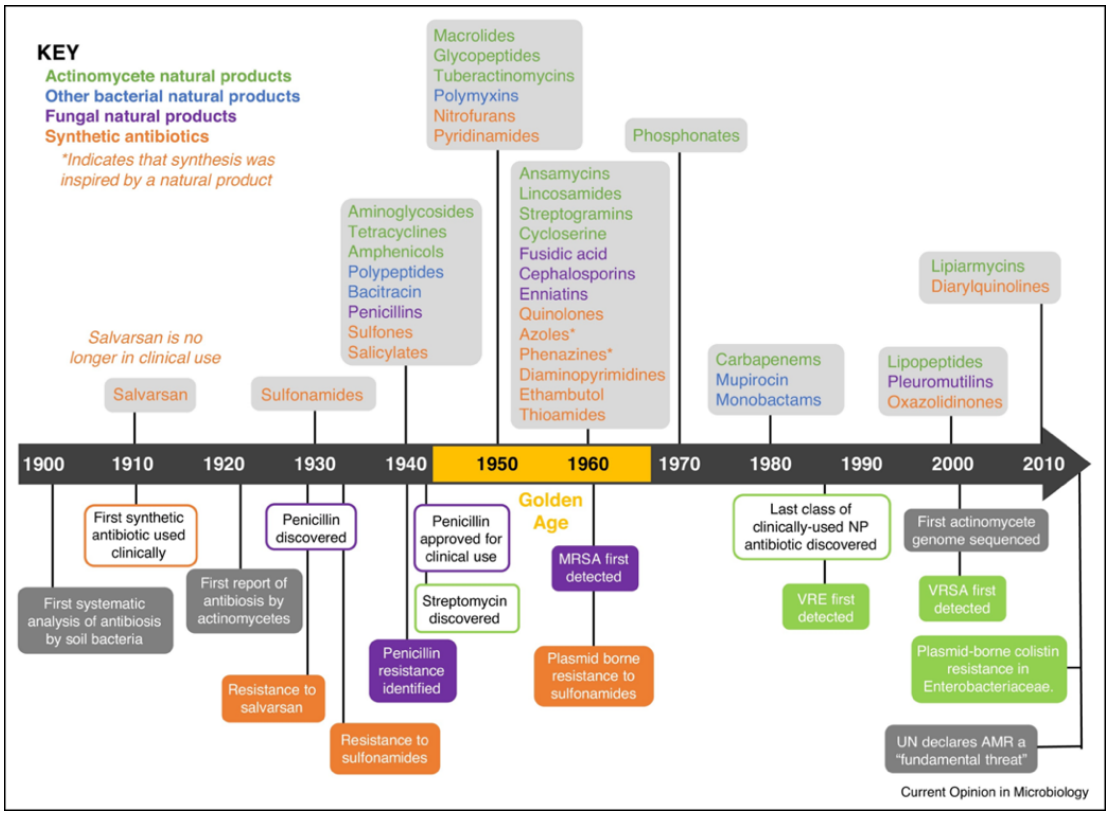

Figure 2.1 Timeline illustrates the decade of antibiotics development. Colors indicate source of each antibiotic – green = actinomycetes, blue = other bacteria, purple = fungi and orange = synthetic. With MDR pathogens reported including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA) and plasmid-borne colistin resistance in Enterobacteriaceae (Hutchings et al., 2019).

2.2 Antibiotics Producing Microorganisms

Following the discovery of penicillin, the first natural antibiotic, many antibiotic production techniques have been established. Scientists continued to seek further new antibiotics to combat infectious diseases. Nowadays most of clinically used antibiotics

are produced/derived from microorganisms including bacteria and fungi. Within the microorganism part, actinomycetes is considered as a major group of antibiotic producers, accounting for approximately 64 percent of all antibiotics producing industries (Figure 2.2). They are gram-positive filamentous bacteria that form a branching network of filaments and produce spores. They grow through both extension and branching of hyphal tip, hence giving them their name “Actinomycete” derived from the Greek words for "ray" (aktis or aktin) and "fungi" (mukēs). Actinomycetes are abundant in soil and marine sediments (Barka et al., 2016).

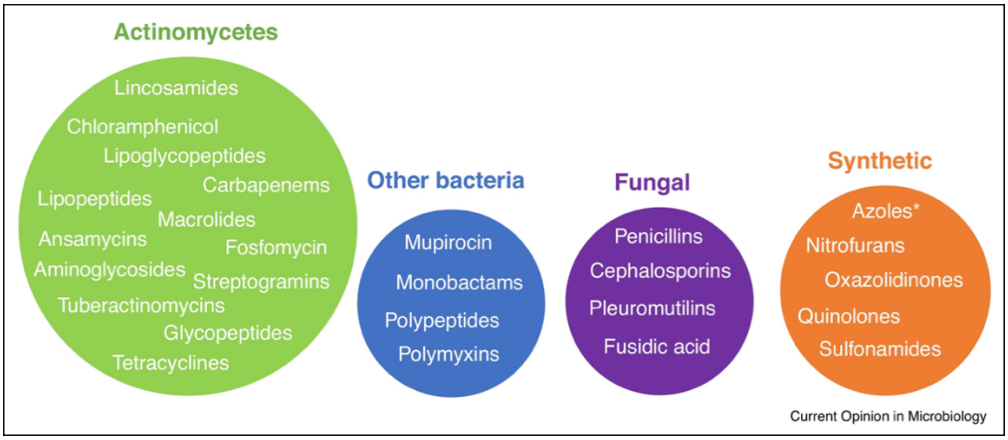

Figure 2.2 The majority of clinically used classes of antibiotics are derived from natural products mainly actinomycetes (Hutchings et al., 2019).

Within the phylum Actinobacteria, the genus *Streptomyces* exhibits a remarkable capacity to produce antibiotics. The attention to *Streptomyces* initially dated back to the discovery of actinomycin and streptomycin in the 1940s (de Lima Procópio et al., 2012) and was further intensified when Benjamin Minge Duggar identified tetracycline, a broad-spectrum antibiotic, from *Streptomyces aureofaciens* isolated from campus soil in the United States (Nelson and Levy, 2011).

Following then, *Streptomyces* remained a promising source of several currently therapeutic antibiotics (Table 2.1).

Table 2.1 List of currently used antibiotics derived from *Streptomyces* species and their mechanism of action (de Lima Procópio et al., 2012).

Antibiotics	***Streptomyces* sp.**	**Mechanism of Action**
Cephalosporin	*S. clavuligerus*	Cell Wall Synthesis Inhibition
Neomycin	*S. fradiae*	
Vancomycin	*S. orientalis*	
Phosphomimic	*S. fradiae*	
Nystatin	*S. noursei*	Cell Membrane Disruption
Daptomycin	*S. rosesporus*	
Novobiocin	*S. niveus*	Inhibits Nucleic Acid Synthesis and Metabolism
Streptomycin	*S. griseus*	Inhibits Protein Synthesis
Chloramphenicol	*S. venezualae*	
Tetracyclin	*S. aurofaciens*	
Viomycin	*S. vinaceus* & *S. capreolus*	
Virginiamycin	*S. pristinaespirallis* & *S. virginae*	
Erythromycin	*S. erythreus*	
Lincomycin	*S. lincolnensis*	
Kanamycin	*S. kanamyceticus*	
Ribostamycin	*S. ribosidificus*	

2.2.1 The Genus *Streptomyces*

Streptomyces is the largest genus of phylum Actinobacteria. They are complex bacteria having various notable characteristics for instance aerobic respiration, gram-positive, high GC content, filamentous, spore-producing bacteria. Cell wall type I distinguished by the presence of *LL* form α, ε-diaminopimelic acid (*LL*-DAP) composition and the lack of characteristic sugars of the cell wall is exclusively found in *Streptomyces* which differ them form other genera of Actinobacteria (Ramasamy and Sudalaimuthu, 2022). They are prevalent in soil microbiomes and marine sediments (Khanna and Solanki, 2012). Another unique trait of streptomycetes is the production of substrate and aerial mycelia, which is comparable to that of fungi. Their aged aerial mycelia (hyphae) will form multinucleated mycelia which could undergo synchronous cell division and differentiate into spores (Law et al., 2019; Ohnishi et al., 2008). When the spores reached the favorable temperature, nutritional, and moisture conditions, the germ tube developed, and a new bacterium emerged (Figure 2.3). The color of the colony with diffusible pigments could be utilized to roughly differentiate the species as well (Figure 2.4). They are well-known for their remarkable bioactive secondary metabolites for instance antifungals, antivirals, antitumoral, anti-hypertensives, and greatly antibiotics and immunosuppressants as well (Khan et al., 2011; Ōmura et al., 2001).

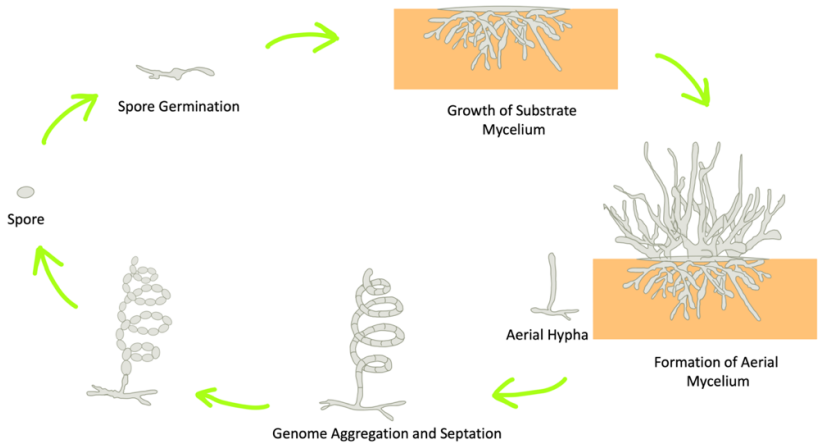

Figure 2.3 Illustration depicts the life cycle of Streptomycetes, adapted figure from Law et al., 2019 and Barka et al., 2016.

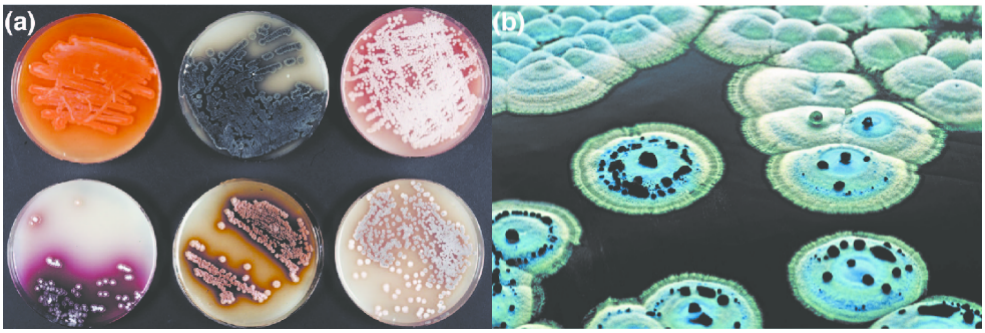

Figure 2.4 *Streptomyces* produce diversified pigments (Thompson et al., 2002).

2.3 Multidrug-Resistant (MDR) Pathogens

MDR bacteria are well-recognized to be one of the most important current public health problems. The Infectious Diseases Society of America (IDSA) identified antimicrobial resistance as "one of the greatest threats to human health worldwide" (Spellberg et al., 2011). For example, only methicillin-resistant *Staphylococcus aureus* (MRSA), ends more Americans' lives each year (~19,000) than emphysema, HIV/AIDS, Parkinson's disease, and homicide all combined (Klevens et al., 2007). In 2017, there are approximately 120,000 MRSA bloodstream infections and 20,000 MRSA-associated deaths in the United States (Kourtis et al., 2019). Failure of primary antimicrobial therapy results from multidrug-resistant bacteria increases the risk of complications and fatality. Second- or third-line drugs, which are far more expensive and toxic, are required to treat patients, yet they still raise treatment expenses (World Health Organization. Regional Office for South-East, 2011). It is estimated that failing to treat MDR infections would result in 10 million deaths per year by 2050, costing nearly 100 trillion US dollars (Neil, 2016). Shifting from non-MDR infection cases to MDR infection cases could raise the expenses for treatment by 42% per patient (Phodha et al., 2019). In Thailand, a study showed that in 2010 the annual cost of antibiotics used in the treatment of MDR infections ranged from 83 to 200 million US dollars and the additional cost due to the morbidity and mortality consequences from MDR infections were at least 1.3 billion US dollars (Phumart et al., 2012). Thus, MDR infection is an

important worldwide public health threat and should be approached abruptly and proactively (van Duin and Paterson, 2016).

2.4 Adaptive Laboratory Evolution: Co-Cultivation Method

Microorganisms can adapt to drastic alterations in their environment by mutation followed by natural selection, this phenomenon is known as “adaptive evolution” (Shi et al., 2021). Adaptive laboratory evolution is the term to describe protocol(s) of forcing the microorganisms to adapt to yield desired results (Boruta, 2021). Based on the fact that evolution and adaptation occur through generations which in higher organisms would take years, in microorganisms it could happen in days. They also have a significantly smaller genome than higher organisms thus one mutation delivers more observable results (Herron and Freeman, 2013). Therefore, the desirable traits can be chosen in the laboratory by manipulating microbial growth conditions. Any microorganisms lacking adaptability to the defined conditions will not be able to reproduce, leaving just the adaptive ones. For example, an experiment was conducted in which the carbon source of *E. coli* was altered by serially lowering the normal carbon supply and gradually increasing the new carbon source (glycerol or lactate) through time; as a result, *E. coli* was able to use glycerol or lactate as the sole carbon source (Conrad et al., 2009; Herring et al., 2006); Adaptive evolution has been applied to examine mechanisms of ethanol (Wang et al., 2011) and osmotic stress tolerance in *E. coli* as well (Stoebel et al., 2009). The competition between microbes for limiting natural resources such as oxygen, nutrients, space, in the microbiomes is believed to be the selective factor that promotes biosynthesis of secondary metabolites especially antimicrobial compounds (Slattery et al., 2001). In laboratory, the contamination from other strain tends to be avoided since pure cultivation is more common approach, to the extent that there are techniques developed by Robert Koch and colleagues to eliminate those contaminations. But interestingly, several studies have found that imitating the natural habitat of bacteria by 'co-cultivating' them with either natural co-

existing bacteria from the same isolated sample or pathogens might trigger/increase the synthesis of bioactive compounds (Harwani et al., 2018; Kurosawa et al., 2008; Oh et al., 2007; White Jr and Torres, 2009).

2.5 Bioinformatical Approach: Whole Genome Sequencing

Whole genome sequencing and adaptive laboratory evolution in combination is a robust technique in order to understand the underlying molecular mechanisms of the adapted strains (Dettman et al., 2012). Whole genome sequencing analysis was used to identify the polymorphism and further possible influence on adapted traits in various adaptive laboratory evolution experiment including the production of antibiotics by *Streptomyces* sp. (Harwani et al., 2022). For instance, the whole genome comparison between *Streptomyces clavuligerus* strain adapted by co-culture with methicillin-resistant *Staphylococcus aureus* N315 found to have 6 mutations different form wild-type. The mutations occur at various locations including loss of megaplasmid, malate dehydrogenase gene, glycosyl hydrolase gene, pyrroloquinoline quinone biosynthesis protein B gene, N-(5-carboxylpentanoyl)-L-cysteinyl-valine synthase gene (Charusanti et al., 2012).

2.6 Related Research

Previously, certain studies, especially from the early 2000s, reported the co-cultivation approach of *Streptomyces* spp. to promote the formation of bioactive metabolites (Harwani et al., 2018; Kurosawa et al., 2008; Oh et al., 2007; Ueda et al., 2000; White Jr and Torres, 2009). In this research, the co-cultivation method will be modified from Wu et al. (2015), and Harwani et al. (2022), both of which are related to co-culture adaptive laboratory evolution of *Streptomyces* spp. in liquid medium (Harwani et al., 2022; Wu et al., 2015). Since, the *Streptomyces* strain of interest, SSUT88A, was previously isolated and purified by a former graduated Ph.D. student in

Microbial Resources Laboratory, Suranaree University, Thailand. Based on 16s rRNA gene analysis, *Streptomyces* sp. SSUT88A had only 98.8% similarity to *Streptomyces chiangmaiensis* TA4-1^{T} and was only be used for single research purpose to mediate the green synthesis of silver nanoparticles (Rosyidah et al., 2022). Thus, neither SSUT88A nor *Streptomyces chiangmaiensis* TA4-1^{T} have been utilized in any adaptive laboratory evolution.