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Streptomyces Cytochrome P450 Enzymes and Their Roles in the Biosynthesis of Macrolide Therapeutic Agents

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Abstract

The study of the genus *Streptomyces* is of particular interest because it produces a wide array of clinically important bioactive molecules. The genomic sequencing of many *Streptomyces* species has revealed unusually large numbers of cytochrome P450 genes, which are involved in the biosynthesis of secondary metabolites. Many macrolide biosynthetic pathways are catalyzed by a series of enzymes in gene clusters including polyketide and non-ribosomal peptide synthesis. In general, *Streptomyces* P450 enzymes accelerate the final, post-polyketide synthesis steps to enhance the structural architecture of macrolide chemistry. In this review, we discuss the major *Streptomyces* P450 enzymes research focused on the biosynthetic processing of macrolide therapeutic agents, with an emphasis on their biochemical mechanisms and structural insights.

Key Words: *Streptomyces*, P450, CYP, Biosynthesis, Macrolide, Secondary metabolite

INTRODUCTION

The phylum actinobacteria is one of the major lineages currently recognized within bacteria (Ventura *et al*., 2007). Actinobacteria are widely distributed in terrestrial, especially soil, and aquatic ecosystems (McCarthy and Williams, 1992; Stach and Bull, 2005). They are important for soil; they use extracellular enzymes to decompose and recycle complex mixtures of polymers in dead plant, animal and fungal materials (McCarthy and Williams, 1992). They also have diverse physiological and metabolic properties, including the ability to produce volatile substrates and secondary metabolites (Gust *et al*., 2003; Berdy, 2005).

Streptomyces is the largest genus among actinobacteria and a genus of the Streptomycetaceae family (Dyson, 2011). Filamentous *Streptomyces* is characterized by a complex life cycle; its morphological differentiation begins as a spore that germinates to produce a substrate (Chater and Chandra, 2006). Actinobacteria produce more than 10,000 bioactive compounds, of which the *Streptomyces* species produces approximately three-quarters (Berdy, 2005). Many of these bioactive compounds are potent antibiotic, antifungal, antitumor, immunosuppressant, antiviral, and antiparasitic agents. These properties have practical and important applications in clinical, veterinary, and agricultural fields. Soil-microorganisms became important to human health with the discovery of penicillin in 1928 by Fleming, and the discovery of the antituberculosis agent streptomycin from *Streptomyces griseus* in 1944 by Waksman (Ikeda, 2017). More recently, the 2015 Nobel prize in Physiology or Medicine was awarded to Omura and Campbell for their contributions to the discovery of the antiparasitic agent avermectin from *Streptomyces avermitilis* (Ikeda, 2017).

STREPTOMYCES P450

The cytochrome P450 enzymes (P450, CYP) are monooxygenases that catalyze a variety of metabolic and biosynthetic chemical reactions. These enzymes are of particular interest to the toxicology, drug metabolism, and pharmacology fields (Guengerich, 2001; Ortiz de Montellano, 2005). They exist throughout various forms of life, including animals, fungi, bacteria, and plants. In many organisms, the progressive completion of genome-sequencing projects has identified more than 15,000 P450 enzyme genes. These are systemically registered in the database (http://drnelson.uthsc.edu/CytochromeP450.html/). The Streptomycetaceae and Mycobacteriaceae genomes are interesting in that they carry an unusually large numbers of P450 genes; they are presumably

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Table 1. *Streptomyces* cytochrome P450 enzymes and their putative reactions

Organisms	P450 names	Putative reactions	References
Streptomyces coelicolor	CYP105N1	Hydroxylation (coelibactin)	Lim *et al*., 2012
	CYP158A1	Biaryl ring coupling (C-C, flaviolin)	Zhao *et al*., 2005, 2007
	CYP158A2	Biaryl ring coupling (C-C, flaviolin)	Zhao *et al*., 2005, 2007
	CYP154C1	Hydroxylation (YC-17, Pikromycin, Narbomycin)	Podust *et al*., 2003
	CYP154A1	Unknown	Lamb *et al*., 2002; Podust *et al*., 2004
	CYP170A1	Hydroxylation Oxidation (Albaflavenone)	Zhao *et al*., 2009; Moody *et al*., 2012
	CYP107U1	Dehydrogenation	Tian *et al*., 2013
	CYP105D5	Hydroxylation	Chun *et al*., 2006, 2007
Streptomyces avermitilis	CYP171A1	Furan ring formation (Avermectin)	Ikeda and Omura, 1997
	CYP107W1	Hydroxylation (Oligomycin A)	Han *et al*., 2015, 2016
	CYP107L2	Hydroxylation (YC-17, Pikromycin, Narbomycin)	Han *et al*., 2017
	CYP105P1	Hydroxylation (Filipin)	Xu *et al*., 2009, 2010
	CYP105D6	Hydroxylation (Filipin)	Xu *et al*., 2009, 2010
	CYP105D7	Hydroxylation	Pandey *et al*., 2010; Xu *et al*., 2015
	CYP158A3	Biaryl ring coup (C-C)	Lim *et al*., 2017
Streptomyces venezuelae	CYP107L1	Hydroxylation (Methymycin, Neomethymycin, Pikromycin)	Xue *et al*., 1998a, 1998b; Dundas *et al*., 2006
Streptomyces rapamycinicus	CYP112A2	Oxidation (Rapamycin)	Graziani, 2009; Molnar *et al*., 1996
	CYP107G1	Hydroxylation (Rapamycin)	Graziani, 2009; Molnar *et al*., 1996
Streptomyces hygroscopicus	CYP105U1	Desaturation (Geldanamycin)	Rudolf *et al*., 2017
	fkbD	Hydroxylation (Ascomycin)	Wu *et al*., 2012
Streptomyces peucetius	CYP129A2	Hydroxylation (Doxorubicin)	Madduri and Hutchinson, 1995; Lomovskaya *et al*., 1999
	CYP107P3	Unknown	Parajuli *et al*., 2004
	CYP107U3	Unknown	Parajuli *et al*., 2004
	CYP107N3	Unknown	Parajuli *et al*., 2004
	CYP105F2	Unknown	Parajuli *et al*., 2004
	CYP105P2	Hydroxylation (Flavone)	Parajuli *et al*., 2004; Lee *et al*., 2016
Saccharopolyspora erythraea	CYP147F1	Hydroxylation (Fatty acids)	Bhattarai *et al*., 2013
	CYP107A1	Hydroxylation (Erythromycin A)	Shafiee and Hutchinson, 1987; Stassi *et al*., 1993
	CYP113A1	Hydroxylation (Erythromycin A)	Shafiee and Hutchinson, 1987; Stassi *et al*., 1993

involved in the biosynthesis of secondary metabolites. The fully sequenced genomes of *Streptomyces* species show that *S. coelicolor* A3(2) has 18 P450 genes, *S. avermitilis*, 33; *S. scabies*, 25; *S. peucetius*, 21; *S. hygroscopicus*, 7; and *Saccharopolyspora erythraea*, 22 within their chromosome sequences (Table 1) (Lamb *et al*., 2011).

Streptomyces coelicolor

S. coelicolor is a soil-dwelling, filamentous, gram-positive bacterium. It is the prototypic strain of the actinomycetes species (Lim *et al*., 2012). Therefore, its 8.7 Mb long chromosome sequence was completed early on; the results indicates a high (72.1%) GC content (Bentley *et al*., 2002). Also, it is predicted to contain 7,825 protein-encoding genes and 18 P450 enzyme genes (Lamb *et al*., 2002, 2010; Lim *et al*., 2012). Many of *S. coelicolor* P450 enzymes are involved in the biosynthesis of antibiotics and pigments. For example, CYP105N1 is an oxidase in the coelibactin siderophore biosynthetic pathway. *S. coelicolor* has a cluster of genes predicted to synthesize a siderophore-related, non-ribosomal-encoded peptide designated as coelibactin (Lim *et al*., 2012). The crystal structures of CYP105N1 were determined by the Waterman group and our group (Lim *et al*., 2012; Zhao *et al*., 2012). Both structures indicate that the wide-open conformation exposes the heme pocket and long I helix to the solvent (Fig. 1). This wide-open binding pocket is located above the heme group with a large, approximately 4299 $Å^3$ volume (Lim *et al*., 2012). Further analyses indicate that the large open pocket to the active site is a key feature for easy accessibility to the peptidyl carrier protein-bound substrate, which performs many P450 catalytic reactions in a series of biosynthetic processes of Streptomycetes (Lim *et al*., 2012).

The *S. coelicolor* genome contains two members of the CYP158A enzymes: CYP158A1, 2, and 3. CYP158A1 and CYP158A2 use oxidative C-C coupling in flaviolin biosynthesis and their structures have been determined bound to flavi-

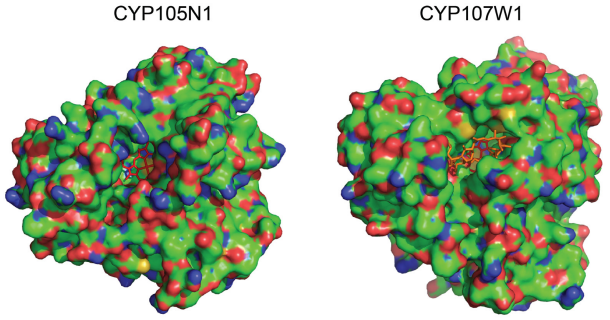

Fig. 1. Wide open conformation of the substrate binding pocket of CYP105N1 and CYP107W1. Electrostatic molecular surfaces of *Streptomyces* P450 enzymes are shown. 3D structural models were constructed using PDB data (CYP105N1, 4FXB; CYP107W1, 4WQ0). The prosthetic heme is labeled in red and oligomycin is labeled in orange.

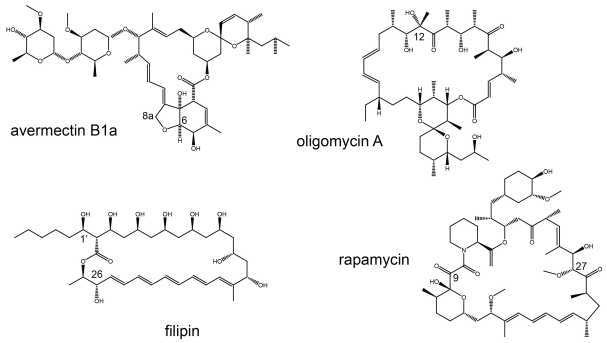

Fig. 2. Chemical structures of metabolites synthesized by *Streptomyces*.

olin. Two flaviolin molecules localize to the active site and are arranged such that dimerization can take place during oxidative metabolism (Zhao *et al*., 2005; Lamb *et al*., 2006; Zhao *et al*., 2007).

The functional interaction of macrolide antibiotics was studied in *S. coelicolor* CYP154C1 (Podust *et al*., 2003). Structural analysis indicates that a novel, open substrate channel into the heme allows substrate access to the active site. This could provide the basis for CYP154C1 catalytic activity toward the macrolide ring system (Podust *et al*., 2003). In addition, structural analysis of CYP154A1 determined an interesting heme orientation in P450 that is inverted 180°. This positions the vinyl and propionic acid side chains of the pyrrole rings to the P450 heme (Podust *et al*., 2004; Lamb *et al*., 2006). Albaflavenone, a tricyclic sesquiterpene antibiotic, is biosynthesized in *S. coelicolor* (Moody *et al*., 2012) by CYP170A1, which catalyzes the final two steps of its biosynthesis. The structural analysis of CYP170A1 determined the bifunctional activity of P450. This CYP170A1 also has a second, completely distinct catalytic activity corresponding to the synthesis of farnesene isomers from farnesyl diphosphate (Zhao *et al*., 2009). Its crystal structure indicates the presence of a novel terpene synthase active site that is moonlighting on the P450 structure (Zhao *et al*., 2009).

The Guengerich group identified a P450 enzyme participating in a sporulation process in Streptomycetes (Tian *et al*., 2013). CYP107U1 from *S. coelicolor* is encoded by a new *bld* gene designated as *sco3099*. Deletion of *sco3099* resulted in a mutant defective in aerial hyphae sporulation, indicating this P450 enzyme plays a key role in the growth and development of *S. coelicolor* (Tian *et al*., 2013). They also studied the electron transport pathways of P450 enzymes in *S. coelicolor*. (Chun *et al*., 2006, 2007) and found that one of the 18 P450s, CYP105D5, tightly binds fatty acids and forms hydroxylated products when electrons are delivered from heterologous systems. The six ferredoxin (Fdx) and four flavoprotein Fdx reductase (FDR) proteins coded by genes in *S. coelicolor* were used to characterize the electron transfer pathway. A primary pathway including FDR1, Fdx4, and CYP105D5 was identified (Chun *et al*., 2007).

Streptomyces avermitilis

S. avermitilis has been named for its ability to produce avermectin, a 16-membered macrocyclic lactone derivative with potent anthelmintic and insecticidal properties. The 2015 Nobel Prize in Physiology or Medicine was awarded to Satoshi Omura and William C. Campbell for their contribution to the discovery of avermectin (http://www.nobelprize.org/). The 9-Mbp *S. avermitilis* genome contains approximately 7,600 open reading frames of proteins (Ikeda *et al*., 2003; Lamb *et al*., 2003) and 33 P450 genes, a relatively high number. These P450 genes are predicted to participate in the production of secondary metabolites, including antibiotics (Lamb *et al*., 2011).

The avermectin biosynthesis pathway contains three stages of processing: the formation of the polyketide-derived initial aglycon, the modification of the initial aglycon to generate avermectin aglycons, and glycosylation of avermectin aglycons to generate avermectins (Ikeda and Omura, 1997). The gene cluster of the second stage include *aveE*, which encodes CYP171A1. CYP171A1 catalyzes the C6-C8a ring cyclase reaction of avermectin (Fig. 2) (Ikeda and Omura, 1997). Efforts to obtain recombinant CYP171A protein by our group and others have not been successful, therefore limited information is available about its biochemistry or catalytic properties.

Oligomycin A, an ATP synthase inhibitor is produced by the *olm* gene cluster of *S. avermitilis* (Fig. 2). The *olmB* gene encodes CYP107W1, which catalyzes the conversion of the macrolide oligomycin C (12-deoxy-oligomycin A) to oligomycin A through a hydroxylation step at C12. Our earlier study characterized the structure of CYP107W1 and the P450 catalytic conversion of oligomycin C to oligomycin A (Han *et al*., 2015, 2016). Mass spectrometry analysis showed that CYP107W1 produced oligomycin A by regioselectively hydroxylating the C12 of oligomycin C (Han *et al*., 2015). Structural analysis of the CYP107W1-oligomycin complex indicated that Trp178, located in the open pocket of the P450active site, is a critical residue that facilitates the productive binding conformation of large macrolide substrates (Fig. 1) (Han *et al*., 2016).

CYP107L2 is an orphan P450 of *S. avermitilis*, meaning its substrate or function is not identified. Its gene is located near a gene encoding a phenylacetate-degrading enzyme. CYP107L2 from *S. avermitilis* shares high sequence similarity with CYP107L1 (PikC) from *S. venezuelae*. Binding of pikromycin and lauric acid yielded the typical type I spectra, but no metabolic product was observed in the enzymatic reaction

(Han *et al*., 2017). Structural analysis of the CYP107L1-lauric acid complex showed that lauric acid is bound mainly by hydrophobic interactions to the carboxylate group of lauric acid. This indicates that significant conformational changes provide sufficient space for lauric acid in the substrate-binding site (Han *et al*., 2017).

The Shoun group characterized two P450 enzymes that catalyse the hydroxylation reaction of the polyene macrolide antibiotic filipin biosynthesis in *S. avermitilis* (Xu *et al*., 2009, 2010). This biosynthetic pathway contains two position-specific hydroxylations of filipin by the C26-specific CYP105P1 and C1'-specific CYP105D6 (Fig. 2) (Xu *et al*., 2009). The crystal structure of the CYP105P1-filipin complex indicated that the distal pocket of CYP105P1 provides a specific environment for the large filipin substrate to bind with its pro-S side of position C26 directed toward the heme iron (Xu *et al*., 2010). A small sub-pocket in CYP105P1, but not in CYP105D6, differentiates the strict regiospecificity of these two P450 enzymes. Therefore, filipin cannot bind to CYP105D6 with the same orientation as in CYP105P1 due to steric hindrance (Xu *et al*., 2010). Furthermore, CYP105D7 from *S. avermitilis* was studied to determine its catalytic power in the metabolism of xenobiotic chemicals (Pandey *et al*., 2010; Xu *et al*., 2015). CYP105D7 catalyzed the 4'-hydroxylation reaction of the anti-inflammatory drug diclofenac and the 3'-hydroxylation reaction of isoflavone daidzein (Pandey *et al*., 2010; Xu *et al*., 2015). Interestingly, structural analysis showed that the active site pocket of CYP105D7 is relatively large and contains two diclofenac molecules, illustrating drug recognition with a double-ligand-binding mode (Xu *et al*., 2015).

One member of the biflaviolin synthase enzymes, CYP158A3, was found in *S. avermitilis*. Its amino acid sequence displays high similarity with CYP158A2, a biflaviolin synthase from *S. coelicolor* (Lim *et al*., 2017). A flaviolin analog displayed similar type I binding upon titration with purified CYP158A3, indicating a P450 enzymatic reaction that forms pigment product *S. avermitilis* (Lim *et al*., 2017).

Streptomyces venezuelae

S. venezuelae is widely recognized for making chloramphenicol, the first antibiotic manufactured synthetically in large-scale. Yet, it also produces secondary metabolites including jadomycin and pikromycin (Bradley and Ritzi, 1968; Jakeman *et al*., 2009). Analysis of the full, approximately 9 Mb genomic sequence of *Streptomyces venezuelae* indicated 71% GC content and 8,080 protein coding genes (Song *et al*., 2016). Like other *Streptomyces*, some of its genome remains clustered among proteins involved in secondary metabolite biosynthesis pathways. The *pik* cluster includes genes which have roles for the biosynthesis of macrolide antibiotics such as narbomycin, pikromycin, methymycin and neomethymycin (Xue *et al*., 1998b). CYP107L1 (also called PikC) is found in the *pik* gene cluster and is a unique P450 in *Streptomyces* in that it can produce a number of related macrolide products from the gene cluster pathway (Xue *et al*., 1998a; Dyson, 2011). Interestingly, PikC can catalyze P450 reactions at different positions of macrolactones, using the 12-membered ring YC17 or the 14-membered ring narbomycin as substrates (Sherman *et al*., 2006; Dyson, 2011). Crystal structure analysis revealed a prominent feature of the PikC-macrolactone substrate interaction, which is the anchoring of the desosamine residue in two alternative binding pockets. This was based on a series of distinct amino acid residues that form a salt bridge and a hydrogen-bonding network with the deoxysugar C3' dimethylamino group (Sherman *et al*., 2006).

Streptomyces rapamycinicus

The *S. rapamycinicus* strain was first isolated from soil on the Easter island and was earlier classified as *Streptomyces hygroscopicus* (Kumar and Goodfellow, 2008; Baranasic *et al*., 2013). This strain is the only organism that produces rapamycin, a macrolide immunosuppressant agent (Vezina *et al*., 1975; Alayev and Holz, 2013). Rapamycin is used to prevent organ transplant rejection, especially useful in kidney transplants, and used to coat coronary stents (Vezina *et al*., 1975). The immunosuppressive function of rapamycin is accomplished by inhibiting T- and B-cell activation by inhibiting mTOR to reduce their sensitivity to interleukin-2 (IL-2) (Mukherjee and Mukherjee, 2009).

In 2013, the Petkovic group obtained a draft genome sequence of the approximately 12.7 Mb *S. rapamycinicus* NRRL 5491 (Baranasic *et al*., 2013). This finding makes it possible to predict that *S. rapamycinicus* contains 10,425 protein-coding genes and 25 modular secondary metabolite clusters (Baranasic *et al*., 2013).

Since rapamycin is widely used in the medical field, the biosynthesis of rapamycin in this organism has been an area of intense focus. The gene cluster in rapamycin biosynthesis includes a type I polyketide synthase in conjunction with a non-ribosomal peptide synthetase often found in other macrolide biosynthesis pathways. Two P450 enzymes are involved in the rapamycin biosynthesis pathway, CYP112A2 (RapJ) and CYP107G1 (RapN) participate in the final series of post-PKS tailoring steps, including oxidation and *O*-methylation reactions (Molnar *et al*., 1996; Graziani, 2009). Furthermore, they catalyze hydroxylation reactions at the C9 and C27 of pre-rapamycin macrolide, respectively (Fig. 2) (Chung *et al*., 2001). Recently, our group successfully obtained the recombinant proteins of these enzymes and their crystal structures have been analyzed (unpublished).

Streptomyces hygroscopicus

S. hygroscopicus has several subspecies and their classification is based on genomic similarity, however it is still difficult to determine features of its genome (Kumar and Goodfellow, 2010). In 2012, genomic analysis of *Streptomyces hygroscopicus* 5008 found that its 10.4 Mb genome has seven genes encoding P450 enzymes (Wu *et al*., 2012). Like other Streptomycetes, *S. hygroscopicus* produces valuable secondary metabolites. Geldanamycin, an antitumor agent, was originally discovered in *S. hygroscopicus* 17997 (He *et al*., 2006). Geldanamycin is a macrocyclic polyketide that is synthesized by a Type I polyketide synthase and a series of post-PKS tailoring steps including hydroxylation, o-methylation, carbamoylation, and oxidation (Lee *et al*., 2006). CYP105U1 (gdmP) is a P450 enzyme that participates in the geldanamycin biosynthesis pathway where it catalyzes the desaturation reaction (Rudolf *et al*., 2017). In addition, the FK520 gene cluster of *S. hygroscopicus* var. *ascomyceticus* (ATCC 14891) includes the *fkbD* gene encoding a P450 hydroxylase involved in ascomycin (FK520) biosynthesis. Ascomycin is an ethyl analog of tacrolimus (FK506) with strong immunosuppressant properties (Wu *et al*., 2012). However, there are no reports on the biochemical study of cytochrome P450 enzyme from *S. hygroscopicus*.

Streptomyces peucetius

S. peucetius strain produces the widely used daunorubicin and doxorubicin, which are clinically important anthracycline chemotherapeutic agents of the polyketide class of antibiotics (Lomovskaya *et al*., 1999). Its 8.7 Mb genome has 7,187 protein coding genes with 21 P450 enzymes including CYP129A2 (DoxA) (Parajuli *et al*., 2004). The *DoxA* gene is found in the DNR-DXR gene cluster and its gene product, the enzyme CYP129A2, participates in the final hydroxylation step of the doxorubicin production process (Madduri and Hutchinson, 1995; Lomovskaya *et al*., 1999). Recently, the Oh group successfully purified the CYP129A2 enzyme and used mass spectrometric analysis to characterize its hydroxylation of resveratrol (Rimal *et al*., 2018).

DnrQ, a member of the DNR gene cluster in *S. peucetius*, shows significant similarity to cytochrome P450, but lacks the heme binding region essential to P450 enzymes (Otten *et al*., 1995). Some P450s (CYP107P3, CYP107U3, CYP107N3, CYP105F2, and CYP105P2) are similar to the P450 enzymes of other organisms, but their functions are unclear (Parajuli *et al*., 2004). Interestingly, recombinant CYP105P2 showed flavone hydroxylase activity when assisted by the redox partner genes camA and camB (Niraula *et al*., 2012). The crystal structure of CYP105P2 indicates it has structural flexibility that can accommodate a broad range of ligands (Lee *et al*., 2016). CYP147F1 has ω-hydroxylation activity on long-chain fatty acids, and is therefore responsible for physiological processes in *S. peucetius* instead of producing secondary metabolites (Bhattarai *et al*., 2013).

Saccharopolyspora erythraea

S. erythraea produces the macrolide antibiotic erythromycin A. Full genomic sequencing revealed *S. erythraea* is an 8.2 Mb long, circular chromosome with 7,198 protein coding genes and at least 25 gene clusters for the biosynthesis of secondary metabolites (Oliynyk *et al*., 2007). Two P450s, CYP107A1 (EryF) as a C6 hydroxylase and CYP113A1 (EryK) as a C-12 hydroxylase, participate in multiple steps leading to the production of erythromycin A (Shafiee and Hutchinson, 1987; Stassi *et al*., 1993). Interestingly, the crystal structure of EryF revealed an alanine residue instead of a threonine at a conserved site that in most P450 enzymes facilitates the involvement of the substrate hydroxy group in O_2 activation (Nagano *et al*., 2005). Furthermore, the crystal structure of the EryF-ketoconazole complex demonstrates the collapse of the I-helix cleft (an unexpected conformational change of the I-helix region of the EryF-ketoconazole complex), giving researchers a new perspective on the structural features of active-site conformational changes due to ligand binding (Cupp-Vickery *et al*., 2001). On the other hand, structural analysis of EryK suggests that it remains in an open conformation without ligand binding in the physical condition. Upon EryK binding substrate, its conformation changes into the closed form (Savino *et al*., 2009). This structural data on the conformational change of EryF and EryK indicates that more effective P450 catalysis occurs during biosynthetic production of erythromycin A.

CONCLUSIONS

The utilization of *Streptomyces* is most attractive for clinical therapeutics and the drug development fields. At present, approximately two-thirds of naturally occurring antibiotics and other bioactive molecules are produced by *Streptomyces* and its related species. Cytochrome P450 enzymes have exquisite regio- and stereo-selectivity for catalysis. They are also versatile in many difficult chemistries. The numerous P450 genes found in *Streptomyces* aids in the unique chemistry required for secondary metabolite synthesis. The study of *Streptomyces* P450 enzymes will be a contributing factor for future bioengineering strategies in the pharmaceutical industry. The sequencing of *Streptomyces* genomes is expanding as our knowledge increases about P450 enzymes in the production of therapeutic drugs. Therefore, we speculate that the rational design and application of P450 enzymes for more effective therapies will be routine for future drugs.

The physiological functions of P450 enzymes in microorganisms are not yet clear. *Streptomyces* P450 enzymes studies will provide novel insights into the function and chemistry of these enzymes. Specifically, they will aid in elucidating P450 functions in existing and novel secondary metabolite gene clusters. Genetic engineering of specific pathways to generate modified natural molecules would be made possible. Finally, these studies will provide fundamental insights into the biochemistry of the P450 superfamily as a whole.

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