CHAPTER 2

LITERATURE REVIEW

2.1 General characteristics of actinomycetes

Actinomycetes are a group of prokaryotic organisms belonging to subdivision of the Gram-positive bacteria phylum. Most of them are in subclass *Actinobacteridae*, order *Actinomycetales*. All members of this order are characterized in part by high G+C content (>55 mol %) in their DNA (Stackbrandt *et al.*, 1997). They are filamentous bacteria which produce two kinds of branching mycelium, aerial mycelium and substrate mycelium. The aerial mycelium is important as the part of the organism that produces spores. For this reason they have been considered as fungi, as is reflected in their name, *akitino* means ray and *mykes* means mushroom/fungus, so actinomycete was called ray fungi. Actinomycetes are the most widely distributed group of microorganisms in nature and are also well known as saprophytic soil inhabitants (Takizawa *et al.,* 1993). The soil actinomycetes produce a volatile compound called geosmin, which literally translates to "earth smell" (Gust *et al.*, 2003). This organic compound is responsible for a contributor to the strong odor that occurs in the air when rain falls after a dry spell of weather. In natural habitats, *Streptomyces* are common and are usually a major component of the total actinomycetes population. Some actinomycete genera such as *Actinoplanes, Amycolatopsis, Catenuloplanes, Dactylosporangium, Kineospora, Microbispora, Micromonospora, Nonomuraea*, which are often very difficult to isolate and cultivate due to their slowly growth, are called rare actinomycetes (Hayakawa, 2008).

2.2 Distribution of actinomycetes in natural habitats

Actinomycetes have proven to be a rich source of important natural products especially antibiotics. Thus far, approximately 10,000 antibiotics have been found, and almost half of them are produced by *Streptomyces* that originated in the soil. (Lazzarini *et al*., 2000). Recently, the rate of discovery of new compounds from existing genera obtained from common soil has decreased therefore it is critical that novel actinomycetes from unexplored habitats such as marine, hot spring be pursued as sources of novel antibiotics and others bioactive compounds. The majorities of actinomycetes are free living and found widely distributed in many natural environments including various soil, freshwater habitat, marine habitat, organic matter habitats and colonizing plants.

2.2.1 Actinomycetes in aquatic environments

Actinomycetes are found to occur in aquatic environments; freshwater and marine habitats (Fenical and Jensen, 2006; Singh *et al*., 2006; Pathom-aree *et al*., 2006). In aquatic habitats, taxonomically diverse of actinomycetes exhibit unique physiological and structural characteristics that enable them to survive in extremes of pressure, salinity and temperature, with the potential production of novel secondary metabolites not observed in actinomycetes, isolated from terrestrial habitats (Radajewski *et al*., 2002). *Micromonospora* are the dominant actinomycetes isolated from several samples from streams, rivers, lake mud, river sediments, beach sands, sponge and marine sediments (Rifaat, 2003; Jensen *et al*., 2005a,b, Eccleston *et al*., 2008). Beside from *Micromonospora*, other actinomycetes genera were found from aquatic habitats such as *Amycolatopsis*, *Marinophilus, Rhodococcus, Salinispora,*

Streptomyces and *Williamsia* (Mincer *et al*., 2005; Williams *et al*., 2005; Kim *et al*., 2006; Kwon *et al*., 2006; Pathom-aree *et al*., 2006). Nowadays, several novel bioactive compounds were discovered from aquatic actinomyctes including rifamycin from *Micromonospora* (Jensen *et al*, 1991), the anticancer metabolite salinosporamide A from a *Salinispora* strain (Fehling *et al*., 2003), marinomycins from *Marinophilus* sp. (Jensen *et al.*, 2005), abyssomicin C from *Verrucosispora* sp. (Riedlinger *et al.*, 2004) and marinopyrroles from *Streptomyces* sp. (Hughes *et al.,* 2008).

2.2.2 Endophytic actinomycetes

Some actinomycetes, called endophytic actinomycetes, are occurring in plant and give beneficial and/or adverse effects to the host plants. The associations between endophytes and host plants can be formed without harming the plant. Endophytes have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens or various environmental stresses. A variety of actinomycetes inhabit a wide range of plants as symbionts, parasites or saprophytes were reported and most of them belong to the genera, *Streptomyces* and *Microbispora* (Matsumoto *et al*., 1998). Endophytic actinomycetes have ability to produce a variety of bioactive metabolites including antibiotics, plant growth promoters, plant growth inhibitors and hydrolytic cell wall-degrading enzymes such as cellulases, hemicellulases, chitinases that can apply to agricultural usages (Getha and Vikineswary, 2002; Igarashi *et al*., 2002; Taechowisan *et al*., 2003; Hasegawa *et al*., 2006).

2.2.3 Actinomycetes in plant rhizosphere soils

Majority of actinomycetes are found in various types of soils such as rice paddy, agricultural fields, tropical forests and natural caves (Gomes *et al*., 2000; Lee and Hwang, 2002; Shirokikh *et al*., 2006; Ilic *et al*., 2007; Jayasinghe and Parkinson, 2008; Nakaew *et al*., 2009). The soil actinomycetes produce a volatile compound called geosmin, which literally translates to "earth smell" (Gust *et al*., 2003). This organic compound is responsible for a contributor to the strong odor that occurs in the air when rain falls after a dry spell of weather. Some of actinomycetes are distributed in plant rhizosphere soils. The term rhizosphere, first used by Hiltner (1904), is defined as the zone of soil that surrounds plant roots influenced by root metabolism. This zone can extend more than 5 mm from the root (Figure 2.1). A diverse range of microorganisms can be found in rhizospheres. The density of these is higher in this zone than in root-free soils (bulk soils) (Lynch, 1990). This difference has been related to the secretion, by roots, of small organic compounds in the form of exudates that supply nutrition and energy sources for microbial growth (Soderberg and Baath, 1998). It has been known for some time that the exudates contain organic acids, amino acids, fatty acids, vitamins and carbohydrate monomers; the composition and quantity of root exudates varies depending on the plant species and abiotic conditions such as temperature and soil moisture (Martin and Kemp, 1980). These microbial flora of the rhizosphere comprises mainly bacteria, fungi and actinomycetes. The interactions between prokaryotic microbes and plant roots may have beneficial, harmful or neutral effects on the plant depending on the type of symbiont interaction and the soil conditions (Smith and Read, 1997).

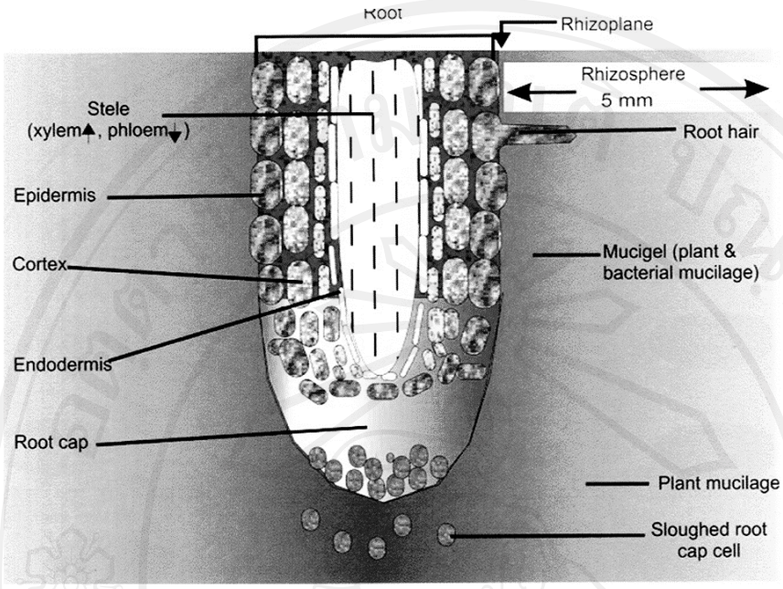


Figure 2.1 Root and corresponding rhizosphere (Kennedy, 2004)

2.2.4 Actinomycetes and plan rhizosphere interactions

Actinomycetes have been found to play an important role in rhizosphere soil (Suzuki *et al*., 2000, El-Tarabilya and Sivasithamparam, 2006; Norovsuren *et al.,* 2007; El-Tarabily *et al.,* 2008). Attention has been paid to the possibility that these microorganisms can protect plant roots from plant pathogen and promote plant growth. For plant root protection, the modes of action of actinomycetes include antibiosis, parasitism, the production of extracellular hydrolytic enzymes and competition for iron (Goodfellow & Williams, 1983; El-Tarabily *et al.,* 2000; Getha *et al.,* 2005; Errakhi *et al*., 2007). Table 2.1 shows a number of soil actinomycetes that produce biocontrol metabolites for plant pathogenic fungi.

Table 2.1 Actinobacteria biocontrol agents for plant pathogenic fungi (El-Tarabily *et al.,* 1997).

Actinobacteria	Pathogen	Host Plant
Streptomyces netropis	*Verticillium* sp.	Cotton
Actinomadura sp.	*Phytophthora cinnamomi*	Snapdragon
Micromonospora carbonacea	*Phytophthora cinnamomi*	Banksia
Micromonospora globosa	*Fusarium udum*	Cotton
Actinoplanes missouriensis	*Phytophthora megasperma*	Soy Bean
Actinoplanes utahensis	*Phytophthora megasperma*	Soy Bean
Amorphosporangium auranticolor	*Phytophthora megasperma*	Soy Bean
	Phytophthora megasperma	Soy Bean
Micromonospora sp.	*Pythium ultimum*	N/A
Actinoplanes sp.	*Pythium aphinidermatum*	N/A

2.2.4.1 Antibiosis

As antimicrobial metabolites producers, actinomycetes especially *Streptomyces* have played an important role in controlling soil-borne plant pathogens in rhizosphere soil. The antagonistic potential of streptomycetes isolated from plant rhizosphere soils to pathogenic fungi, involving the production of antifungal compounds, has been reported. Crawford *et al.* (1993) found that 12 actinomycete strains isolated from *Taraxicum officinale* rhizosphere were active against *Pythium ultimum. Streptomyces rochei* and *S. rimosus* from the chickpea rhizosphere were strong antagonists of *Fusarium oxysporum* (Bashar and Rai, 1994). Ouhdouch *et al.* (2001) found 10 isolates of actinomycetes from medicinal plant rhizosphere soils, most of which were *Streptomyces* spp. After testing for antifungal activity against *Candida albicans* and *C. tropicalis*, they found that all *Streptomyces* had antifungal activity. Thangapandian *et al.* (2007) isolated *Streptomyces* from medicinal plant rhizosphere soils and 8 isolates had antipathogenic activity and six strains of

Streptomyces and *Micronomospora* isolated from rhizosphere soil of *Vitis vinifera* L. produced antifungal metabolites strongly inhibited *Botrytis cinerea* (gray mold of grapevines) (Loqman *et al.*, 2008).

2.2.4.2 Parasitism and production of extracellular hydrolytic enzymes

Actinomycetes have ability to parasitize and degrade spores or the cell-wall of fungal plant pathogens and it is assumed that nutrients pass from the pathogen to them so fungal growth is inhibited (El-Tarabily *et al.,* 1997). The spectrum of parasitism could range from attachment of cell to fungal hyphae so cell-wall degrading enzymes are needed. Actinomycetes can produce a variety of extracellular hydrolytic enzymes such as cellulase, chitinase, amylase etc. (Beyer and Diekmann, 1985; Hopwood, 1990). A role of cell-wall degrading enzymes producing actinomycetes for control plant pathogen has been reported. The cellulase-producing strain, *Micromonospora carbonacea*, was used for control *Phytophthora cinamomi* (root rot of *Banksia grandis* Willd.) (El-Tarabily *et al.,* 1997) and η-1,3-glucanase-producing actinomycetes were used for control of *Phytophthora fragariae* var. *rubi* (raspberry root rot) (Valois *et al*., 1996). Singh *et al.* (1999) used a chitinolytic *Streptomyces* sp. to control cucumber wilt caused by *Fusarium oxysporum* and chitinase produces by *S. hygroscopicus* APA14, isolated from crop rhizosphere soil from center of Thailand, showed activity against *Collectotrichum gloeosporioides* and *Sclerotium rolfsii* (Prapagdee *et al.,* 2008).

2.2.4.3 Competition for iron

Iron is an essential trace element for most microorganisms. It is used as cofactor for several electron transport proteins, reduction of ribotide precursors of DNA, for formation of heme and other essential purposes (Neilands, 1995). In soils, iron is found as ferric ion (Fe^{3+}) that microorganism cannot directly assimilate so microbes produce iron chelating compounds or siderophores. These compounds have a high affinity for Fe^{3+} and form complexes which are imported into the cells (Figure 2.2).

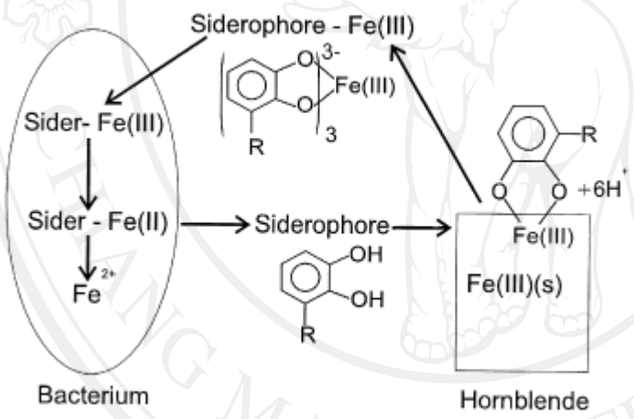


Figure 2.2 Mechanism of iron acquisition by siderophore in bacteria (Kalinowski *et al.,* 2000)

Siderophores are low molecular weight molecules (300-2000 daltons), water soluble and have the property to bind iron. Microbial siderophores are generally classified as hydroxamates, catecholates and carboxylates according to main chelating groups (Figure 2.3). The functional group in hydroxamates is hydroxamic acid, which is a carbonyl oxygen combined with an amino group. The catecholate ligands have adjacent hydroxyl oxygens on an aromatic ring. Most of siderophores which are produced from bacteria are hydroxamate and catecholate types and few bacteria

(*Rhizobium meliloti* and *Staphylococcus hyicus*) produce carboxylates (Drechsel *et al.,* 1995).

Catecholate **Hydroxamate**

Figure 2.3 The two main bidentate chelating groups in siderophores (Pattus and Abdallah, 2000)

The soil actinomycetes, especially genus *Streptomyces* have been reported to produce siderophores that could inhibit the growth of phytopathogens by competition for iron in plant rhizosphere soils (Muller *et al.*, 1984; Muller and Raymond, 1984; Tokala *et al*., 2002).

2.2.4.4 Plant growth hormone production

Actinomycetes may also promote plant growth directly through production of plant growth promoter substances e.g auxin or gibberellin (Merckx *et al.*, 1987). Auxin represent wide group of the derivatives of indole ring compounds. This compound has the ability to improve plant growth by stimulating cell elongation, root initiation, increase seed germination and seedling growth (El-Tarabily, 2008). Indole acetic acid (IAA) is the common natural auxin and tryptophan has been identified as a main precursor for IAA biosynthesis pathways in microorganisms. Microbial IAA biosynthesis pathways are shown in Figure 2.4.

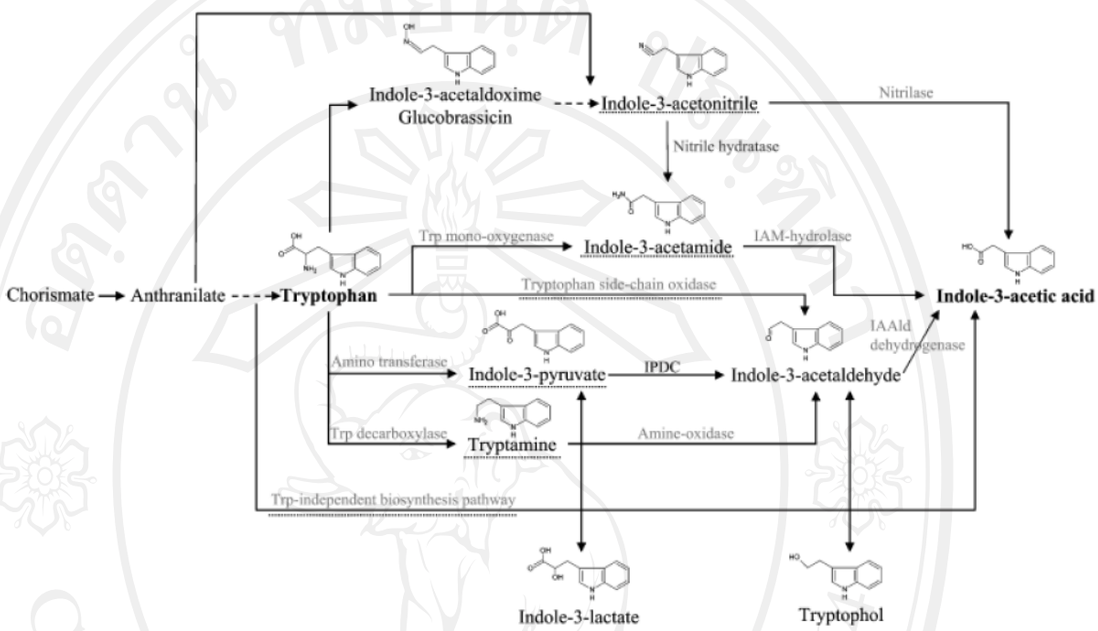


Figure 2.4 The different pathways to synthesize IAA in microorganism (Spaepen *et al.*, 2007)

Approximately 80% of rhizosphere bacteria can secrete IAA (Leinhos and Vace, 1994). Several actinomycetes especially in genus *Streptomyces*, such as *S. olivaceoviridis, S. rimosus, S. rochei, S. lydicus* WYEC108 and *Streptomyces* spp. from tomato rhizosphere, have the ability to produce IAA and improve plant growth (Aldesuquy *et al.,* 1998; Tokala *et al.,* 2002; El-Tarabily, 2008).

Nowadays**,** some rhizosphere actinomycetes were studied and developed to commercial product. For example, Mycostop, based on strain K61 of *S. griseoviridis* and *S. lydicus* WYEC108, can control some root rot and wilt diseases caused by *Pythium* spp*., Fusarium* spp*., Rhizoctonia* spp. and *Phytophthora* spp. The antagonistic effect seems to be based on several mode of action including parasitism, antibiosis and auxin excretion (Mahadevan and Crawford, 1997).

Medicinal plant rhizospheres are the attractive sources for isolation actinomycetes. Active actinomycetes were found from these soils and had the ability to produce bioactive compounds. Ouhdouch and Barakate (2001) found 10 isolates of actinomycetes from medicinal plant rhizosphere soils, most of which were *Streptomyces* spp. After testing for antifungal activity against *Candida albicans* and *C. tropicalis*, they found that all *Streptomyces* had antifungal activity. Thangapandian *et al.* (2007) isolated *Streptomyces* spp. from medicinal plant rhizosphere soils and 8 isolates had antipathogenic activity. Crawford *et al.* (1993) found that 12 actinomycete strains isolated from *Taraxicum officinale* rhizosphere were active against *Pythium ultimum*.

2.3 Isolation of soil actinomycetes

Members of the actinomycetes especially *Streptomyces* and *Micromonospora* have long been recognized as major producers of useful natural secondary metabolites (Miyadoh, 1993). Thus, the rate of discovery of new metabolites from these common actinomycetes has declined, so the selection of improved methodologies for isolating the uncommon and rare actinomycetes is required to avoid re-isolating strains that produce known bioactive metabolites and to improve the quality of the natural products screened (Lazzarini *et al*., 2001; Takahashi and Omura, 2003; Berdy, 2005). Various media and methods including the techniques that enhance the desirable actinomycetes in natural habitat samples (enrichment) or eliminate undesirable *Streptomyces* and other contaminants from the isolation plate media (pretreatment) for isolating novel actinomycetes from natural habitats, especially from various types of soil, were improved and developed.

2.3.1 Isolation media

The isolation media were designed to reduce the development of competing soil microoganisms without adversely affect to actinomycete. Hayakawa and Nonomura (1987) formulated humic acid-vitamin (HV) agar, a medium containing soil humic acid as the sole carbon and nitrogen source. This medium was useful for recovery and adequate growth of *Streptomyces* and various rare actinomycetes, while restricting growth of non-filamentous bacteria colonies. For eliminate bacterial and fungal contaminants, HV agar was supplemented with synthetic antibacterial agents such as nalidixic acid and cycloheximide (Hayakawa *et al*., 1996). Moreover, the addition of some antibiotics and some chemicals into HV medium enabled to recover some rare genera of actinomycetes, the data was shown in Table 2.2.

Table 2.2 Variation of antibiotics and some chemicals for soil actinomycetes isolation (Takahashi and Omura, 2003)

Addition of antibiotics and some chemicals	**Genera selected**
Antibiotics:	
Novobiocin	*Actinoplanes, Kitasatospora*
Tunicamycin	*Micromonospora*
Rifamycin	*Actinomadura*
Chlortetracycline	*Nocardia*
Macrolide or aminoglycoside	Macrolide or aminoglycoside producers
Chemicals:	
Humic acid	Rare actinomycetes
Proline	Rare actinomycetes
Gellan gum	*Actinobispora*

In addition, another medium, LSV-SE agar containing a Kraft lignin as the carbon and nitrogen sources was formulated (Hayakawa *et al*., 1996) to facilitate an increased recovery of *Microtetraspora* spp. and minimal medium (MM) agar containing glucose, yeast extract and mineral salts was formulated to increase recovery of *Actinomadura, Amycolatopsis* from desert soil (Hozzein *et al*., 2008).

2.3.2 Physical or chemical pretreatment method

The soil samples were pretreated with chemical reagents or pretreated by heating for reducing the numbers of filamentous bacteria and streptomycetes and increased the number of uncommon *Streptomyces* and various rare actinomycetes genera.

2.3.3 Enrichment method

For increasing the rate of discovery of rare soil actinomycetes genera, enrichment method were used including chemical treatment, chemotactic method and the rehydration and centrifugation (RC) method.

2.3.3.1 Chemotactic method

This method was used for recovering some motile soil actinomycetes by treating soil samples with some chemicals such as ν-collidine or vanillin (Hayakawa *et al.*, 1991c; Tamura *et al*., 2001).

2.3.3.2 Rehydration and centrifugation (RC) method

This method is a simple enrichment method incorporating differential centrifugation for the isolation of motile actinomycetes (Hayakawa *et al*., 2000). The RC method was consistently achieved at 37 to 86% selective isolation of motile actinomycetes from various soil samples. The most frequently isolated motile actinomycetes were *Actinoplanes* and *Dactylosporangium*.

The various pretreatment methods and enrichment methods were summarized in Table 2.3.

Table 2.3 Summary of methods developed for the selective isolation of uncommon actinomycetes from soil (Hayakawa, 2008)

Pretreatment	Culture media	Genera selected	Reference
Physical:			
None	HV agar with or without nalidixic acid and trimethoprim	*Streptomyces* and other genera	Hayakawa & Nonomura (1987a,b), Hayakawa *et al.* (1996a)
Dry heat at 120 °C for 1 h	HV agar with nalidixic acid	Many rare actinomycete genera including *Spirilliplanes***	Hayakawa *et al.* (1991a) Tamura *et al.* (1997)
Chemical:			
SDS 0.05% and Yeast extract 5%	HV agar with nalidixic acid	*Streptomyces* and other genera	Hayakawa & Nonomura (1989)
Phenol 1.5%	HV agar with nalidixic acid and tunicamycin	*Micromonospora*	Hayakawa *et al.* (1991a)
Phenol 1.5%	HV agar	*Streptomyces violaceusniger* cluster	Hayakawa *et al.* (2004)
Chloramine-T	HV agar with nalidixic acid	*Herbidospora*, *Microbispora*, *Microtetraspora*, *Nonomuraea* and *Streptosporangium*	Hayakawa *et al.* (1997)
Physical and chemical:			
Dry heat at 110 °C for 1 h and phenol 1.0%	HV agar with kanamycin, josamycin,lysozyme and nalidixic acid	*Actinomadura viridis*	Hayakawa *et al.* (1995a)
Dry heat at 120 °C for 1 h and phenol 1.5%-CG 0.01%	HV agar with nalidixic acid	*Microbispora*	Hayakawa *et al.* (1991a)
Dry heat at 120 °C for 1 h and BC 0.01% (or 0.03%)	HV agar with nalidixic acid and leucomycin (or tunicamycin)	*Streptosporangium* or *Dactylosporangium*	Hayakawa *et al.* (1991b)
Dry heat at 110 °C for 1 h and BC 0.05%	LSV-SE agar with kanamycin, nalidixic acid and norfloxacin	*Microtetraspora*	Hayakawa *et al.* (1996b)
Enrichment:			
Chemotaxis (γ-collidine, vanillin)	HV agar with nalidixic acid	*Actinoplanes*, *Catenuloplanes*, *Dactylosporangium*, *Virgosporangium***	Hayakawa *et al.* (1991c, 1995b) Tamura *et al.* (2001)
Enrichment and physical:			
Pollen-baiting and drying	HV agar with nalidixic acid	*Actinoplanes*	Hayakawa *et al.* (1991d), Nonomura *et al.* (1979)
Rehydration (30 °C, 90 min) and centrifugation (1,500 × g, 20 min)	HV agar with nalidixic acid and trimethoprim	*Actinoplanes*, *Actinokineospora*, *Actinosynnema*, *Catenuloplanes*, *Cryptosporangium***, *Dactylosporangium*, *Geodermatophilus* *Kineosporia*, *Sporichthya*	Hayakawa *et al.* (2000) Tamura *et al.* (1995, 1998, 1999)
$CaCO_3$, rehydration and centrifugation	HV agar with fradiomycin, kanamycin, nalidixic acid and trimethoprim	*Actinokineospora*	Otoguro *et al.* (2001a,b)
Sucrose-gradient centrifugation (240 × g, 30 min)	HV agar with nalidixic acid and chlortetracycline	*Nocardia*	Yamamura *et al.* (2003a,) b, 2004, 2005)
Moist incubation and drying	HV agar with nalidixic acid and trimethoprim	*Streptomyces* and other genera	Matsukawa *et al.* (2007a,b)

*Both HV agar and LSV-SE agar contain the antifungal antibiotic cycloheximide.
**Proposed as new genera.

2.3.4 Other method for selective isolation of soil actinomycetes

Beside from pretreatment and enrichment methods, other methods were used for isolating actinomycetes from soil such as trap *in situ* cultivation method or Extremely High Frequency Radiation (EHF) method. The trap *in situ* cultivation method was used for the isolation of filamentous actinomycetes from soil samples. This method is based on placing actinomycetes in a diffusion chamber which is mixed with agar and diluted environmental sample sandwiched between two semipermeable membranes glued onto a washer. The trap is placed on top of soil, and filamentous microorganisms selectively penetrate into the device and form colonies. The trap recovered more filamentous actinobacteria. The trap cultivation resulted in the isolation of unusual and rare actinomycetes; *Actinoplanes, Kribbella, Nocardioides* and *Promicromonospora* (Gavrish *et al.,* 2008). EHF radiation method is applied for the selective isolation of actinomycetes from soil by pretreating soil samples with different EHF wavelengths. Many rare genera of soil actinomycetes including *Actinomadura, Microtetraspora, Nonomuraea, Micromonospora, Amycolatopsis,Pseudonocardia, Saccharotrix*, and *Streptosporangium* were recovered from this method (Li *et al*., 2002; Li *et al.,* 2003).

2.4 Identification and classification of actinomycetes

Chemotaxonomic characterization, morphological observation and molecular techniques have been used to identify and classify actinomycetes to genus level. Chemotaxonomy is the study of chemical variation in cell compositions in microorganisms. One of the quickest methods for preliminary identification of actinomycetes to genus level is the demonstration of the presence of diaminopimelic

acid isomers (DAP) and sugar composition in the whole-cell hydrolysates (Hesagawa *et al*., 1983). The presence of DAP isomers is one of most important cell-wall properties of actinomycetes and gram-positive bacteria. These amino acids mostly located in the peptidoglycan of bacterial wall envelope are generally contained as one of the isomers, LL-form or *meso*-form. Cells of the actinomycetes contain glucosamine and muramic acid, the monomers of peptidoglycan. Most components of the cell wall and whole-cell of actinomycetes are shown in Table 2.4.

Table 2.4 Type of cell wall diaminopimelic acid isomers (DAP) and whole-cell sugars of actinomycetes (Lechevalier and Lechevalier, 1970)

	Cell-wall types			
	I	**II**	**III**	**IV***
DAP isomers	LL	meso	meso	meso
Glycine	+	+	-	-
	Whole-cell sugars pattern (WCSP)			
	A	**B**	**C**	**D**
Arabinose	+	-	-	+
Galactose	+	-	-	-
Xylose	-	-	-	+
Madurose	-	+	-	-

*Type IV was differentiated from type III by the presence of arabinose and galactose in the whole cell hydrolysates.

The range of DNA base compositions of most actinomycetes is 63-78 mol% G+C. The value of mol% G+C content has been used to classify group of actinomycetes (Madigan and Martinko, 2006). Moreover, compositions of phospholipids, fatty acids and menaquinones in the cell have also been used (Boone and Pine, 1968; Lechevalier and Lechevalier, 1980). The phospholipids composition in the actinomycetes was classified into 5 types as in Table 2.5.

Table 2.5 Type of phospholipids of actinomycetes (Lechevalier, 1977)

Type	PIM	PI	PC	PG	PE	PME	GluNU	APG	DPG
I	+	+	-*	V	-*	-	-*	V	V
II	+	+	-*	V	+*	-	-*	V	+
III	V	+	+*	V	V*	+	-*	V	V
IV	?	+	-*	-*	V*	V	+*	-	+
V	?	+	-*	-*	V*	-	+*	V	+

* = diagnostic phospholipids
PIM = phosphatidylinositolmannosides
PI = phosphatidylinositol
PC = phosphatidylcholin
PG = phosphatidylglycerol
PE = phosphatidylethanolamine
PME = phosphatidylmethylethanolamine
GluNU= unknown glucosamine-containing phospholipids
APG = acyl phosphatidylglycerol
DPG = diphosphatidylglycerol

Morphology has been an important characteristic to identify actinomycete isolates. It was this characteristic that was used in the first descriptions, especially of *Streptomyces* species. This study is best made by using a variety of standard culture media, including those recommended for the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966). For non-streptomycetes or rare actinomycetes, strains were cultivated on ATCC Medium No.172 (NZ-amine glucose starch agar) (American Type Culture Collection, 1982). Morphological observations including germination of spores, elongation and branching of vegetative mycelium, formation of aerial mycelium (Figure 2.5), color of aerial and substrate mycelium and pigment production have been used to identify actinomycetes (Holt *et al.,* 1994). Formation of aerial mycelium, substrate mycelium and spores were studied by light microscopy and the spore surface and spore structure by scanning electron microcopy. Table 2.6 shows the distribution of genera of the actinomycetes by cell wall type, including a description of morphology (Labeda, 1986).

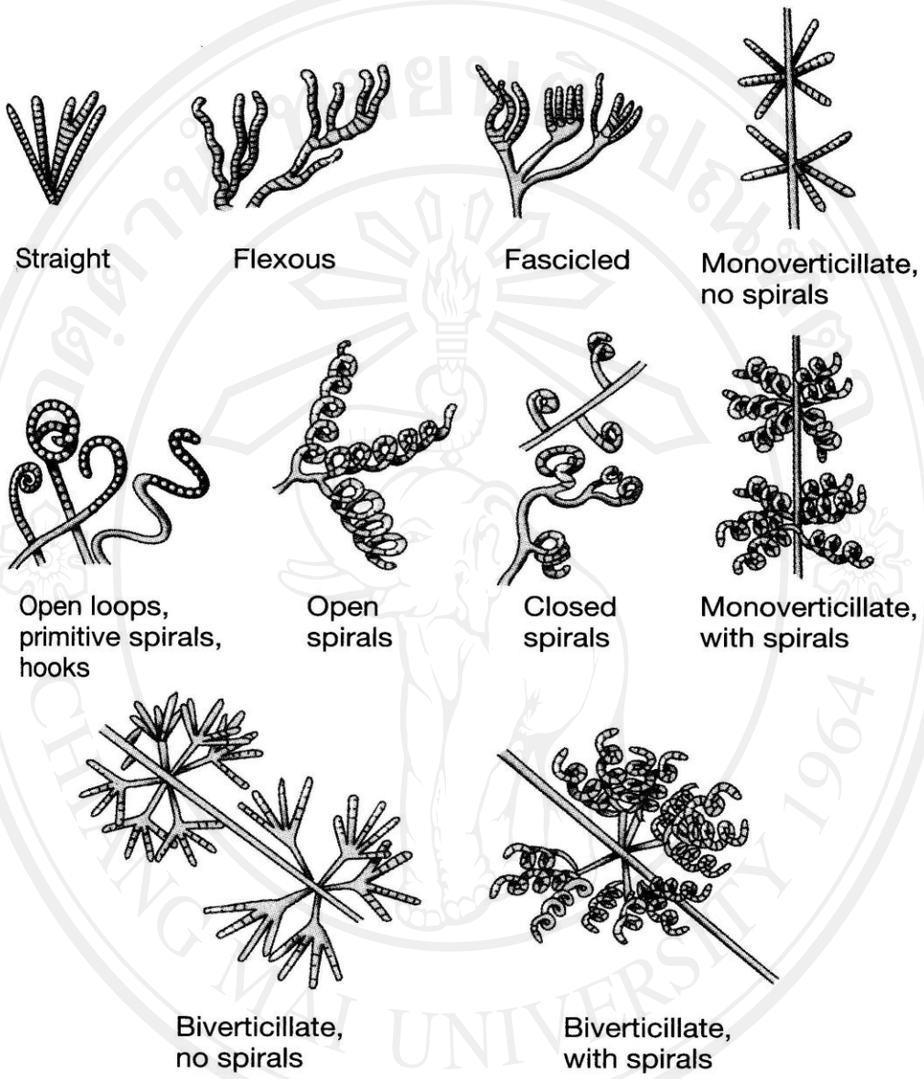


Figure 2.5 Various type of spore-bearing structure in streptomycetes (Madigan and Martinko, 2006)

Table 2.6 Distribution of genera of the actinomycetes by cell wall type, including a description of morphology (Labeda, 1986).

Type I cell wall

Streptomyces – chains of conidia on aerial mycelium.
Streptoverticillium – chains or umbels of conidia on verticils formed on aerial mycelium.
Nocardioides – substrate and aerial mycelium fragment into coccoid elements.
Actinopycnidium – same as *Streptomyces* but pycnidia-like structures formed.
Actinosporangium – same as *Streptomyces* but spores accumulate in drops.
Chainia – same as *Streptomyces* but sclerotia are also formed.
Elytrosporangium – same as *Streptomyces* but merosporangia are also produced on vegetative mycelium.
Intrasporangium – no aerial mycelia; substrate mycelium forms vesicles.
Microellobosporia – merosporangia produced on both aerial and substrate mycelia.
Sporichthya – no substrate mycelium; aerial chains of motile conidia held to surface of substrate by holdfasts.
Kitasatoa – single spores in sporangia on aerial and substrate mycelia; spores motile.

Type II cell wall

Micromonospora – no aerial mycelium; single conidia produced.
Actinoplanes – globose-to-lageniform sporangia; motile spores.
Amorphosporangium – same as *Actinoplanes* but irregular sporangia; spores generally non-motile.
Ampullariella – lageniform-to-globose sporangia; motile rod-shaped spores.
Dactylosporangium – claviform sporangia containing one chain of motile spores.
Glycomyces – aerial mycelium with chains of non-motile conidia.

Type III cell wall

No characteristic whole cell sugars
Actinosynnema – synnemata with chains of motile conidia.
Geodermatophilus – hyphae divide in all planes, forming packets of motile coccoid conidia.
Nocardiopsis – long chains of conidia on aerial mycelium.
Thermomonospora – single conidia formed on aerial and substrate mycelia.
Thermoactinomyces – single heat-resistant endospores produced on aerial and substrate mycelium.

Madurose as characterisitic whole cell sugar
Actinomadura – short chains of conidia on aerial mycelium.
Dermatophilus – same as *Geodermatophilus*.
Exellospora – short chains of conidia on aerial and substrate mycelia.
Microbispora – longitudinal pairs of conidia on aerial mycelium.
Microtetraspora – chains of four to six conidia on aerial mycelium.
Planobispora – cylindrical sporangia, each containing two motile spores.
Planomonospora – cylindrical sporangia, each containing one motile spore.
Spirillospora – globose sporangia with rod-shaped motile spores.
Streptosporangium – globose sporangia with nonmotile spores.

Rhamnose and galactose as characteristic whole cell sugars
Saccharothrix – long chains of conidia on aerial mycelium.

Type IV cell wall

Nocardia – abundant filamentation, often fragmenting into coccoid rods; aerial mycelium and chains of conidia sometimes formed.
Actinopolyspora – long chains of conidia on aerial mycelium; substrate mycelium may fragment.
Amycolata – abundant filamentation, chains of conidia formed on aerial mycelium; substrate mycelium may fragment.
Amycolatopsis – same as *Amycolata*.
Micropolyspora – short chains of conidia formed on aerial and substrate mycelia.
Faenia – same as *Micropolyspora*.
Pseudonocardia – long, cylindrical conidia formed on aerial mycelium, dividing into shorter coccoid elements.
Saccharomonospora – single spores primarily on aerial mycelium.
Saccharopolyspora – similar to *Nocardiopsis*.
Kibdelosporangium – chains of conidia produced on aerial mycelium; sporangia-like structures also produced.

Type X cell wall (cell walls contain glycine and *meso* and LL isomers of DAP).

Kitasatosporia – long chains of conidia produced on aerial mycelium.

At present, the molecular biological identification is based on 16S rDNA sequences which are the most significance for actinomycetes identification (Yokota, 1997). The phylogenetic tree constructed from 16S rDNA sequences allows the investigation of actinomycetes evolution. Based on the 16S rRNA gene sequencing, actinomycetes are separated into over 100 genera (Figure 2.6).

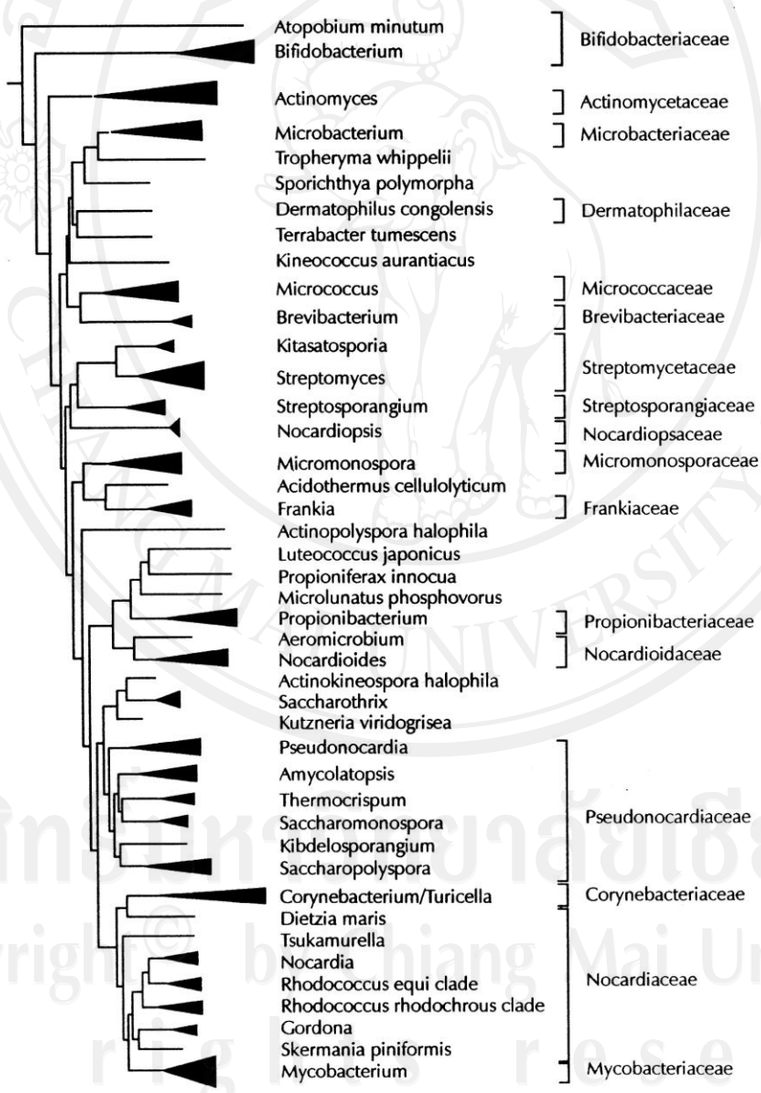


Figure 2.6 Phylogenetic relationship of actinomycetes based on 16S rRNA sequences (Goodfellow, 1989).

2.5 Bioactive metabolites from actinomycetes

Soil actinomyctes, especially *Streptomyces* represent an important source of biologically active compounds with high commercial value and important applications in human and livestock medicine and agriculture (Watve *et al*., 2001; Berdy, 2005). The biological active compounds produced by actinomycetes are antibiotics, immunosuppressant, extracellular hydrolytic emzymes, plant growth promoters and siderophores.

2.5.1 Antibiotics

Approximately 80% of world's antibiotics are produced by actinomycetes, mostly by the genus *Streptomyces* and *Micromonospora* (Pandey *et al*., 2004). A range of useful actinomycete antibiotics were reported (Table 2.7).

Table 2.7 Actinomycete antibiotics for medical applications (Kieser *et al.,* 2000).

Antibiotic	**Producer**	**Application**
Erythromycin	*Saccharopolyspora erythraea*	Antibacterial
Gentamicin	*Micromonospora* sp.	Antibacterial against gram-negative bacteria
Nocardicin A	*Nocardia uniformis*	Antibacterial
Nystatin	*Streptomyces noursei*	Antifungal against fungi especially *Candida* sp.
Rifamycin	*Amycolatopsis mediterranei*	Antibacterial against *M. tuberculosis*
Ristocetin	*Amycolatopsis lurida*	Antibacterial against *Streptococcus* sp.
Spinosyns	*Saccharopolyspora spinosa*	Insecticidal
Streptomycin	*Streptomyces griseus*	Antibacterial against gram-positive and gram-negative bacteria
Teicoplanin	*Actinoplanes teichomyceticus*	Antibacterial against gram-positive bacteria
Vancomycin	*Amycolatopsis orientalis*	Antibacterial against *Streptococcus* sp.

Microbial metabolites which have application as antibiotics are synthesized from intermediates of primary metabolites such as amino acids, aliphatic acids, sugars, nucleotides and lipids. The most important groups of secondary metabolites which have found application as lead molecules are the peptides and polyketides.

2.5.1.1 Peptide antibiotics

Peptide antibiotics are synthesized from amino acids and form a diverse and clinically important class of antibiotics. Most peptide antibiotics are synthesized by group of enzymes, the non-ribosomal peptide synthetases (NRPS) (Ylihonko *et a*l., 1996). Many peptide antibiotics from actinomycetes, for example, penicillin, streptomycin, tetracycline, nystatin and candicidin are important antibacterial, antifugal or antiviral agents. The NRPS enzyme has the potential to develop new libraries of antimicrobial therapeutics by bringing together theamino acid precursors in diverse chemical structures. These enzymes are very large, modular proteins; the order of the modules is corresponding with the order of the amino acids incorporated in final product (Metsa-Ketala, 2003). The number of modules in NRPS varies greatly, for example, 3 modules with multiple enzymatic activities are involved in penicillin synthesis but 11 modules are involved in cyclosporine synthesis. Figure 2.7 shows a model for NRP synthesis from surfactin pathway (Mootz *et al.,* 2002). According to the model, amino acid is first activated at the adenylation (A) domain to form an aminoacyl adenylate, and the activated amino acid is bound to a 4'-phosphopantethine (Ppant) prothetic group at a peptidyl carrier (PCP) domain by a thioester linkage. The consecutive amino acid is transferred to the condensation (C) domain, which is located between the combination of A and PCP domains, and they

catalyze the formation of peptide bonds between amino acid bound to neighboring PCP domains. In most NRPS synthesis, the C-terminal end of the last module is modified as a thioesterase (TE) domain. The function of this domain is to catalyze a variety of termination reactions in different NRPS systems including hydrolysis, cyclization and oligomerization of the compound.

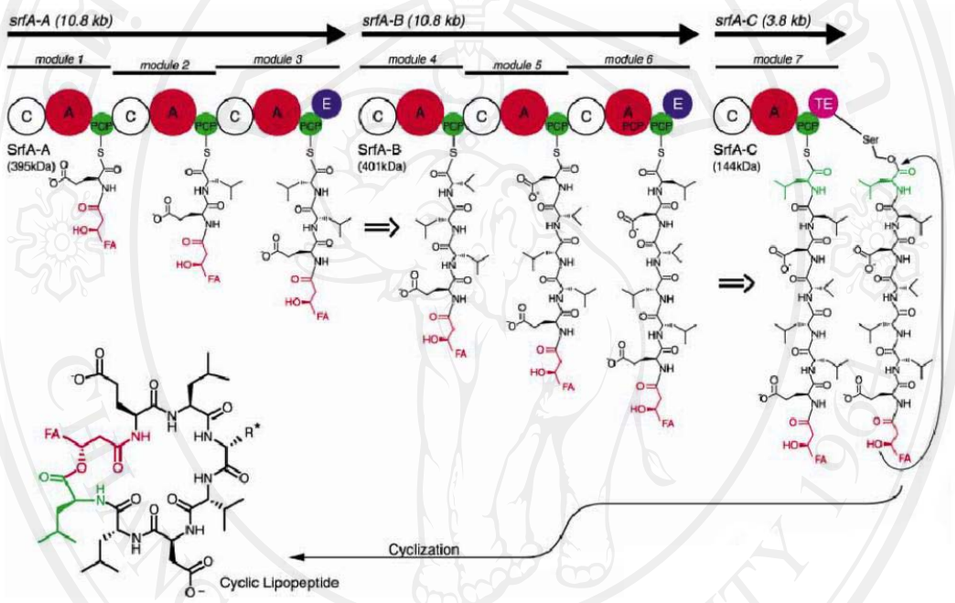


Figure 2.7 A model for NRP synthesis in surfactin pathway (Mootz *et al.,* 2002).

2.5.1.2 Polyketides

Polyketides are a group of natural products including antibiotics, antifungals, anthelmintics, immunosuppressants, anticholesterolemics, antiparasitics and natural insecticides. The group is highly diverse and the molecules are derived from the successive condensation of small carboxylic acids in a process closely analogous to fatty acid biosynthesis (O'Hagan, 1995). Some species of actinomycetes are a major source of polyketide compounds. Polyketides are used in pharmacological and agricultural situations, for example, erythromycin is active against gram-positive

cocci and penicillin is used against streptococcal and pneumococcal infections. Figure 2.8 represent some polyketides from actinomycetes. Assembly of the initial carbon skeleton of a polyketide is catalysed by a large enzyme known as polyketide synthase (PKS). PKS are structurally similar to the fatty acid synthase (FAS) enzymes and may share a common evolutionary origin (Hutchinson and Fuji, 1995). PKS are classified as type I, II and III based on how closely they mimic the architecture of type I or type II fatty acid synthase (Hopwood and Sherman, 1990).

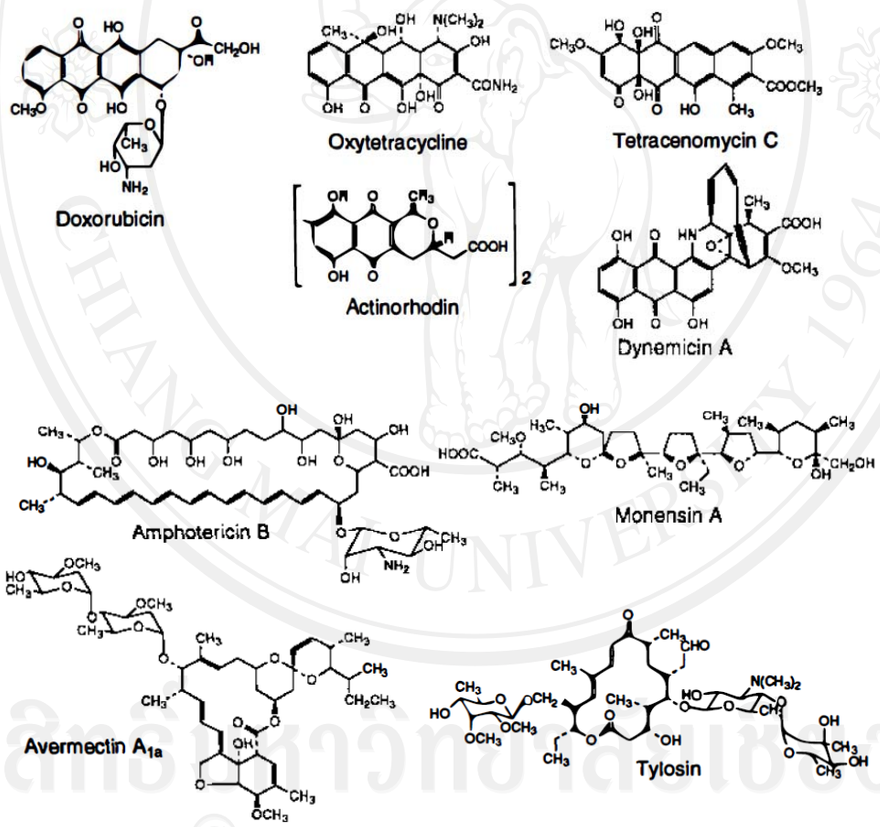


Figure 2.8 Representative polyketides from actinomycetes (Hutchinson, 1995)

-Type I polyketides

The type I polyketide synthases (PKS-I) are multidomain mega-polypeptides (often >300 kDa), in which each domain is usually used only once in the biosynthetic

process. In one module, type I enzymes contain obligatory three domains, a ketosynthase (KS) domain, an acyl transferase (AT) domain and an acyl carrier protein (ACP) (Katz, 1997). The KS domain is responsible for the catalysis of a condensation reaction between the growing chain and an extender unit. The AT domain selects the type of CoA and transfer to the 4'-phosphopantetheine arm and ACP domain are responsible for synthesize polyketide chain. Moreover, type I enzymes contain other domains including, keto-reductase (KR), dehydratatase (DH), enoyl-reductase (ER) and thio-esterase (TH) domains.

A type I polyketide is synthesized in three stages (Figure 2.9), starting stage, elongation stages and termination stage. For the starting stage, the starter group, usually acetyl-CoA or malonyl-CoA, is loaded onto the ACP domain of the starter module catalyzed by the starter module's AT domain. In the elongation stages, the polyketide chain is transfered from ACP domain of the previous module to KS domain of the current module and catalyzed by the KS domain. After that, the elongation group is loaded onto the current ACP domain catalyzed by the current AT domain, the ACP-bound elongation group reacts in a Claisen condensation with the KS-bound polyketide chain under CO_2 evolution, leaving a free KS domain and an ACP-bound elongated polyketide chain. The reaction takes place at the KSn-bound end of the chain, so that the chain moves out one position and the elongation group becomes the new bound group. The new fragment of the polyketide chain can be altered stepwise by additional domains. The KR domain reduces the β-keto group to a β-hydroxy group, the DH domain eliminates H_2O, resulting in the α-β-unsaturated alkene, and the ER domain reduces the α-β-double-bond to a single-bond. The cycle is repeated for each elongation module. Finally, in termination stage, the TE domain

hydrolyzes the completed polyketide chain from the ACP-domain of the previous module. Figure 2.10 shows type I polyketide synthesis from erythromycin A pathway.

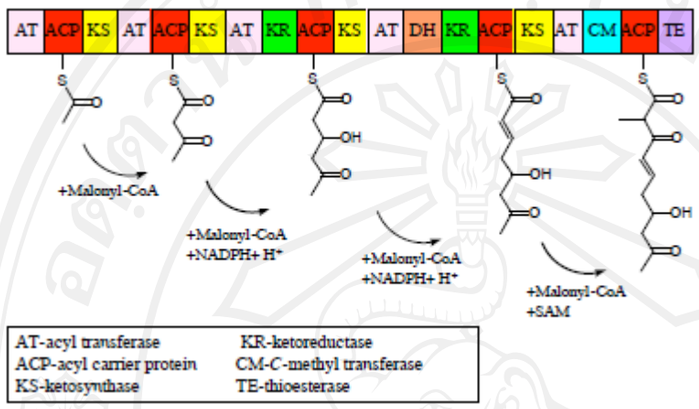


Figure 2.9 Type I polyketide biosynthesis (Khosla, 1997)

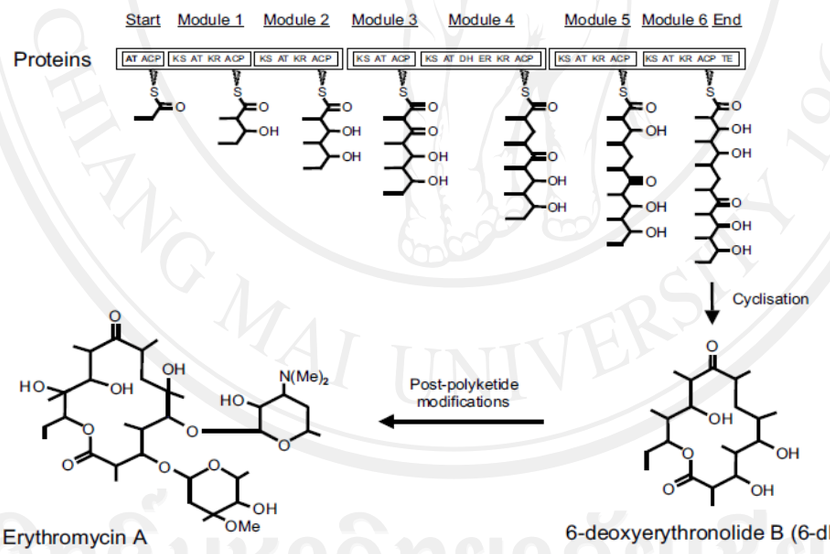


Figure 2.10 A model for type I polyketide synthesis from erythromycin A pathway (Hranueli *et al.,* 2001)

It was found that many strains of actinomycetes produce type I polyketide antibiotics such as erythromycin produced by *Saccharopolyspora erythraea* (Donadio

et al., 1991), piromycin and methymycin produced by *Streptomyces venezuelae* (Xue *et al.*,1998) and candicidin produced by *S. griseus* (Aparicio *et al*., 2000).

-Type II polyketides

Assembly of the initial carbon skeleton of a type II polyketide, aromatic polyketide, is catalysed by the type II polyketide synthase (PKS-II). PKS-II is an aggregate of monofunctional proteins.

The type II polyketide synthesis follows three stages, firstly, a polyketide chain is formed by the minimal polyketide synthase (minPKS) complex, which is comprised ofh ketosynthase ζ, ketosynthase η (KS_ζ, KS_η) and the acyl carrier protein (ACP). Secondly, a polyketide chain is changed into different aromatic compounds by ketoreductases (KR), cyclases (CYC) and aromatases (ARO). Finally, deoxysugars are attached by some chemical reaction, such as methylation and oxygenation. Figure 2.11 shown type II polyketide synthesis from actinorhodin produced by *S. coelicolor* A3 (2) pathway.

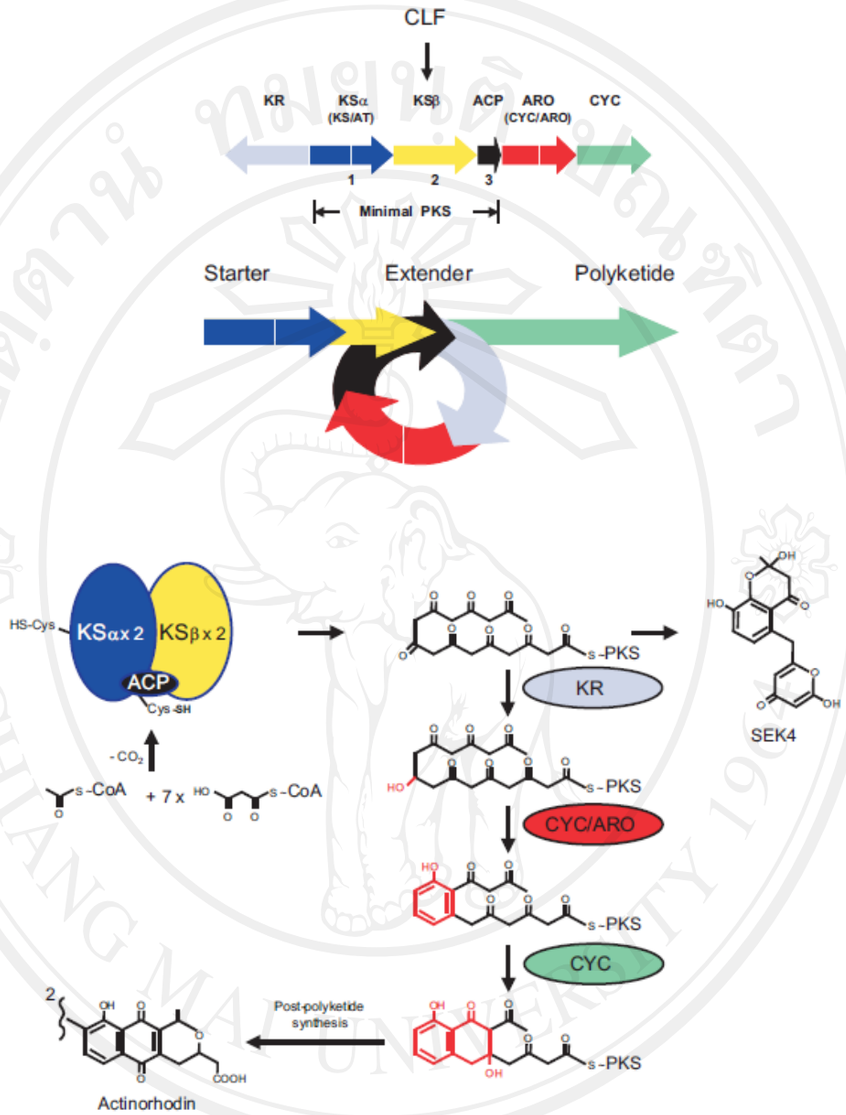


Figure 2.11 A model for type II polyketide synthesis from actinorhodin pathway. (Hranueli *et al.,* 2001)

Many *Streptomyces* spp. produce type II polyketide antibiotics, such as actinorhodin produced by *S.coelicolor* A3(2) (Fernandez-Moreno *et al.,* 1992) and griseorhodin produced by *Streptomyces* sp. JP95 (Li and Piel, 2002).

The ability to manipulate actinomycete genes mainly using the model species *Streptomyces coelicolor* A3(2) was combined with chemical and biochemical

experiments to begin to crack this polyketide code. Nowadays, many studies use molecular techniques to discover new polyketides and peptide antibiotics by using primer synthesized from PKS and NRPS genes (Bull *et al.,* 2000). Metsa-Ketela (2003) used specific primers to screen for PKS genes of *Streptomyces* and found new polyketide compounds. Sciara *et al.* (2003) used molecular techniques to modify actinorhodin and produced a new molecule. Specific primers were successfully used for screening PKS-I and NRPS in group of actinomycetes (Ayuso-Sacido and Genilloud, 2005; Savic and Vasiljevic, 2006)

2.5.2 Extracellular enzymes

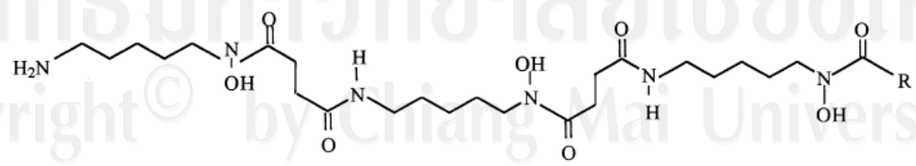
Actinomycetes can produce a variety of extracellular hydrolytic enzymes such as cellulase, chitinase, amylase which can be applied for agriculture (Beyer and Diekmann, 1985; Hopwood and Sherman, 1990, Taechowisan *et al.,* 2003). Theses enzymes have ability to degrade cell wall and spore of fungal pathogen so the fungal loss ability to infected host plant. Beside from agricultural usages, some enzymes from actinomycete were applied for medical treatment. L-asparaginase (L-asparagine amido hydro-lase, E.C.3.5.1.1) has been widely used as a therapeutic agent in the treatment of certain human cancers, mainly in acute lymphoblastic leukemia (Gallagher *et al.,* 1989; Verma *et al.*, 2007). This enzyme acts as a catalyst in the breakdown of asparagine to aspartic acid and ammonia. Tumor cells, especially lymphatic tumor cells, require L-asparagine for growth. When this amino acid was converted into aspartic acid and ammonium ion by this enzyme, the malignant cells would die and be destroyed. The production of L-asparaginase has been studied in actinomycetes (De Jong, 1972; Mostafa and Salama, 1979; Narayana *et al.,* 2007).

2.5.3 Plant growth promoters

Some actinomycetes have ability to produce plant growth promoter substances which important for plant growth and can apply for agriculture. Several researches reported that actinomycetes can produce auxins, gibberellins and cytokinins (El-Tarabily and Sivasithamparam, 2006).

2.5.4 Siderophores

Siderophores are the compounds that have ability to bind Fe^{3+}. The soil actinomycetes, especially species of *Streptomyces*, have been reported to produce siderophores (Tokala *et al*., 2002). Siderophores were used for agriculture and medical treatment. In agriculture, actinomycetes produce these compounds for compete iron with plant pathogenic fungi (Muller *et al.*, 1984; Muller and Raymond, 1984). Nowadays, siderophores from actinomycetes were used for clinical application. The siderophores, desferrioxamine, from *S. pilosus* (Figure 2.12) and oxachelin, from *Streptomyces* sp. GW9/1258 were used for treatment of iron overload and removal of other toxic metal from human tissue (Neilands, 1995, Sontag *et al*., 2006).



Desferrioxamine B R = CH_3

Figure 2.12 Structure of desferrioxamine B produce by *S. pilosus* (Mucha *et al*., 1999)