Review

Biosynthesis of Polyketides in *Streptomyces*

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Abstract: Polyketides are large group of secondary metabolites that have notable variety in their structure and function. Polyketides exhibit a wide range of bioactivities such as antibacterial, antifungal, anticancer, antiviral, immune-suppressing, anti-cholesterol and anti-inflammatory activity. Naturally, they are found in bacteria, fungi, plants, protists, insects, mollusks and sponges. *Streptomyces* is a genus of Gram-positive bacteria that has a filamentous form like fungi. This genus is best known as one of polyketides producers. Some examples of polyketides produced by *Streptomyces* are rapamycin, oleandomycin, actinorhodin, daunorubicin and caprazamycin. Biosynthesis of polyketides involves a group of enzyme activities called polyketide synthases (PKSs). There are three types of PKSs (type I, type II, and type III) in *Streptomyces* that responsible for producing polyketides. This paper focuses on biosynthesis of polyketides in *Streptomyces* with three structurally different types of PKSs.

Keywords: *Streptomyces*, polyketides, secondary metabolite, polyketide synthases (PKSs)

1. Introduction

Polyketides, large group of secondary metabolites, are known possessing remarkable variety not only in their structure and but also in their function [1,2]. Polyketidesexhibit a wide range of bioactivities such as antibacterial (e.g., tetracycline), antifungal (e.g.,amphotericin B), anticancer (e.g., doxorubicin), antiviral (e.g., balticolid), immune-suppressing (e.g., rapamycin), anti-cholesterol (e.g., lovastatin) and anti-inflammatory activity (e.g., flavonoids) [3–9]. Some organisms such as bacteria, fungi, plants, protists, insects, mollusks and sponges can produce polyketides naturally [10–12]. In order to survive, these polyketide-producing organisms could use polyketides that they generate to protect themselves in their environment [13].

Since the beginning of 1940's, the history of antibiotic is much related to microorganisms. One of the groups of bacteria which produce many important antibiotics is Actinobacteria. Actinobacteria are Gram-positive bacteria, have high GC content and comprise various genera known for their secondary metabolite production, such as *Streptomyces*,*Micromonospora*, *Kitasatospora*, *Nocardiopsis*, *Pseudonocardia*, *Nocardia*, *Actinoplanes*, *Saccharopolyspora* and *Amycolatopsis* [14,15]. The most important genus of them is *Streptomyces* which has a filamentous form like fungi and recently becomes a source of 80% of the antibiotics since the discovery strepthothricin within this genus in 1942 [16–18]. Among the antibiotics produced by *Streptomyces,* polyketides are one group of the very important compounds. Some examples of polyketides produced by *Streptomyces* are rapamycin (produced by *Streptomyces hygroscopicus*), oleandomycin (produced by *Streptomyces antibioticus*), actinorhodin (produced by *Streptomyces coelicolor*A3(2)), daunorubicin (produced by *Streptomyces peucetius*)and caprazamycin (produced *by Streptomyces sp.* MK730-62F2) [19–23].

Biosynthesis of polyketides is very complex because the process involves multifunctional enzymes called polyketide synthases (PKSs). The mechanism of PKS is similar to fatty acid synthase (FAS) which includes acyltransferase (AT) that has a role in catalyzing the attachment of the

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substrate (e.g., acetyl or malonyl) to the acyl carrier protein (ACP), ketosynthase (KS) which catalyzes condensation of substrates attached in ACP. For the subsequent steps, polyketide intermediate is processed by ketoreductase (KR), dehydratase (DH) and enoylreductase (ER) as shown in Figure 1. Unlike in FAS, the three remaining process are optional in PKSs that can give the various structures of polyketides [24–26]. In *Streptomyces*, there are three types of PKSs (type I, type II, and type III) [27–29]. This review describes the biosynthesis of polyketides in *Streptomyces* with three distinct types of PKSs.

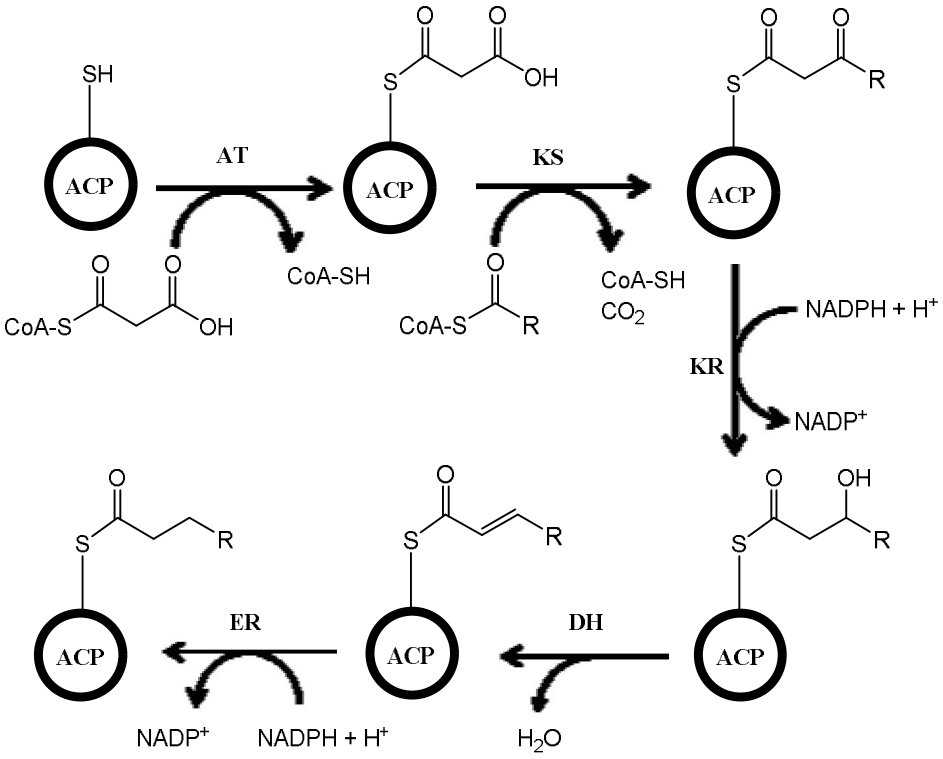

Figure 1. Scheme of reaction occurred in polyketide synthases (PKSs).

2. Polyketide Synthases Type I

The type I polyketide synthases (type-I PKSs) involve huge multifunctional proteins that have many modules containing domains, in which a particular enzymatic reaction occur. Each module has responsibility to perform one condensation cycle in a non-iterative way. Because this system works with some modules, hence it is also called as modular PKS. The essential domains exist in each module are acyltransferase (AT), keto synthase (KS) and acyl carrier protein (ACP) that collaborates to produce β-keto ester intermediate. In addition, the other domains that may be present in the module are β-ketoreductase (KR), dehydratase (DH) and enoyl reductase (ER) which are responsible for keto group modification. In the process of producing polyketide, the expanding polyketide chain is transferred from one module to other module until the completed molecule is liberated from the last module by a special enzyme [2,26,30].

Furthermore, type-I PKSs are responsible for producing macrocyclic polyketides (macrolides). Macrolide belongs to polyketide compound characterized by macrocyclic lactone ring containing between 12 and 16 atoms which has various bioactivities such as antibacterial, antifungal, immunosuppressant and anticancer. As an antibacterial agent, macrolide works by inhibiting protein synthesis by binding to the 50S ribosomal subunit and blocking translocation steps of protein

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synthesis [8,27,31]. Some examples of macrolides produced by *Streptomyces* are rapamycin, FK506, spiramycin, avermectin, methymycin, narbomycin and pikromycin as shown in Figure 2 [32–36].

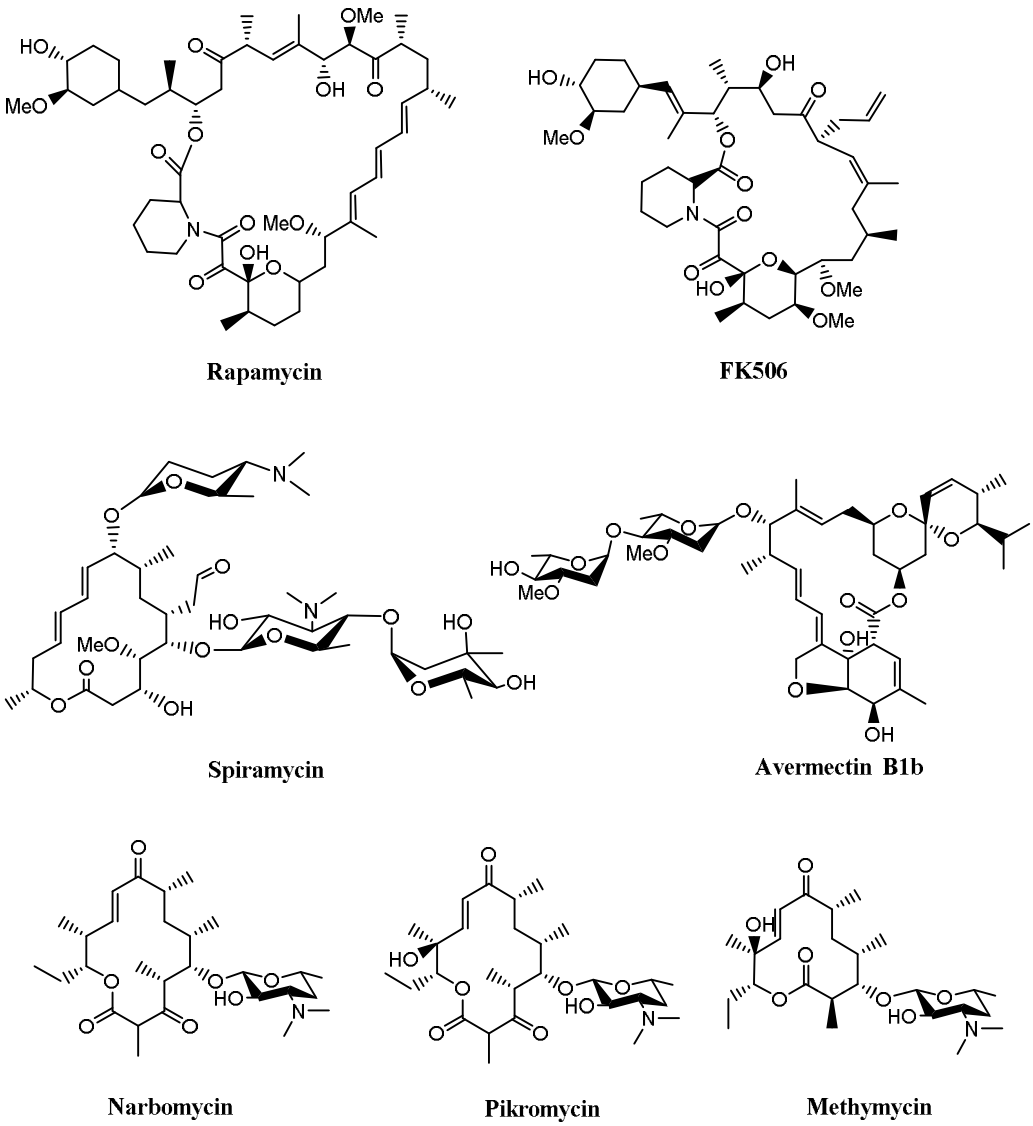

Figure 2. Some of macrolides produced by *Streptomyces*.

3. Biosynthesis of Rapamycin

Rapamycin is 31-membered ring macrolide produced by *Streptomyces hygroscopicus* isolated firstly from a soil of Easter Island (Chile) in South Pacific Ocean. It is a hydrophobic compound and was discovered as antifungal compound against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Fusarium oxysporum*, and some pathogenic species from genus *Penicillium*. The antifungal mechanism of this compound has been described by diffusing into the cell and attaching to intracellular receptor FKB12. Moreover, the complex of FKB12-rapamycin inhibits the TOR (target of rapamycin) kinases that has important role in cell cycle progression. Interestingly, rapamycin has not only antifungal activity but also anticancer and immunosuppressant activity [8,27,37,38].

Rapamycin is synthesized by type-I PKSs rapamycin synthase (RAPS) [39]. Rapamycin-PKS gene cluster (*rapPKS*) is 107.3 kb in size and has 3 remarkable large ORFs (open reading frames),

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rapA, *rapB* and *rapC* which encode multifunctional protein RAPS1 (~900 kDa), RAPS2 (~1.07 MDa) and RAPS3 (~660 kDa), respectively. Protein RAPS1 comprises four modules for polyketide chain extension, protein RAPS2 contains six modules responsible for continuing the process of polyketide chain elongation until C-16, and RAPS3 possesses four modules which have role in completing the polyketide fraction of rapamycin molecule. Overall, these three giant proteins encompass 70 domains or enzymatic functions and because of this, rapamycin PKSs are considered as the most complex multienzyme system discovered so far [26,27,32].

In rapamycin PKSs, there is a loading domain (LD) before the first module involving three domains, i.e. coenzyme A ligase (CL), ER and ACP domain, which are considered to play in role of activating and reducing a free shikimic-acid-derived moiety starter unit and finally passing it to the KS domain of the first module. The extender units incorporated for growing chain are malonyl-CoA and methylmalonyl-CoA. Rapamycin PKSs has special characteristic not only in the starting process but also in the finishing process which the mechanism of transferring from the last domain in rapamycin PKSs and cyclisation of polyketide molecule is assisted by pipecolate-incorporating enzyme (PIE) as depicted in Figure 3. This enzyme (170 kD) is encoded by gene *rapP* which is also located in the *rapPKS* gene cluster and considered has the similarity to genes encoding nonribosomal peptide synthethases (NRPSs) [26,27,32].

4. Polyketide Synthases Type II

The type II polyketide synthases (type-II PKSs) are responsible for producing aromatic polyketide. Based on the polyphenolic ring system and their biosynthetic pathways, the aromatic polyketides produced by type-II PKSs are classified into three groups, i.e. anthracyclines, angucyclines, aureolic acids, tetracyclines, tetracenomycins, pradimicin-type polyphenols, and benzoisochromanequinones. Some examples of aromatic polyketide produced by *Streptomyces* are actinorhodin, doxorubicin, jadomycin B, oxytetracycline, mithramycin, tetracenomycin C, and benastatin A (Figure 4) [28,40–45].

Unlike type-I PKSs that involve huge multifunctional proteins that have many modules containing domains and perform the enzymatic reaction in a non-iterative way, the type-II PKSs have monofunctional polypeptides and work iteratively to produce aromatic polyketide. However, like the type-I PKS, the type-II PKSs also comprise acyl carrier protein (ACP) that functions as an anchor for the nascent polyketide chain. In addition to possessing ACP, the type-II PKSs also consist of two ketosynthases units (KS_α and KS_β) that work cooperatively to produce poly-β-keto chain. KS_α unit catalyze condensation of the precursors, on the other hand, the role of KS_β in the type-II PKSs is as a chain length-determining factor. The three major systems (ACP, KS_α and KS_β) are called 'minimal PKS' that work iteratively to produce aromatic polyketide. The other additional enzymes such as ketoreductases, cyclases and aromatases cooperate together to transform the poly-β-keto chain into the aromatic compound core. Furthermore, the post-tailoring process is conducted by oxygenases, glycosyl and methyl transferases [40,46–48].

5. Biosynthesis of Doxorubicin

Doxorubicin was isolated from *Streptomyces peucetius* in the early of 1960s. It belongs to anthracyclines that has tetracyclic ring containing quinone and hydroquinone group in its structure. Doxorubicin is one of the important drugs for treatment of cancer such as breast cancer, childhood solid tumors, soft tissue sarcomas, and aggressive lymphomas. There are some proposed mechanisms how doxorubicin kills the cancer cells, i.e. (i) DNA intercalation, (ii) topoisomerase II poisoning, (iii) oxidative stress, and (iv) ceramide overproduction [49–51].

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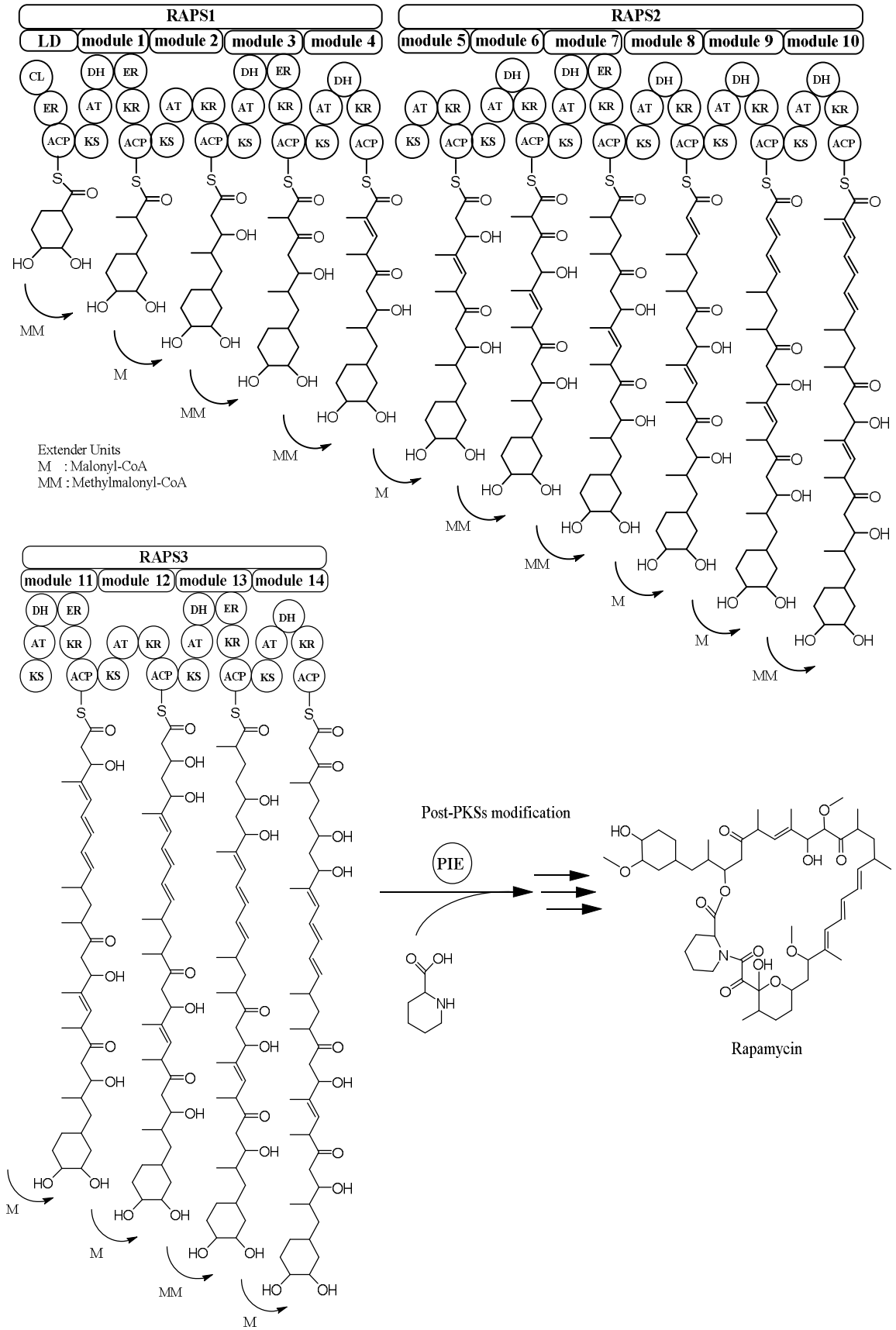

Figure 3. Biosynthesis of rapamycin.

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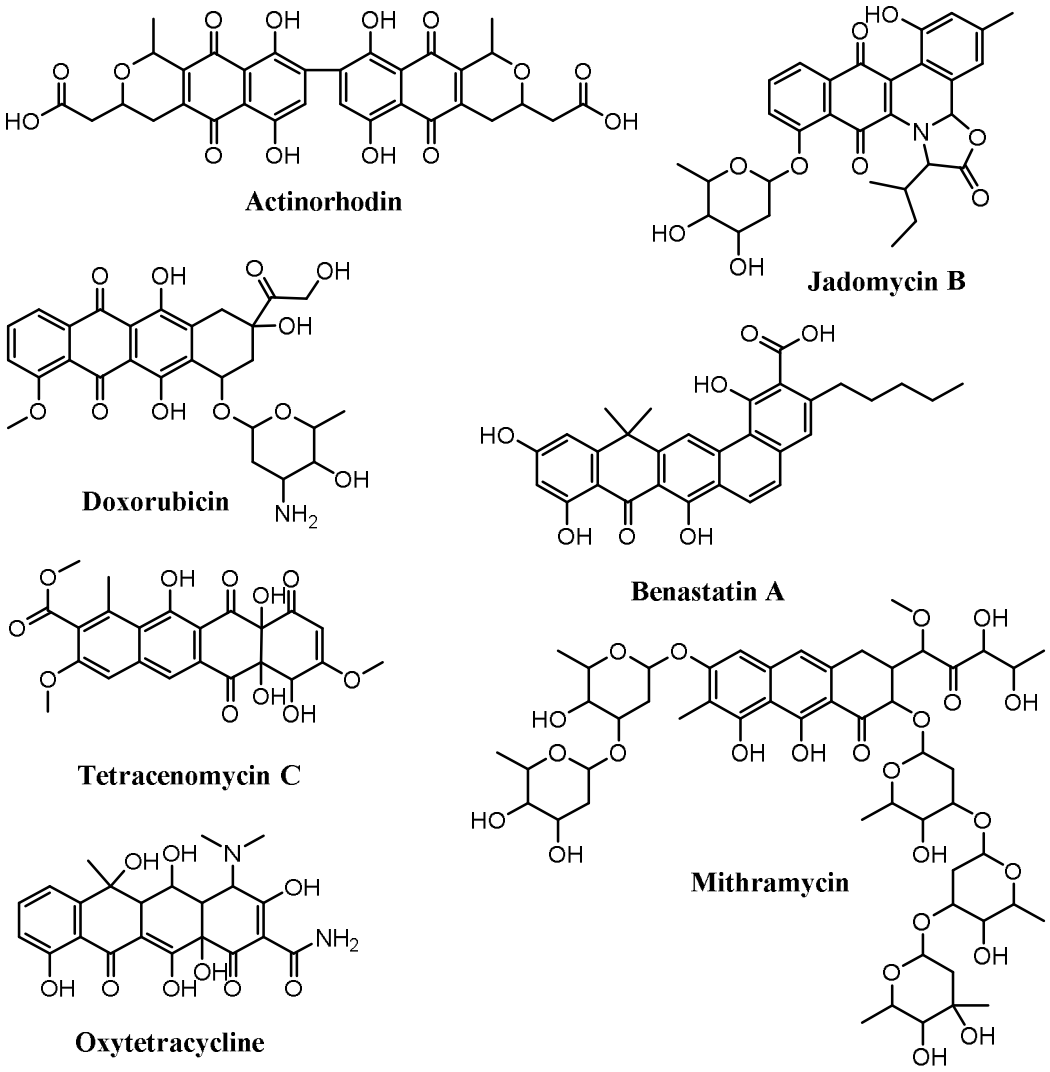

Figure 4. Some aromatic polyketides produced by *Streptomyces*.

Daunorubicin (DNR)-doxorubicin (DXR) type-II PKSs, encoded by *dps* genes in *Streptomyces peucetius*, are involved in the formation of doxorubicin. The biosynthesis of doxorubicin requires one of propionyl-CoA as the starter unit and nine of malonyl-CoA as the extender units. The process involves two 'minimal PKS' expressed by *dpsABCDG* genes to produce a 21-carbon decaketide as an intermediate compound. The repetitive process is conducted by KS_{α} (DpsA), KS_{β} (DpsB) and ACP (DpsG). The next process employs several enzymes such as ketoreductase (DpsE), cyclases (DpsF, DpsY and DnrD), oxygenase (DnrG and DnrF), and methyl transferase (DnrC) to produce ε-rhodomycinone, an important intermediate of doxorubicin biosynthesis. The remaining steps to synthesize doxorubicin utilize glycosyltransferase (DnrS) with the thymidinediphospho (TDP) derivative of L-daunosamine, methyl esterase (DnrP), oxygenase (DoxA) and methyl transferase (DnrK) (Figure 5) [48,52–56].

6. Polyketide Synthases Type III

Unlike the type-I and type II PKSs, the type-III PKSs do not utilize ACP as an anchor for the production of polyketide metabolite. In this case, acyl-CoAs are used directly as substrates for generating polyketide compounds. In order to create polyketides, this system contains enzymes that construct homodimers and catalyzes many reactions such as priming, extension, and cyclization in the iterative way. With this fact, the type-III PKSs are the simplest structure among the other type PKSs. The type-III PKSs founded in bacteria was first time reported in 1999 and before that time the type-III PKSs were known only could be detected in plants [57–59].

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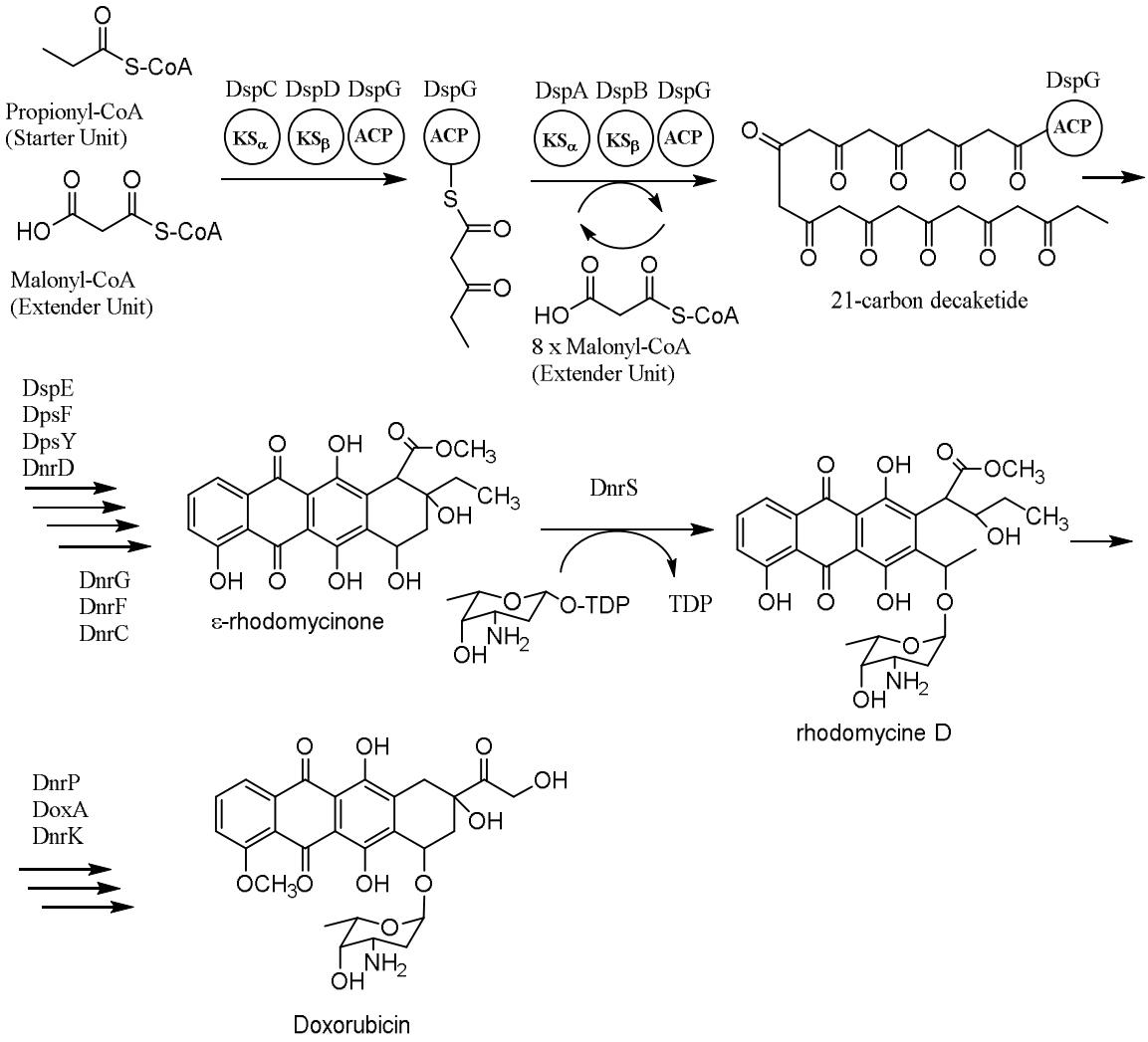

158
159

160 **Figure 5.** Biosynthesis of doxorubicin.

161 Some studies previously revealed that type-III PKSs could also be identified in the *Streptomyces*
162 such as RppA, founded in *Streptomyces griseus*, that is responsible in the synthesis of
163 1,3,6,8-tetrahydroxynaphthalene (THN), which is the intermediate compound in the synthesis of
164 flaviolin and hexahydroxyperylenequinone (HPQ) melanin [60]. Gcs, identified in *Streptomyces*
165 *coelicolor* A3(2), is reported has an important role in the biosynthesis of germicidin [61]. SrsA,
166 encoded by *srsA* gene and isolated from *Streptomyces griseus*, is known to have an important role in
167 the biosynthesis of phenolic lipids, i.e. alkylresorcinols and alkylpyrones [29]. The type-III PKS
168 Ken2, isolated from *Streptomyces violaceoruber*, was suggested to be involved in the production of
169 3,5-dihydroxyphenylglycine (3,5-DHPG). This compound is nonproteinogenic amino acid needed
170 for formation of kendomycin and several other glycopeptide antibiotics such as balhimycin,
171 chloroeremomycin and also vancomycin [62]. Cpz6, encoded by *cpz6* gene and isolated from
172 *Streptomyces sp.* MK730–62F2, was reported to be engaged in the biosynthesis of caprazamycins by
173 producing a group of new triketidepyrenes (presulficidins) [63]. Moreover, other finding also
174 suggested that DpyA, encoded on a linear plasmid of *Streptomyces reveromyceticus*, catalyzes the
175 formation of the alkyldihydropyrones (Figure 6) [64].

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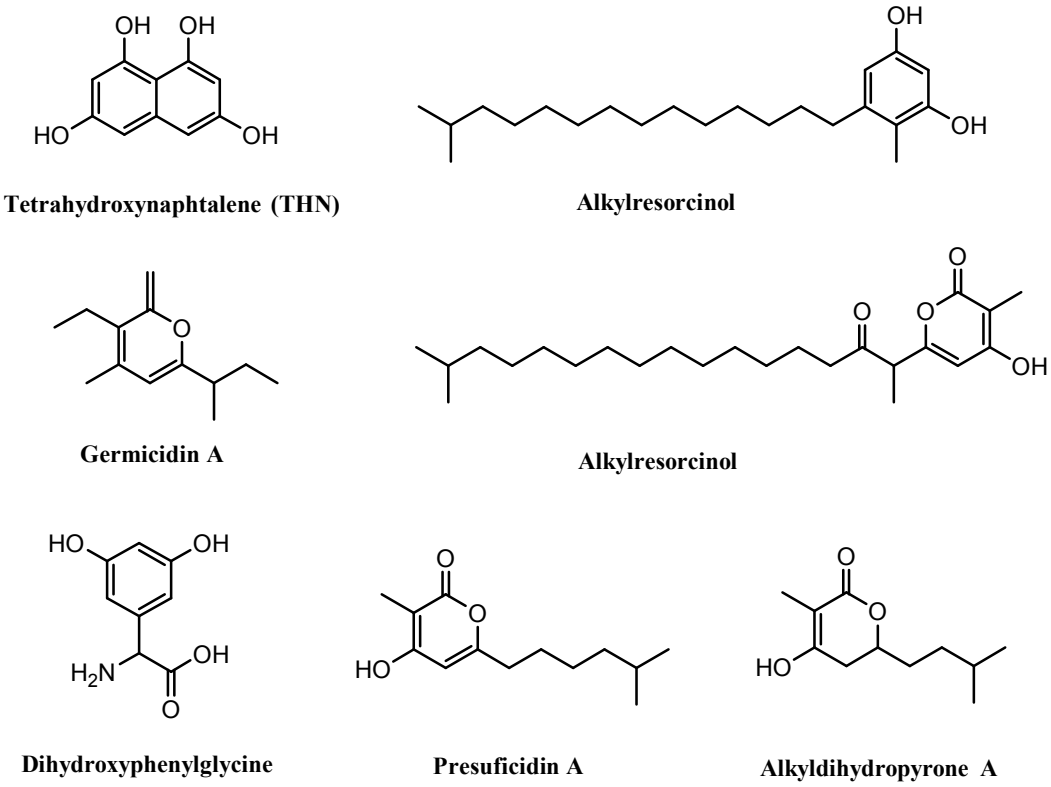

Figure 6. Some compounds produced by type-III PKSs.

7. Biosynthesis of Germicidin

Germicidin, a pyrone-derived polyketide, is produced by a type-III PKSs germicidin synthase (Gcs) and is known to inhibit spore germination. Germicidin A, produced by *Streptomyces viridochromogenes* and *Sreptomyces coelicolor*, prevents the spore germination reversibly at very low concentration (40 pg/ml). The mechanism of inhibition is suggested by affecting the sporal respiratory chain and blocking Ca^{2+}-activated ATPase, thus resulting inadequate energy for spore gemination. Furthermore, germicidin A also has antibacterial properties against various Gram-positive bacteria [65,66].

Although, many bacterial type-III PKSs use only malonyl-CoA as both starter and extender units, the type-III PKS Gcs, which is responsible in germicidin biosynthesis, is suggested having ability to utilize either acyl-ACP or acyl-CoA as a starter unit [67]. Moreover, for extender units, Gcs may involved malonyl-CoA and either methylmalonyl-CoA or ethylmalonyl-CoA in order to produce many types of germicidins [68]. In the first step, the starter unit is transacylated onto the cystein residue of Gcs and then Gcs catalyzes the condensation reaction between starter unit and extender unit concomitantly with decarboxylation process resulting β-ketoacyl-thioester of CoA. The procces continues with β-ketoacyl-CoA that transacylates back onto the cysteine residue of Gcs (repetitive process) and subsequently undergoes condensation reaction with either methylmalonyl-CoA or ethylmalonyl-CoA simultaneously with decarboxylation to formulate β,δ-diketothioester of CoA. In the end of the reaction, cyclization of the β,δ-diketothioester of CoA is catalyzed also by Gcs to produce varios type of germicidins (Figure 7) [61].

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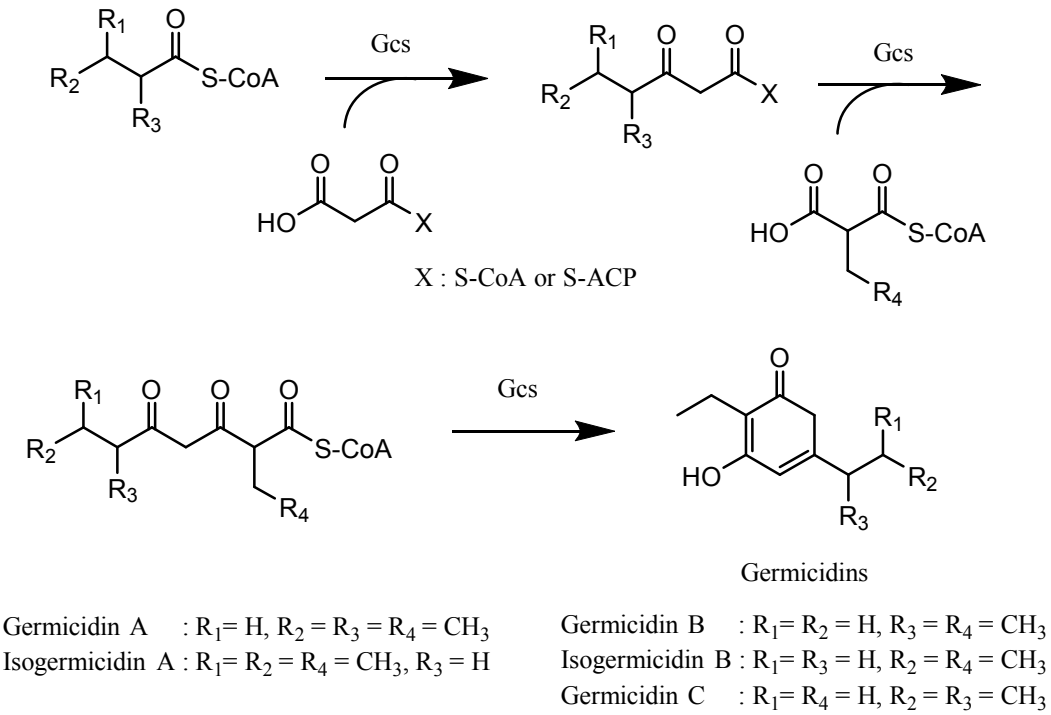

Figure 7. Biosynthesis of Germicidins.

8. Conclusions

There are three types of PKSs (type I, type II, and type III) in *Streptomyces* which is responsible in the biosynthesis of polyketides. Type I polyketide synthases (type-I PKSs) are huge multifunctional proteins that have many modules containing different domains. Each module has responsibility to perform one condensation cycle in a non-iterative way and in each domain, a particular enzymatic reaction is occured. Type-I PKSs are responsible for producing macrocyclic polyketides (macrolides) such as rapamycin, FK506, spiramycin, avermectin, methymycin, narbomycin and pikromycin. The type-II PKSs have monofunctional polypeptides and synthesize iteratively aromatic polyketide such as actinorhodin, doxorubicin, jadomycin B, oxytetracycline, mithramycin, tetracenomycin C, and benastatin A. Unlike the type-I and type II PKSs, the type-III PKSs do not utilize ACP as an anchor for the production of polyketide and use acyl-CoAs directly as substrates for generating polyketide compounds. Type-III PKSs contain enzymes that construct homodimers and catalyze many reactions in the iterative way in the biosynthesis of some compounds such as tetrahydroxynaphthalene (THN), alkylresorcinols, alkylpyrones, dihydroxyphenylglycine, germicidins, presulficidins, and alkyldihydropyrones. The type-III PKSs are also considered as the simplest structure among the other type PKSs.

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