Comparative analysis of cytochrome P450 monooxygenases between the genera *Streptomyces* and *Mycobacterium*

By

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DECLARATION

I, **SENATE MOSHOESHOE** (Lesotho ID number:), hereby certify that the thesis submitted by me for the degree DOCTOR OF HEALTH SCIENCES in BIOMEDICAL TECHNOLOGY is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology (Free State). I hereby declare that this research project has not previously been submitted to any university or faculty for the attainment of any qualification. I further waive copyright of the dissertation in favour of the Central University of Technology (Free State). I also state that this work was carried out in collaboration with my colleague, Mr Martin Tjatji.

12/04/2019

SENATE MOSHOESHOE **DATE**

ABSTRACT

Cytochrome P450 monooxygenases (CYPs/P450s) are found in all domains of life and are known for their catalytic versatility and stereo- and regio-specific activity. While the impact of lifestyle on P450 evolution has been reported in many eukaryotes, this remains to be addressed in bacteria. In this report, *Streptomyces* and *Mycobacterium*, belonging to the phylum *Actinobacteria*, were studied owing to their contrasting lifestyles and impact on human beings.

Genome-wide data mining and annotation of P450s in 48 *Streptomyces* species revealed the presence of 1 625 P450s in their genomes. Of these 1 625 P450s, all had characteristic glutamic acid and cysteine at the EXXR and CXG motifs, respectively; however, 34 P450s lacked one or both P450 characteristic motifs, EXXR and CXG, owing to their short amino acid sequences and were thus regarded as fragment/pseudo-P450s. Comparison of P450s revealed that species belonging to the genera *Streptomyces* and *Mycobacterium* had almost the same patterns in terms of the average number of P450s in their genomes. All 1 625 P450 identified in 48 *Streptomyces* species were grouped into 144 P450 families and 377 P450 subfamilies. Among the families and subfamilies, 66 new P450 families and 144 new P450 subfamilies were identified in *Streptomyces* species. Analysis of P450 family conservation across 48 *Streptomyces* species revealed that among 144 P450 families identified, only two P450 families, the CYP107 and CYP157 P450 families, are conserved across all *Streptomyces* species.

Comparative analysis of P450 family dynamics between the genera *Streptomyces* and *Mycobacterium* revealed the presence of the highest number of P450 families and P450 subfamilies in *Streptomyces* species. *Streptomyces* species have 144 P450 families and 377 P450 subfamilies, compared to 77 P450 families and 132 P450 subfamilies in mycobacterial species. *Streptomyces* species also have the highest number of new P450

families (66 families) and new P450 subfamilies (144 subfamilies) in their genomes compared to mycobacterial species. Interestingly, only two P450 families (CYP107 and CYP157) were conserved in *Streptomyces* compared to mycobacterial species where 10 P450 families, namely CYP51, CYP123, CYP125, CYP130, CYP135, CYP136, CYP138, CYP140, CYP144 and CYP1128, were conserved. Furthermore, P450 diversity percentage analysis between two genera revealed that *Streptomyces* species had almost double the P450 diversity percentage (0.18%) than mycobacterial species (0.07%). Comparative analysis of P450 profiles between the genera *Streptomyces* and *Mycobacterium* revealed that species belonging to these genera have different P450 profiles with few similarities. Despite both genera belonging to the same phylum, *Actinobacteria*, only 21 P450 families were found to be common and quite a large number of P450 families were found to be unique to *Streptomyces* (123 P450 families) and *Mycobacterium* (56 P450 families). In the 21 P450 families commonly found between the two genera, an interesting feature was observed in terms of the number of member P450s.

A significant difference in the number of member P450s in the commonly shared P450 families was observed between *Streptomyces* and *Mycobacterium*. Differences were also observed in the number of dominant P450 families in the two genera. Only seven P450 families, namely CYP107, CYP105, CYP157, CYP154, CYP156, CYP147 and CYP183, contributed 62% of all P450s in *Streptomyces* species, whereas 15 P450 families, namely CYP125, CYP189, CYP150, CYP136, CYP135, CYP138, CYP140, CYP123, CYP143, CYP142, CYP144, CYP124, CYP108, CYP51 and CYP187, contributed 60% of all P450s in *Mycobacterium*. Furthermore, differences in P450 profiles between the two genera were observed in terms of type of dominant P450 families. A comparison of the dominant P450 families between the two genera revealed that none of the dominant P450 families was common between them. This strongly supports the concept that these *Streptomyces* P450s play a key role in the production of chemically diverse secondary metabolites, as a large number of P450

families were found in 48 *Streptomyces* species. P450 families, namely CYP105, CYP107, CYP161, and CYP183, which are highly populated in *Streptomyces* species compared to mycobacterial species, were found to be involved in secondary metabolite production. In contrast to the P450 families highly populated in *Streptomyces* species, P450 families that are highly populated in mycobacterial species, such as CYP125, CYP124, CYP108, CYP140 and CYP268, are involved in steroid (cholesterol) and hydrocarbon (lipids, alkenes, long chain acetate and ketone) hydroxylation, suggesting that these P450 families possibly help mycobacterial species to assimilate the host compounds.

It is clear that different lifestyles influenced the P450 profiles in *Streptomyces* and *Mycobacterium*, hence the differences observed between the two genera in terms of number of P450s, P450 family and subfamily diversity, dominant and unique P450 families types and differences in number of P450s in common P450 families. Furthermore, functional analysis of P450s suggests that in *Streptomyces*, P450s are destined for secondary metabolite production, whereas in *Mycobacterium* they are destined for utilisation of host lipids or synthesis of novel lipids. Based on the evidence presented in this study, the researcher hereby proposes that lifestyle or ecological niches play a key role in the evolution of P450 profiles in species belonging to the genera *Streptomyces* and *Mycobacterium*.

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DEDICATION

With gratitude I dedicate this work to my supervisor, Prof. Syed, without whom this thesis would not have been completed.

Next, to my beloved family, Benjamin, Prince and Precious, for believing in and praying for me.

Lastly, in commemoration of my late mothers, Mathesele Moshoeshoe and Sarah Phakisi – my memories of you inspired me to reach for the sky.

"The greater the difficulty, the more glory in surmounting it" – Epicurus

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LIST OF ABBREVIATIONS AND ACRONYMS

ABBA	α-β-β-α barrel fold
ATCC	American Type Culture Collection
bp	base pairs
BLAST	Basic Local Alignment Search Tool
CYP/P450s	Cytochromes P450 monooxygenases
CDC	Center for Disease Control
GC	Guanine-Cytosine
KEGG	Kyoto Encyclopedia of Genes and Genomes
Mbp	Mega base pairs
Nm	Nano meter
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NCBI	National Centre for Biotechnology and Information
NO	Nitric Oxide
O_2	Oxygen
ε-PL	ε-Poly-L-lysine
PI	Pristinamycin I
PII	Pristinamycin II

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Streptomyces is the largest genus in the phylum *Actinobacteria* (Ventura *et al*., 2007). *Streptomyces* species are spore-forming filamentous bacteria and commonly known to produce various secondary metabolites including antibiotics (de Lima Procópio *et al*., 2012). It is estimated that more than 50% of commercially available antibiotics are produced by *Streptomyces* species (de Lima Procópio *et al*., 2012). A survey of the characteristics of *Streptomyces* species revealed that most of the species belonging to this genus produce chemically diverse secondary metabolites that are used in human medicine as antibiotics, anti-infective, anti-fibrotic, anti-tumour and immunosuppressant drugs. Much of the chemical diversity of secondary metabolites produced by *Streptomyces* species has been attributed to their symbiotic lifestyle, apart from their well-known saprophytic lifestyles (Seipke *et al*., 2012). Symbiosis of *Streptomyces* species with plants, fungi, and animals has been reported and in some cases parasitic symbiosis was identified (Seipke *et al*., 2012). *Streptomyces* species are known to produce geosmin, a volatile metabolite that is responsible for their characteristic "earthy" smell, and P450s were found to be involved in the production of this metabolite (Lamb *et al*., 2003). On the other hand, the genus *Mycobacterium* includes pathogens known to cause serious diseases (Ventura *et al*., 2007) Generally, mycobacterial species are rod-shaped and some species show pleomorphism (Scherr and Nguyen, 2009). The *Mycobacterium* genus is well known for its human and animal pathogens, especially *M. tuberculosis*, a deadly human pathogen that is responsible for the death of millions of people across the world (Quan *et al*., 2017). In a recent study mycobacterial species were classified into six different categories

based on their lifestyles, site of infection, and other characteristics (Parvez *et al*., 2016). While most of the mycobacterial species are pathogens, some are saprophytes with potential biotechnological applications, such as bioremediation. Comprehensive comparative analysis of P450s in the genus *Mycobacterium* revealed that progression from soil mycobacteria into human pathogens, such as those living in human blood and ultimately adapted as a lung pathogen, resulted in gradual loss of a considerable number of P450s (Parvez *et al*., 2016). Furthermore, species belonging to each of the six categories were found to have category-specific P450s that can be used as a diagnostic marker in the detection and distinction of these species (Parvez *et al*., 2016).

1.2 Problem statement and rationale for the study

Cytochrome P450 monooxygenases (P450s/CYPs) are ubiquitously distributed in all domains of life, and even found in some non-living entities such as viruses (Lamb *et al*., 2009, Nelson, 2018). P450s are catalytically versatile and perform stereo- and regio-specific enzymatic reactions (Bernhardt, 2006, Sono *et al*., 1996).

Because of this unique nature, P450s have been in focus for more than five decades (Yamazaki, 2014). Whole genome sequencing of various organisms belonging to different biological domains and kingdoms have resulted in identification of more than 300 000 P450s (Nelson, 2018). Analyses of P450 evolutionary patterns with respect to species and their ecological niches is gaining great momentum. This type of study has been reported involving eukaryotes, animals (Feyereisen, 2011), plants (Du *et al*., 2016, Hamberger and Bak, 2013, Ma *et al*., 2014), fungi (Jawallapersand *et al*., 2014, Kgosiemang *et al*., 2014, Matowane *et al*., 2018, Ngwenya *et al*., 2018, Parvez *et al*., 2016, Qhanya *et al*., 2015, Suzuki *et al*., 2012, Syed *et al*., 2014) and oomycetes (Sello *et al*., 2015). In the reported studies researchers observed blooming of certain P450 families, the presence of unique P450 families or changes in the

number of P450s with respect to species adaptation to specific ecological niches or lifestyles. However, to date, this type of evolutionary analysis has not been reported for bacteria. To address this research gap, the researcher selected two genera, *Streptomyces* and *Mycobacterium*, belonging to the phylum *Actinobacteria* (Ventura *et al*., 2007), and assessed the impact of lifestyle on the evolution of P450s.

1.3 Aims and objectives of the study

1.3.1 Aims of the study

- Genome data mining, identification, annotation and phylogenetic analysis of P450s in 48 *Streptomyces species*.
- Comparative analysis of P450s between the genera *Streptomyces* and *Mycobacterium*.

1.3.2 Objectives of the study

- Genome data-mining and identification of P450s in 48 *Streptomyces* species.
- Annotation of P450s in 48 *Streptomyces* species.
- Phylogenetic analysis of P450s in 48 *Streptomyces* species.
- P450 diversity analysis.
- Functional prediction of *Streptomyces* P450s.
- Comparative analysis of P450s between the genera *Streptomyces* and *Mycobacterium*.

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CHAPTER 2

LITERATURE REVIEW

2.1 *Streptomyces*

Among living organisms are many species of different nature, which are classified into groups based on very basic, shared characteristics. The two basic types of cells, eukaryotes and prokaryotes, are used to classify living organisms (Black, 2008) and are further organised into three domains: *Archea*, *Eubacteria* and *Eukaryote* (Pang *et al*., 2004, Woese, 2000, Woese *et al*., 1990). Organisms in each group are divided into smaller groups. One of the commonly studied groups is the bacterial kingdom. The bacterial kingdom, being prokaryotes, is further divided into different phyla, including the *Actinobacteria*, which contain diverse groups of bacteria, such as the medically important bacteria belonging to the genus *Streptomyces* (Sun *et al*., 2018, Ventura *et al*., 2007), which will be the focus of this study.

Streptomyces is the largest genus in the phylum *Actinobacteria* (Panpatte *et al*., 2018, Qin *et al*., 2016, Ventura *et al*., 2007). Members of the genus *Streptomyces* are the most complex: they grow as a mycelium with branching hyphal filaments (between 0.5-2.0 µm in diameter), and reproduce in a mould-like manner by sending up aerial branches that turn into chains of spores. Although the aerial hyphae that arise from them are motile, mobility is achieved by dispersion of spores. Spore surfaces may be hairy, rugose, smooth or warty. They demonstrate a complex morphology; their life cycle involves formation of aerial mycelium or filaments from a substrate mycelium when their spores germinate. This process occurs during morphological differentiation (Chater, 1998, Claessen *et al*., 2006, Dyson, 2011, Flärdh and Buttner, 2009). These filamentous Gram-positive actinomycete bacteria live in soil and water as decomposers of organic material. In soil, they behave much like fungi, helping to decompose

the organic matter of dead organisms so the molecules can be taken up anew by plants. Found predominantly in soil and decaying vegetation, most Streptomycetes produce spores (Chater and Merrick, 1979:107), and are noted for their distinct "earthy" odour that results from the production of a volatile compound, geosmin (Gerber and Lechevalier, 1965, Jiang *et al*., 2007, Maheshwari, 2014).

Streptomycetes play an important role in carbon recycling. They act as versatile decomposers through their expression and secretion of a large number of hydrolases, such as chitinase and cellulase, and many other enzymes, to the extracellular environment. These enzymes degrade organic matter to simpler forms, especially biopolymers such as lignocellulose and chitin, from the insoluble remains of other organisms (Chater, 1984, Gilbert *et al*., 1995, Schirawski and Perlin, 2018, Vetrivel *et al*., 2001, Zhou *et al*., 2016b). Their genome consists of a huge number of hydrolases, which constitute 211 gene families that act on a wide range of substrates. The cluster consists of 38 families that hydrolyse peptide bonds, 22 families that act on carbon-nitrogen (but not peptide) bonds, 19 families that hydrolyse O- glycosyl compounds, 12 families that act on ester bonds, 10 families that act on acid anhydrides, and seven families that hydrolyse N-glycosyl compounds (Zhou *et al*., 2016a). Initially, over 500 species of *Streptomyces* were described (Euzeby, 2008). Later, close to 576 *Streptomyces* species were mentioned (Labeda, 2011). With the number increasing every year, Parte (2014) then described 778 species of *Streptomyces*. Since then new additions have been made and to date 848 species of *Streptomyces* have been described in the list of prokaryotic names with standing in nomenclature (Parte, 2018).

Some Actinomycetes form symbioses with plants, while a few are pathogenic to plants, humans and animals (Kers *et al*., 2005, VanMeter and Hubert, 2015, Zhang and Loria, 2017). Examples of plant-pathogenic *Streptomyces* spp. include *S. scabies, S. acidiscabies, S. turgidiscabies,* and *S. ipomoeae,* which have been found to share virulence factors, including

thaxtomins (Huguet-Tapia and Loria, 2012). In humans a few, such as *S. somaliensis* and *S. sudanensis* (Quintana *et al*., 2008) causing mycetoma, have been described.

As with the other *Actinobacteria*, Streptomycetes have genomes with high G+C content and are also known to have a linear chromosome (Madigan and Martinko, 2006, Meurant, 2012). *Streptomyces* microorganisms are known to carry interesting gene clusters of secondary metabolites (Gomez-Escribano and Bibb, 2011, Gomez-Escribano *et al*., 2015, Ikeda *et al*., 2003, Medema *et al*., 2011, Ōmura *et al*., 2001). Many of these secondary metabolites have been isolated and developed as highly successful drugs in the treatment of human diseases (Al- Dhabi *et al*., 2018, Chater, 2006, de Lima Procópio *et al*., 2012, Demain, 1999, Olano *et al*., 2009, Ōmura *et al*., 2001). *Streptomyces* species use these metabolites to compete with fungi and other bacteria for resources in the environment (Hibbing *et al*., 2010, Kinkel *et al*., 2014, Slattery *et al*., 2001, Van der Meij *et al*., 2017). *Streptomyces* species products are also widely used in agriculture as herbicides and pesticides, and in veterinary practice (Dayan *et al*., 2009, Dayan *et al*., 2012, Duke and Dayan, 2011, Korres *et al*., 2018, Ōmura *et al*., 2001, Senseman, 2007). They are also used in the production of volatile substances (Gust *et al*., 2003, Li *et al*., 2012) and liquefaction of coal (Ghani *et al*., 2015, Gupta *et al*., 1988, Sekhohola *et al*., 2013, Torzilli and Isbister, 1994).

A review revealed that genes for the synthesis of such secondary metabolites probably developed millions of years ago (Baltz, 2006). The common ancestor of modern Streptomycetes is said to have lived about 440 million years ago, when it was probably involved in the colonisation of the land by green plants (Chater and Chandra, 2006). However, it was only in the 1940s that their antibiotic properties were discovered (Waksman and Woodruff, 1941). The first true antibiotic identified was from *S. antibiotics* (formerly known as *Actinomyces antibioticus*) and its product was termed actinomycin. Actinomycin could

display both bacteriostatic and bactericidal properties. Waksman and co-workers discovered new natural inhibitory substances, including streptomycin and neomycin (Kingston, 2004). They proposed the standard term “antibiotics” for this class of natural growth inhibitors produced by Actinomycetes, including Streptomycetes (Kingston, 2004). Antibiotics are defined as low-molecular-weight organic natural products (secondary metabolites) made by microorganisms that are active against other microorganisms at low concentration. Since 1940, the majority of antibiotics discovered have been those produced by the Streptomycetes (Gómez *et al*., 2012, Norouzi *et al*., 2018), making *Streptomyces* the largest antibiotic-producing genus of *Actinobacteria* (Figure 2.1) (de Lima Procópio *et al*., 2012).

Figure 2. 1. List of antibiotics produced by Streptomycetes (taken from de Lima Procópio *et al*, 2012).

Streptomycetes are also an important source of clinically important immunosupressants, including sirolimus (also known as rapamycin) and tacrolimus that are used extensively in transplant operations (Kunz and Hall, 1993). Because of the commercial

importance of the secondary metabolites produced by Streptomycetes, there has been considerable investment in the development of methods for molecular genetic manipulation of Streptomycetes (Berdy, 1995, Demain, 1999).

Many decades after the first successful use of antibiotics in the treatment of infections, bacterial infections now pose a serious concern (Spellberg and Gilbert, 2014). The current antibiotic resistance has rendered a large number of these compounds ineffective (McArthur *et al*., 2013). Antibiotics that were discovered are no longer considered effective for treatment owing to resistance. Figure 2.2 illustrates the year of discovery of antibiotics and their current status with regard to resistance. The antibiotic resistance crisis is mostly attributed to the overuse (Read and Woods, 2014) and misuse (Luyt *et al*., 2014) of these medications, as well as lack of new drug development by the pharmaceutical industry (Bartlett *et al*., 2013). These mediations have been used so widely and for so long that infectious organisms have adapted to the antibiotics that are intended for their destruction (Read and Woods, 2014, Ventola, 2015), making the drugs less effective. According to the Centre for Disease Control (CDC, 2017), each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics, resulting in the death of at least 23 000 people.

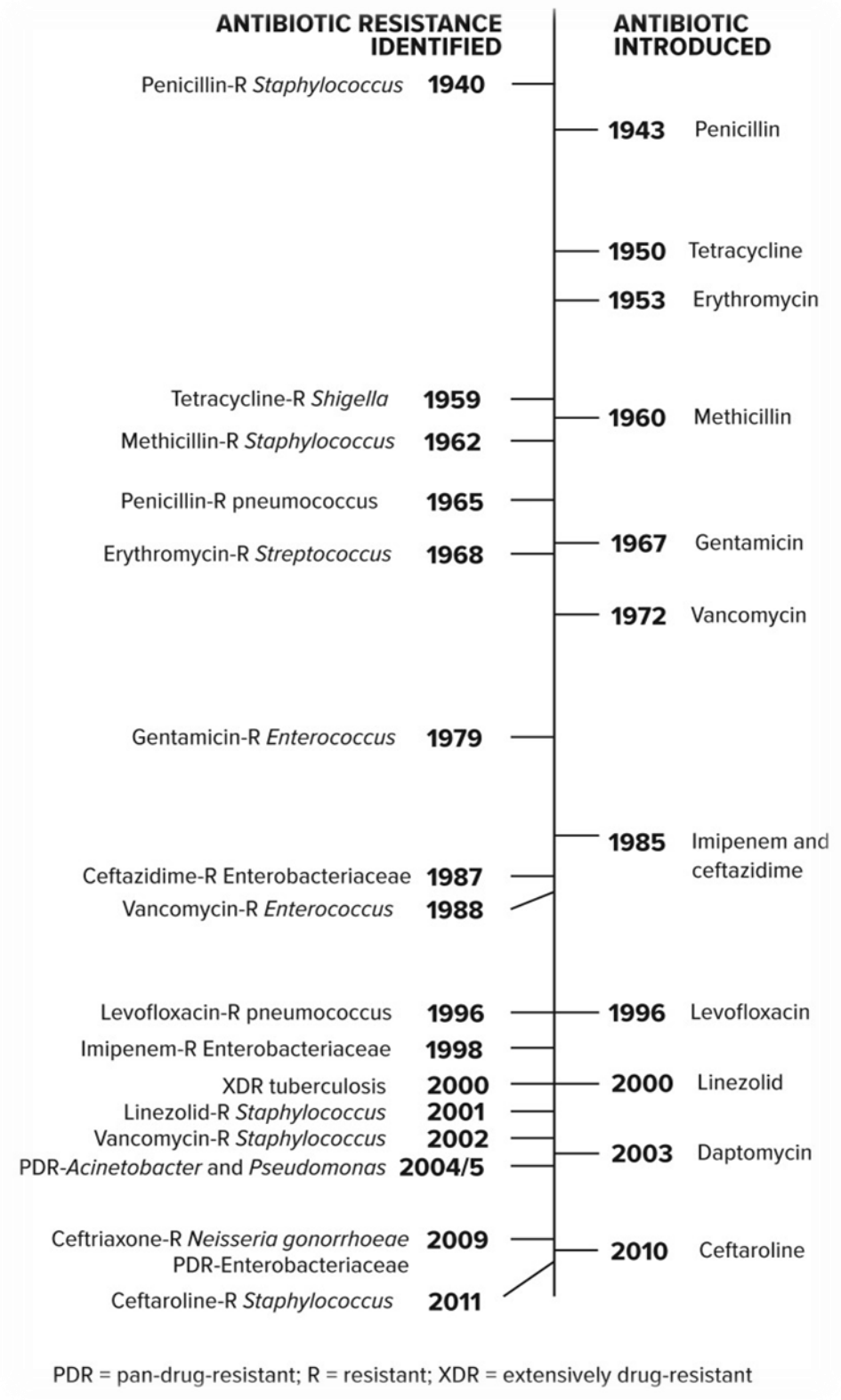


Figure 2. 2. Timelines of antibiotic discovery and development of drug resistance by bacterial species (taken from Spellberg and Gilbert, 2014).

In addition, a number of the antibiotics produced by Streptomycetes have demonstrated toxicity when used in humans. For example actinomycin is toxic to dividing cells, therefore it has been deployed as an anti-cancer drug (Philips *et al*., 1960). Doxorubicin, made by *S. peucetius*, is used as a treatment for several types of cancers (Arcamone *et al*., 1969), even though it has been reported for cardio-toxicity (Thorn *et al*., 2011).

Even though the antibacterial market has entered a critical phase, one that poses numerous challenges, at the same time it also offers opportunities (Gould and Bal, 2013). When the *Streptomyces coelicolor* genome was decoded in 2001 (Bentley *et al*., 2002a), it revealed that there were many previously unknown potentially bioactive substances that have the potential to produce new antibiotics. Since then work on different *Streptomyces* species genome sequencing has been published to identify gene clusters that produce antibiotics (Table 2.1) (Harrison and Studholme, 2014). It is organisms such as *Streptomyces* species that give room and potential for the development of innovative new medicines and other products (de Lima Procópio *et al*., 2012). Recently, three *Streptomyces* species with antibacterial activity against multi-drug-resistant bacteria, especially methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*, were identified (Norouzi *et al*., 2018). In one study, three species of *Streptomyces* demonstrated strong inhibitory activity against quite a number of other bacterial species (Risdian *et al*., 2018). In one study, *Streptomyces* species was found to inhibit the growth of disease causing pathogens such as carbapenem-resistant *Acinetobacter baumannii*, vancomycin-resistant *Enterococcus faecium*, and methicillin-resistant *Staphylococcus aureus* (Terra *et al*., 2018).

Table 2. 1. Information on some *Streptomyces* species and their well-known functions (taken from Harrison and Studholme, 2014).

Species and strain	Well-known for	Reference
Streptomyces albulus CCRC 11814	Produces ε-poly-L-lysine antibiotic	Dodd *et al*., 2013
Streptomyces albus J1074	Widely used host for heterologous expression of bioactive natural products.	Zaburannyi *et al*., 2014
Streptomyces albulus PD-1	Produces ε-poly-L-lysine and poly-L-diaminopropionic acid antibiotics.	Xu *et al*., 2014
Streptomyces bottropensis ATCC 25435	Produces bottromycin antibiotics.	Zhang *et al*., 2013
Streptomyces collinus Tu 365	Producer the elfamycin-family antibiotic kirromycin.	Rückert *et al*., 2013
Streptomyces exfoliatus DSMZ 41693	Degrades poly (3-hydroxyalkanoate).	Martínez *et al*., 2014
Streptomyces fulvissimus DSM 40593	Produces the ionophore antibiotic valinomycin.	Myronovskyi *et al*., 2013
Streptomyces mobaraensis DSM 40847	Industrial producer of transglutaminase.	Yang *et al*., 2013
Streptomyces niveus NCIMB 11891	Produces novobiocin, an aminocoumarin antibiotic.	Flinspach *et al*., 2014
Streptomyces rapamycinicus NRRL 5491	Produces the immunosuppressant drug rapamycin.	Baranasic *et al*., 2013
Streptomyces rimosus ATCC 10970	Oxytetracycline	Pethick *et al*., 2013
Streptomyces roseochromogenes subsp. *oscitan* DS 12.976	Produces clorobiocin, an aminocoumarin antibiotic.	Rückert *et al*., 2014
Streptomyces sp. Mg1	Causes lysis and degradation of *Bacillus subtilis* cells and colonies.	Hoefler *et al*., 2013
Streptomyces sp. PRh5	An endophyte isolated from wild rice root.	Yang *et al*., 2014
Streptomyces violaceusniger SPC6	Tolerant to multiple stresses. Small genome.	Chen *et al*., 2013
Streptomyces viridochromogenes Tu57	Produces the oligosaccharide antibiotic avilamycin.	Grüning *et al*., 2013
Streptomyces viridosporus T7A	Degrades lignin.	Davis *et al*., 2013

2.2.1 *Streptomyces coelicolor*

S. coelicolor is involved in breaking down organic material in the soil. It is similar to fungi in its structure and can adapt to environmental stress. Its potential to cause disease in humans, plants, or animals has not been reported. This bacterium has a very similar core genome to *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*, as well as some similarity to *Mycobacterium leprae*, being members of the same taxonomic order Actinomycetales (Bentley *et al*., 2002a). It comprises a wealth of genes, allowing the organism a more complex life cycle, which allows it to adapt to a wider range of environmental conditions and exploit a greater variety of nutrient sources. It is responsible for producing most of the antibiotics in use today, as well as some immunosuppressant and anti-tumour agents. This organism produces not only antibiotics (Chater and Merrick, 1979:97-98), but also other secondary metabolites, including siderophore pigments and lipids. Figure 2.3 illustrates antibiotics (a), siderophores (b), pigments (c), lipids (d), and other molecules (e) grouped according to their putative function (Bentley *et al*., 2002a). *S. coelicolor* has also been used in genetic engineering studies to express gene clusters of secondary metabolites (Bilyk *et al*., 2013, Gomez-Escribano and Bibb, 2011).

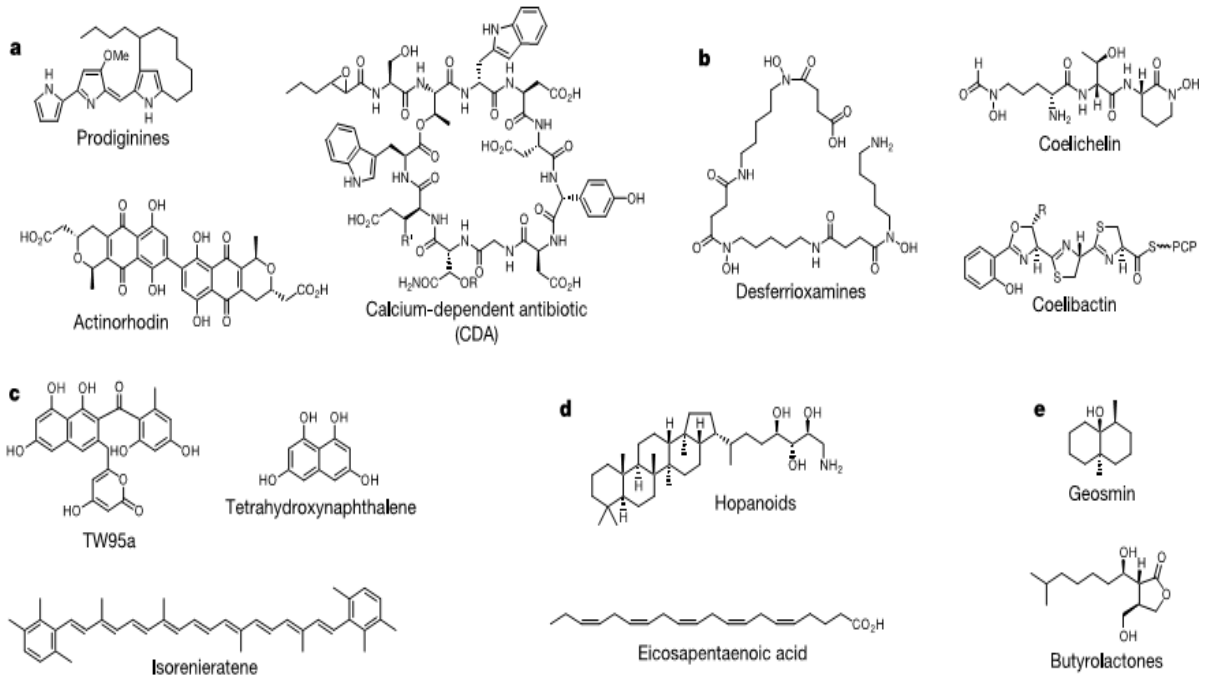


Figure 2. 3. Secondary metabolites known or assumed to be made by *S. coelicolorA3*. (taken from Bentley *et al*., 2002a)

2.2.2 *Streptomyces avermitilis*

S. avermitilis was isolated from a soil sample collected in Shizuoka Prefecture, Japan in 1978 by one of the screening programs for the discovery of antiparasitic metabolites (Burg *et al*., 1979). It is found in terrestrial soils. Its sporophores form spiral side branches on aerial mycelia. They form conidial spores during their life cycle (Witt and Stackebrandt, 1990). *S. avermitilis* produces certain secondary metabolites, namely avermectin, which is a potent agent against a wide array of nematodes and arthropods parasites (Burg *et al*., 1979, Gao *et al*., 2009, MacNeil *et al*., 1992). The complete genome sequence was described by Ikeda *et al*. (2003), who found the strain *S. avermitilis* MA-4680 to be 9.0 Mega base pairs (Mbp) in size. Genome availability is significant, as it allows for comparison of pathogen sequences and secondary metabolite producers within the Actinobacteria. Genome mining of *S. avermitilis* revealed that this organism has at least 38 secondary metabolic gene clusters for at least eight non-ribosomal

peptides, six terpenoids, and assorted pigments; osmolytes, siderophores, and bacteriocins were identified (Ikeda *et al*., 2003). Emamectin (Figure 2.4) which is a derivative of avermectin B1 (Figure 2.4) (Bentley *et al*., 2002b), has also been described.

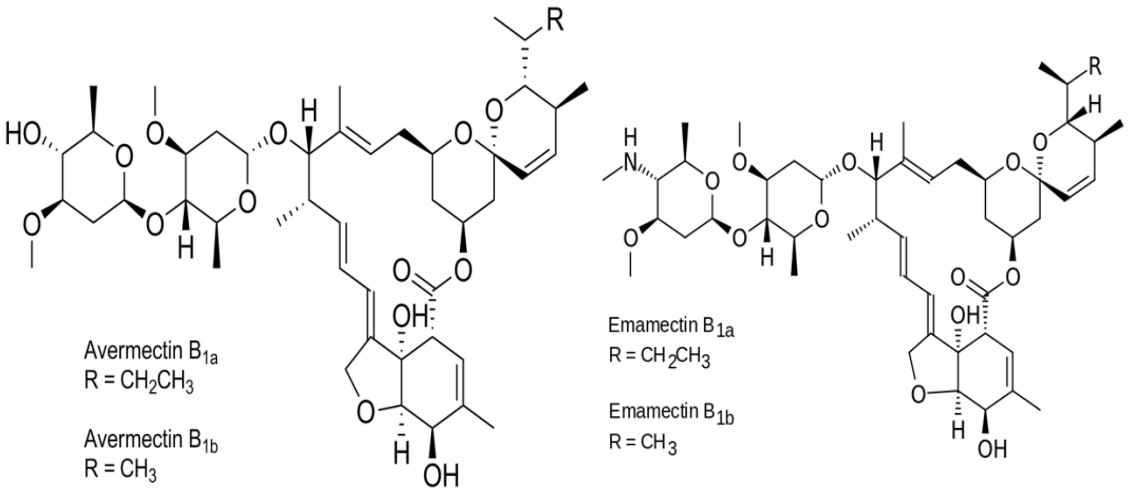


Figure 2. 4. Chemical structure of avemectin and emamectin (taken from Bently *et al*., 2002b).

2.2.3 *Streptomyces griseus*

S. griseus produces streptomycin (Ohnishi *et al*., 2008), a broad-spectrum antibiotic that has been used for the treatment of various diseases, such as tuberculosis and the plague, caused by *Yersinia pestis* (Distler *et al*., 1992). Production of a class III lantipeptide AmfS in *S. griseus* has also been reported (Takano *et al*., 2017) and the organism has been reported to contain a gene cluster that produces fredericamycin, an anticancer drug (Wendt-Pienkowski *et al*., 2005). Genes for streptomycin production in *S. griseus* were described as well (Mansouri and Piepersberg, 1991).

2.2.4 *Streptomyces bingchenggensis* 3

S. bingchenggensis was isolated in Harbin, China from a soil sample. It produces anthelmintic macrolide compounds, which are widely used in the agricultural industry. *S. bingchenggensis* also produces other commercial products, such as milbemycins, a family of macrolide antibiotics used in veterinary medicine and crop protection. In addition, polyether nanchangmycin and cyclic pentapeptides are examples of other natural products made by *S. bingchenggensis* (Wang *et al*., 2013). Its complete genome sequence has been described (Wang *et al*., 2010).

It has a sequenced genome of 11 936 683 bp, which is longer than the other genomes such as *S. avermitilis* MA-4680 with 9.0 Mbp (Ikeda *et al*., 2003), followed by *S. coelicolor A3* with 8.7 Mbp (Bentley *et al*., 2002a) and *S. griseus* with 8.5 Mbp (Ohnishi *et al*., 2008). This is regarded as huge coding potential. *S. bingchenggensis* has a huge number of protein-coding genes and a large number of transport proteins (Zhou *et al*., 2016b), which make compounds that are important for metabolism and enable their adaptation to complex environments.

Some of its proteins, namely bingchamides A (1) and B (2), isolated from the organic extracts of the mycelium, have been found to be cytotoxic to the human colon carcinoma cell line HCT-116 (Xiang *et al*., 2009). In addition to this antitumour activity, compounds with acaricidal and nematocidal activity were further isolated (Xiang *et al*., 2009). Other additional products, such as milbemycins, bingchamides and nanchangmycin produced by *S. bingchenggensis*, were also identified (Wang *et al*., 2015b).

2.2.5 *Streptomyces albus* J1074

S. albus J1074 is described as a derivative of *S. albus* G, because of the absence of restriction and modification enzymes of the SalI system (Chater and Wilde, 1976) The *S. albus* J1074 strain is one of the most widely used strains for the heterologous production of bioactive natural products. It was discovered that, because of its fast growth and genetic system, this strain can be an attractive model for expressing undiscovered biosynthetic pathways to aid in drug discovery (Zaburannyi *et al*., 2014). It produces versatile secondary metabolites ranging from marine micromonospora secondary metabolites to potent anticancer agents.

2.2.6 *Streptomyces rapamycinicus*

During a screening for antifungal compounds, *S. rapamycinicus* was eventually isolated from a soil sample collected from Rapa Nui (Easter Island) (Baranasic *et al*., 2013). It was previously classified as *S. hygroscopicus* (Kumar and Goodfellow, 2008, Sehgal *et al*., 1975) and is often referred to in literature as ATCC 29253. So far it is the only organism known to produce rapamycin, which was initially isolated as an antifungal agent (Sehgal *et al*., 1975). However, it proved to be a potent inhibitor of the mTOR ("target of rapamycin") signalling pathway in mammalian cells, which groups it with a wide range of potential clinical applications (Alayev and Holz, 2013). This compound is used as immunosuppressant in renal transplants. *S. rapamycinicus* also produces azalomucin C, a macrodiolide antibiotic used as anthelmintic in animal feed (Baranasic *et al*., 2013). This compound is used as an immunosuppressant in renal transplants. It is also used for the prevention of restenosis after stent insertion for the treatment of coronary heart disease (Slavin *et al*., 2007). A second gene cluster, similar to the rapamycin gene cluster, has also been reported in the strain (Ruan *et al*.,

1997). It has a large genome of 12.7 Mb, of which over 3 Mb consists of 48 secondary metabolite biosynthesis clusters (Baranasic *et al*., 2013).

2.2.7 *Streptomyces albulus* NK660

This strain was isolated from Gutian, Fujian Province, China. It produces ε-Poly-L-lysine (ε- PL). ε-PL exhibits antimicrobial activity against a wide spectrum of microorganisms, including Gram-positive and Gram-negative bacteria; it also exhibits anti-phage activity. In addition, because of its safety and biodegradability, ε-PL has been used as a food preservative. The genome of *S. albulus* NK660, with a guanine-cytosine (GC) content of 72.32%, comprises a 9 360 281-bp linear chromosome and a 12 120-bp linear plasmid (Gu *et al*., 2014).

2.2.8 *Streptomyces lividans*

S. lividans is a Gram-positive, filamentous soil bacterium. With its high GC content, this species is known for its genomic plasticity (Volff *et al*., 1997), which entails large deletions and extensive amplifications (Dyson and Schrempf, 1987, Eichenseer and Altenbuchner, 1994, Hornemann *et al*., 1993). *S. lividans* is used as hosts for the synthesis and secretion of homologous and heterologous proteins of industrial interest (Anné *et al*., 2014, Jayapal *et al*., 2007, Sianidis *et al*., 2006, Valverde *et al*., 2018, Vierling *et al*., 2000, Nevalainen and Peterson, 2014).

2.2.9 *Streptomyces glaucescens*

S. glaucescens is known for its production of tetracenomycin C, tetracenomycin D and tetracenomycin E antibiotics (Decker *et al*., 1993, Thompson *et al*., 2004). See figure 2.5 below for the structure of tetracenomycin.

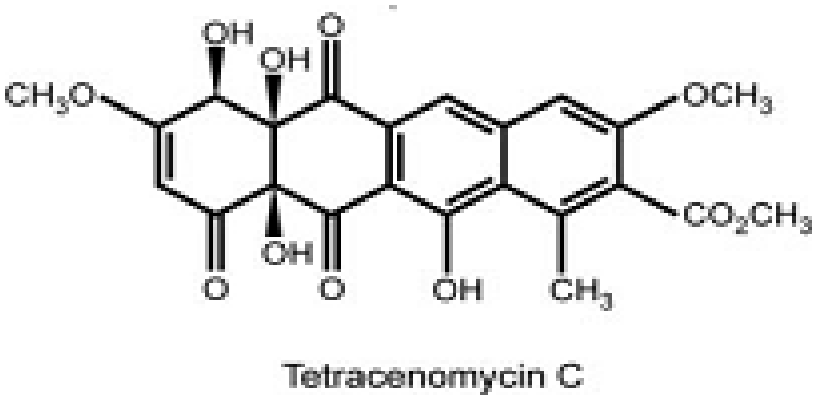


Figure 2. 5. Chemical structure of tetracenomycin (taken from Decker *et al*., 1993).

2.2.10 *Streptomyces cyaneogriseus*

This is a thermotolerant bacterium isolated in soil, producing a commercially important compound, which has been widely used as a bio-pesticide (Wang *et al*., 2015a, Li *et al*., 2019). It produces chitosanase enzyme, which allows it to degrade chitosan as a sole carbon source (El-Sherbiny, 2011). The organism is known to produce the antibiotic nemadectin (Wang *et al*., 2010).

2.2.11 *Streptomyces lydicus*

This bacterium species has been isolated from soil in the United States. *S. lydicus* produces actithiazic acid, natamycin, lydimycin, streptolydigin and1deoxygalactonojirimycin (Atta *et al*., 2015, Gómez *et al*., 2012). A study demonstrated that *S. lydicus* can be used as an anti-fungal plant pathogen (Yuan and Crawford, 1995). It has been shown that *S. lydicus* WYEC108 is a strong *in vitro* antagonist against a variety of fungal plant root rot and white-rot pathogens and various wood decay fungi.

2.2.12 *Streptomyces xiamenensis*

This bacterium has been isolated from mangrove sediments in Xiamen in the Fujian Province in China (Xu *et al*., 2009). It produces the antifibrotic benzopyran compound xiamenmycin (Figure 2.6), used for treating fibrotic diseases such as idiopathic pulmonary fibrosis, liver cirrhosis, systemic sclerosis, progressive kidney disease and cardiovascular fibrosis (Liu *et al*., 2013a, Min-Juan *et al*., 2016, Yang *et al*., 2014, You *et al*., 2013). The assembled genome comprises a linear chromosome as a single strand of 5 961 401bp (Lei *et al*., 2015). Moreover, gene clusters for capsimycin and capsimycin B and their derivatives (Figure 2.7) have been identified in this strain (Yu *et al*., 2006).

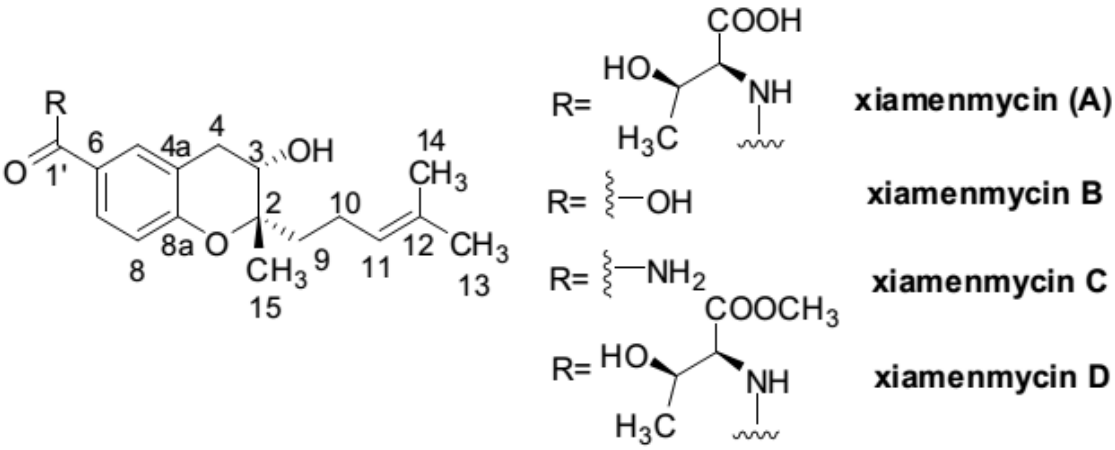


Figure 2. 6. Chemical structure of xiamenmycins A–D (taken from Lei *et al*., 2015).

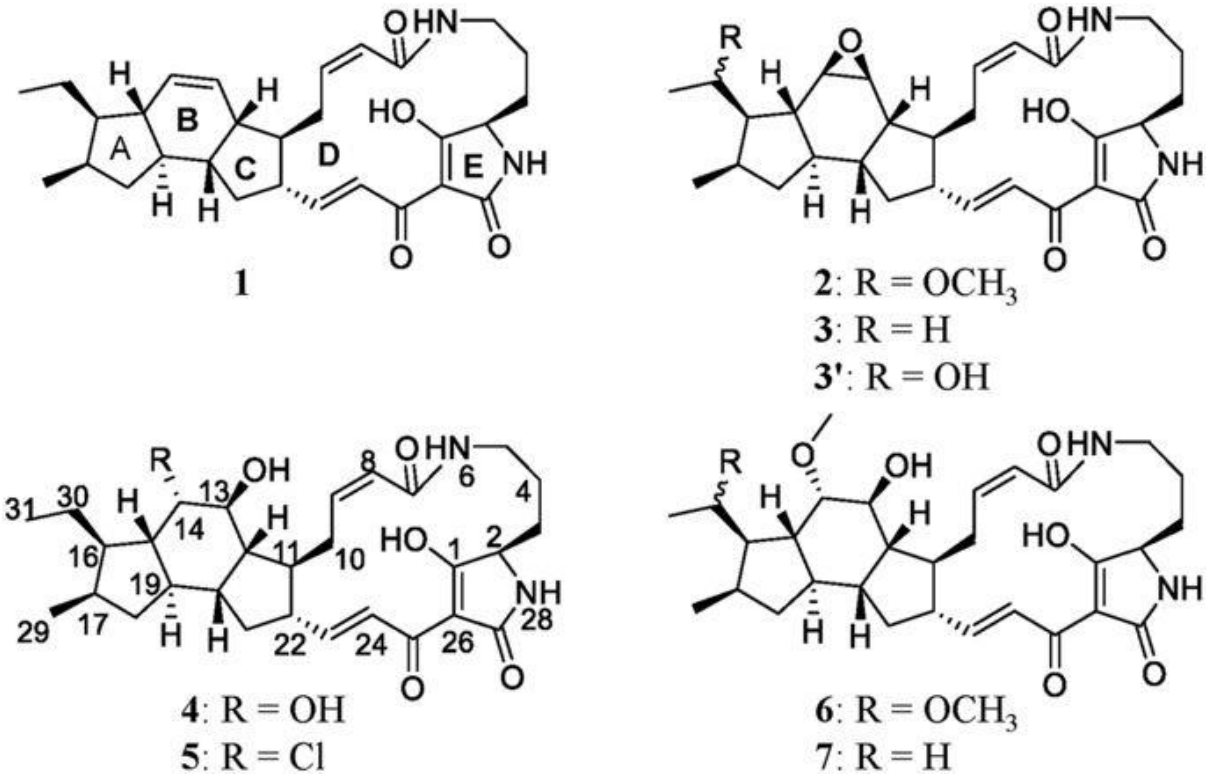


Figure 2. 7. Chemical structure of ikarugamycin (**1**), capsimycin (**2**), capsimycin B (**3**), capsimycin C (**4**), capsimycin D (**5**), capsimycin E (**6**), capsimycin F (**7**), and capsimycin G (**8**) (taken from Yu *et al*., 2017).

2.2.13 *Streptomyces ambofaciens*

S. ambofaciens ATCC 23877 is known to produce two antibiotics: spiramycin, a polyketide derivative (Karray *et al*., 2007), and congocidin (or netropsin) (Juguet *et al*., 2009). Sequencing revealed the presence of a further 23 gene clusters with the potential for production of other secondary metabolites such as kinamycins, stambomycins (Figure 2.8) and antimycins (Aigle *et al*., 2014). Another antibiotic, ambobactin (Figure 2.9), was isolated from the metabolites of *Streptomyces ambofaciens* F3 (Wei *et al*., 2015).

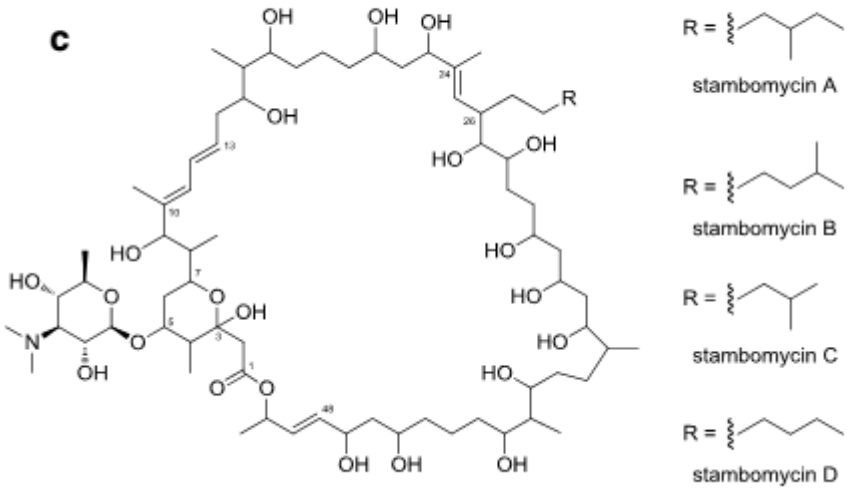


Figure 2. 8. Chemical structure of stambomycins (taken from Aigle *et al.*, 2013).

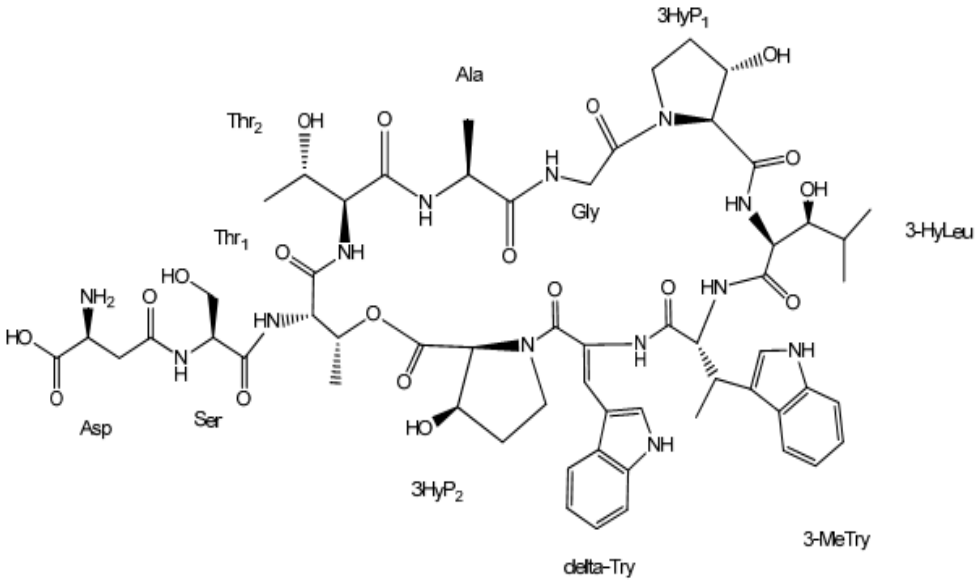


Figure 2. 9. Chemical structure of ambobactin antibiotic (taken from Wei *et al.*, 2015).

2.2.14 *Streptomyces pristinaespiralis*

S. pristinaespiralis produces two chemically diverse antibiotics: the cyclohexadepsipeptide pristinamycin I (PI) and the polyunsaturated macrolactone pristinamycin II (PII) (Figure 2.10). PI and PII are coproduced in a 3:7 ratio (Mast and Wohlleben, 2014). In combination, the pristinamycins exhibit strong synergistic antibacterial activity against a wide range of Gram-positive bacteria, including methicillin-resistant staphylococci, drug-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus faecium* (Qadri *et al*., 1997) and some Gram-negative bacteria such as *Haemophilus* spp. (Mast *et al*., 2011).

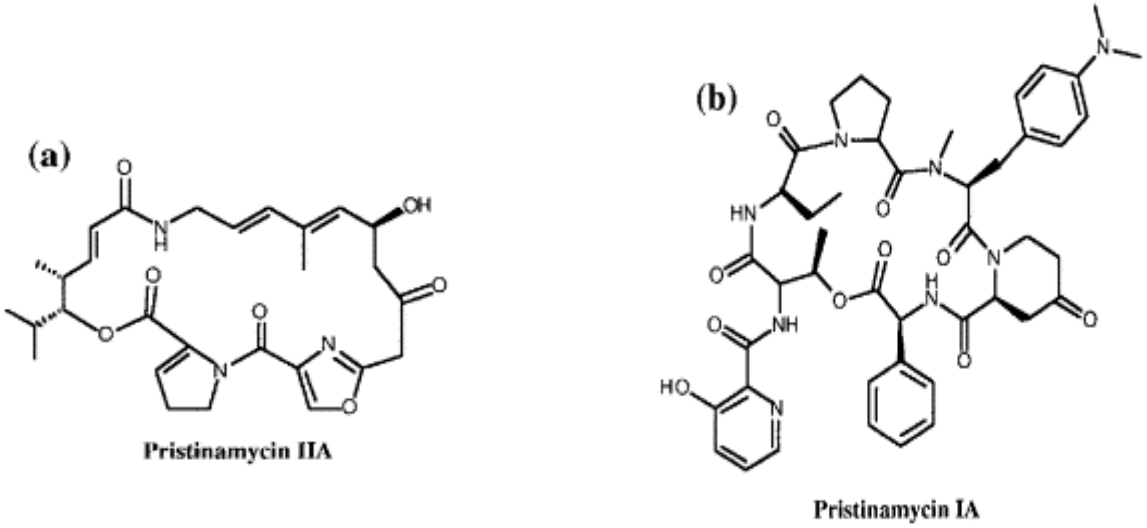


Figure 2. 10. Chemical structure of pristinamycin I (a) and pristinamycin II (b) (taken from Mast *et al.*, 2011).

2.2.15 *Streptomyces globisporus*

S. globisporus produces the antitumour angucyclines landomycin A (LaA) and landomycin E (LaE) (Rebets *et al*., 2003) (Figure 2.11). *S. globisporus* encodes genes for production of the enediyne antitumour antibiotic C-1027 (Liu and Shen, 2000).

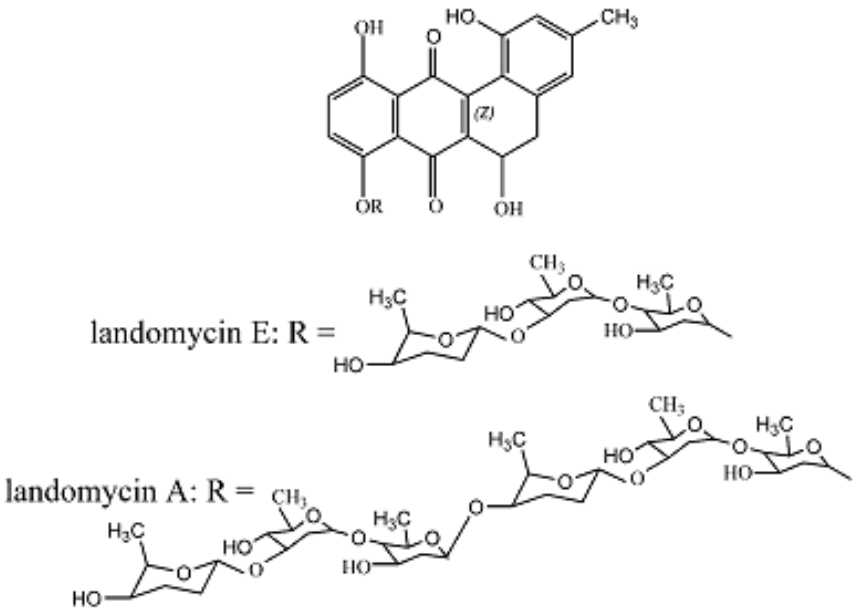


Figure 2. 11. Chemical structure of landomycins (taken from Rebets *et al.*, 2003).

2.2.16 *Streptomyces scabiei*

This bacterium is found in soil around the world. It is a plant pathogen responsible for the formation of lesions on tuber and root crops, as well as decreasing the growth of their seedlings (Song *et al*., 2004). Along with other closely related species, it causes the potato disease common scab (Braun *et al*., 2017, Khatri *et al*., 2010). *S. scabies* can produce several related toxins that are responsible for its pathogenicity (Lerat *et al*., 2009).

2.2.17 *Streptomyces* sp. SirexAA-E

Streptomyces sp. SirexAA-E is an aerobic microbe that has the capacity to hydrolyse complex carbohydrates such as cellulose and hemicellulose (Book *et al*., 2014, Takasuka *et al*., 2013). This aerobic microbe is a prominent member of a bacterial/fungal symbiotic community and is often associated with the invasive woodwasp *Sirex noctilio* (Takasuka *et al*., 2013). *Streptomyces* sp. SirexAA-E is responsible for the secretion of enzymes such as endocellulase,

exocellulases, hemicellulases, pectinases, and polysaccharide monooxygenases (Takasuka *et al*., 2013) and β-mannanase (Takasuka *et al*., 2014)

2.2.18 *Streptomyces violaceusniger*

S. violaceusniger strain SPC6, isolated from the Linze Desert in China, is a halotolerant microorganism. This halotolerant strain can survive in conditions of high salinity (up to 1M sodium chloride). In addition, the strain is remarkably resistant to osmotic, heat and ultraviolet stress compared with other *Streptomyces* species. *S. violaceusniger strain* SPC6 has a very high growth rate and short life cycle compared with other *Streptomyces* species, including the model organism, *S. coelicolor*. It can produce antibiotics that inhibit the growth of other bacteria. The draft genome of *S. violaceusniger strain* SPC6 consists of one chromosome of 6 457 341 bp with 73.37% GC content (Chen *et al*., 2013).

Hayakawa and colleagues (Hayakawa *et al*., 2004) conducted a study and discovered spores of the *S. violaceusniger* cluster strains that were resistant to phenol; about 10–80% of the spore populations survived. In the same study, 100% of the *S. violaceusniger* isolates demonstrated broad antimicrobial spectra, as they inhibited the growth of all the tested Gram-positive bacteria (*Bacillus. subtilis, Micrococcus luteus, Streptomyces murinus* and *Staph. aureus*), yeasts *(Candida albicans and Saccharomyces cerevisiae*) and filamentous fungi (*Aspergillus niger*, *Aspergillus oryzae* and *Penicillium chrysogenum*), while 43% *S. violaceusniger* isolates inhibited Gram-negative bacteria. *S. violaceusniger*-cluster isolates were also found to have an excellent ability against carcinoma cells.

One strain, *S. violaceusniger* YCED-9, has been shown to produce at least three different antifungal antibiotics, including nigericin, geldanamycin, and a polyene-like compound similar to guanidylfungin A (Trejo-Estrada *et al*., 1998). Another strain, *S. violaceusniger* WYE53, also expresses strong antibiotic activities against turfgrass fungal

pathogens, including *Pythium ultimum, Fusarium oxysporium, Rhizoctonia solani, Sclerotina homeocarpa, Gaeumannomyces graminis* and *Microdochium nivale*. These fungi cause diseases in golf course turfgrass; the *S. violaceusniger* strains WYE53 and YCED-9 were found to be effective in controlling these diseases (Chamberlain and Crawford, 2000).

2.2.19 *Streptomyces cattleya* NRRL 8057 = DSM 46488

S. cattleya is a producer of the antibiotics thienamycin (Figure 2.12) (Núñez *et al*., 2003) and cephamycin C (Bushell and Fryday, 1983, Khaoua *et al*., 1991). It is one of the rare bacteria known for the production of fluorinated metabolites. Its genome consists of two linear replicons (Barbe *et al*., 2011). The genes involved in fluorine metabolism and in the biosynthesis of antibiotics such as thienamycin and penicillin were mapped (Barbe *et al*., 2011, Huang *et al*., 2006). Through sequencing, the genome of *S. cattleya* NRRL 8057 was found to comprise one linear chromosome SCAT, which is 6 283 062 bp long and has 72.94% GC content and one linear megaplasmid pSCAT, which is 1 809 491 bp long and has 73.21% GC content. Six rRNA operons, 64 tRNA genes and 5 779 protein-coding genes were also found on the chromosome (Barbe *et al*., 2011).

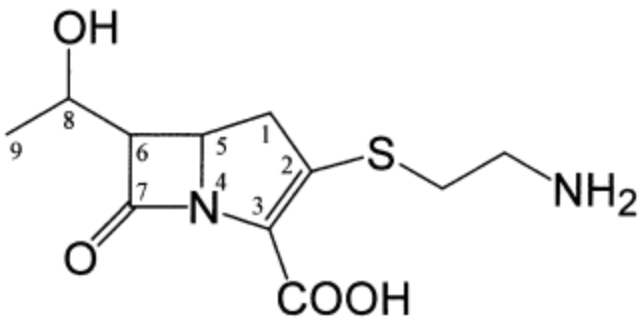


Figure 2. 12. Chemical structure of thienamycin (taken from Núñez *et al*., 2003).

2.2.20 *Streptomyces hygroscopicus* subsp. *jinggangensis* 5008

S. hygroscopicus subsp. *jinggangensis* 5008 (*S. hygroscopicus* 5008 hereafter), isolated from the Jinggang Mountain area of China in 1974 (Xia and Jiao, 1986) produces at least two antibiotics of agricultural importance, jingangmycin (Figure 2.13) and jingsimycin. Jingangmycin is a weakly basic water-soluble aminocyclitol antibiotic, which was later proven to be identical to validamycin. Jingangmycin has been widely used in agriculture for the control of sheath blight disease of rice plants and damping-off of cucumber seedlings. Meanwhile, its transformed product, valienamine, is a pharmaceutically important precursor for the synthesis of voglibose, a highly effective drug for insulin-independent diabetes (Matsumoto *et al*., 1998). The other antibiotic, jingsimycin, is an acidic polypeptide similar to saramycetin and is active against various fungi. The 10 383 684-bp genome of *S. hygroscopicus* 5008 was completely sequenced. It is composed of a linear chromosome, a 164.57-kb linear plasmid, and a 73.28-kb circular plasmid (Wu *et al*., 2012). *S. hygroscopicus* 5008 was also found to produce antibiotic valindamycin, which is similar to jingangmycin (Tan *et al*., 2013). See Figure 2.13 for the structure of jingangmycin.

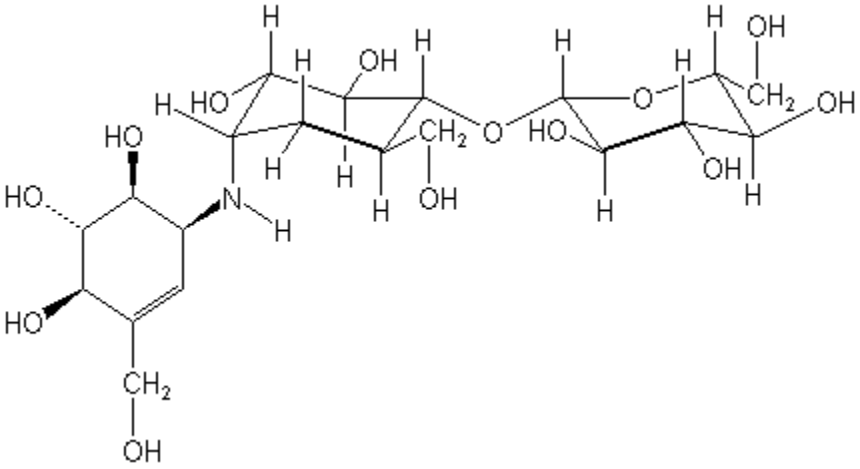


Figure 2. 13. Chemical structure of jingangmycin (taken from http://www.rayfull.com/Productshows.asp?ID=15#.WKBk8lV97IU).

2.2.21 *Streptomyces hygroscopicus* subsp. *jinggangensis* TL01

This species produces antimycin A. Antimycin A is widely used as a pesticide in the catfish farming industry and also has potent killing activity against insects, nematodes and fungi (Seipke and Hutchings, 2013). See figure 2.4 below for the chemical structure of antimycins.

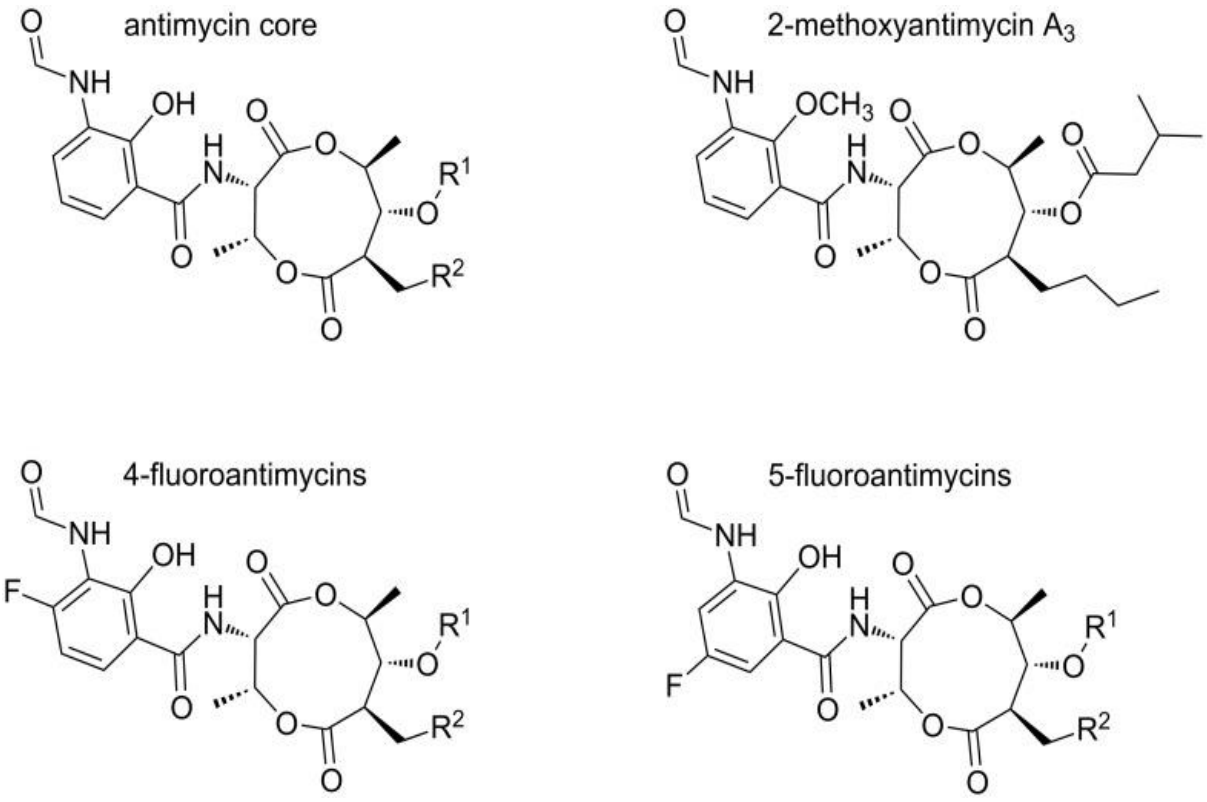


Figure 2. 14. Chemical structure of antimycins (taken from Seipke and Hutchinhs, 2013).

2.2.22 *Streptomyces venezuelae*

The organism was isolated from a soil sample in Venezuela. Chloramphenicol is produced by several Gram-positive soil actinomycetes, but its biosynthesis has been analysed mostly in the *S. venezuelae* strain (Vining and Stuttard, 1995). Chloramphenicol, initially called chloromyeetin, was first isolated in *S. venezuelae* (Ehrlich *et al*., 1947). Another type of antibiotic, jadomycin, is also produced by *S. venezuelae* ATCC 10712 (Ehrlich *et al*., 1947).

See figure 2.15 below for the chemical structure of chloramphenicol and jadomycin (Sekurova *et al*., 2016).

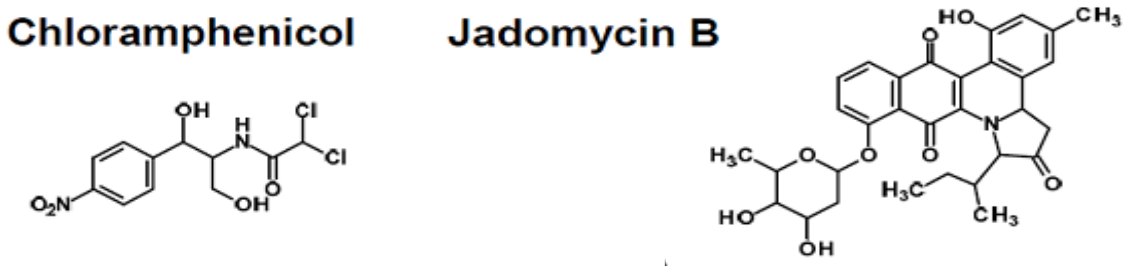


Figure 2. 15. Chemical sructure of antibiotics produced by *S. venezuelae* (taken from Sekurova *et al.*, 2016).

2.2.23 *Streptomyces davawensis*

This non-pathogenic Gram-positive soil bacterium produces the riboflavin analogs roseoflavin and 8-demethyl-8-amino-riboflavin (Figure 2.16) (Otani *et al*., 1974). Both compounds show antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis*, but also against Gram-negative bacteria if uptake systems for flavins/flavin analogs are present (Grill *et al*., 2007).

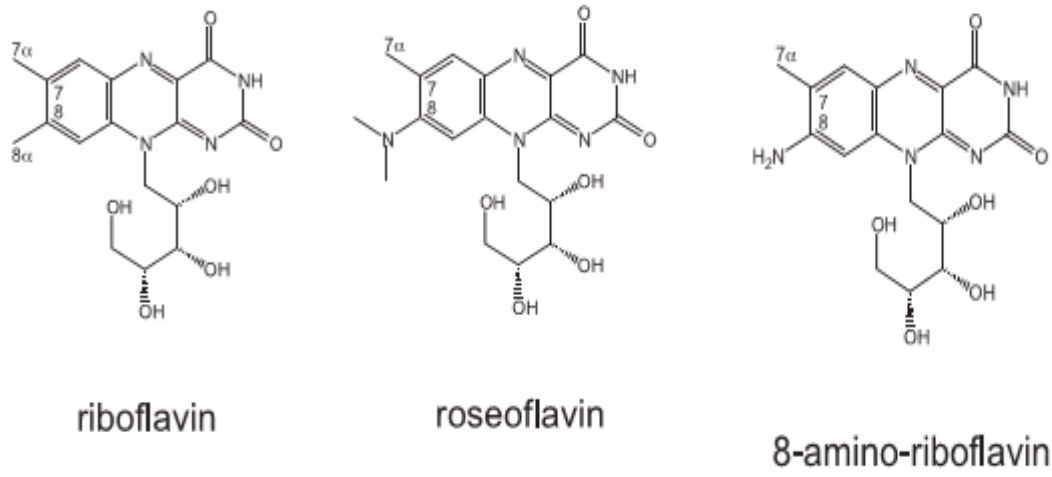


Figure 2. 16. Chemical structure of rivoflavin analogs (taken from Otani *et al.*, 1974).

2.2.24 *Streptomyces albus* DSM 41398

S. albus DSM 41398 produces the antibiotic salinomycin (Jiang *et al*., 2007, Yurkovich *et al*., 2012). Salinomycin has selective activity against cancer (Kim *et al*., 2014, Mai *et al*., 2017), as well as therapy-resistant cancer cells (Dewangan *et al*., 2017). However, it has been widely used as veterinary medicine in animal husbandry as food additive and growth promoter for years (Gumila *et al*., 1997). See figure 2.17 below for the structure of salinomycin.

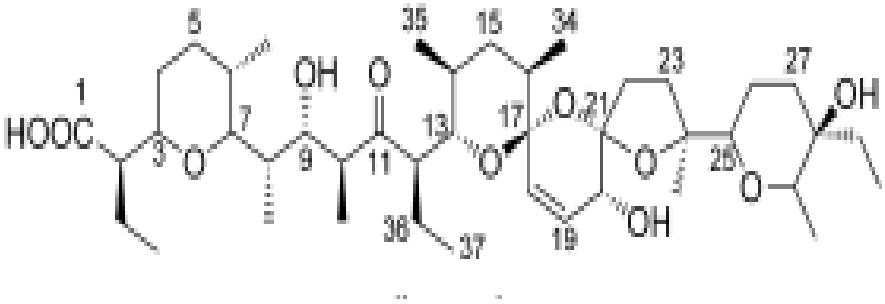


Figure 2. 17. Chemical structure of salinomycin (taken from Jiang *et al*., 2015).

2.2.25 *Streptomyces sp.* PAMC 26508

Streptomyces sp. PAMC 26508 was isolated from the Antarctic lichen *Cladonia borealis*. This endosymbiotic bacterium has a 7 526 197 bp linear chromosome with 70.89% GC content, and one plasmid with 104 048 bp. It has demonstrated antibacterial activities in different studies (Shin *et al*., 2013). In one such study, the strain showed antibacterial activity against all target bacteria (Kim *et al*., 2014).

2.2.26 *Streptomyces fulvissimus*

The *S. fulvissimus* genome consists of a single linear chromosome of 7 905 758 bp (71.5% GC content) with no plasmids. Analysis of the genome sequence indicated the presence of 32 putative gene clusters involved in the biosynthesis of different natural products (Myronovskyi *et al*., 2013). *S. fulvissimus* was found to secrete an antibacterial protein inhibitory to *Micrococcus luteus, Bacillus. subtillis, Bucillus. cereus,* and methicillin resistant *Staphylococcus aureas* strains (Malik *et al*., 2008).

2.2.27 *Streptomyces collinus*

S. collinus was isolated in 1972 from Kouroussa (Guinea). It is best known as producer of the antibiotic kirromycin, an inhibitor of the protein biosynthesis interacting with elongation factor EF-Tu (Rückert *et al*., 2013). Additional products produced by this bacterium were discovered; genome mining revealed 32 gene clusters encoding the biosynthesis of diverse secondary metabolites in the genome of *S. collinus* Tü 365 (Iftime *et al*., 2016). Figure 2.18 below indicates secondary metabolites produced by this strain.

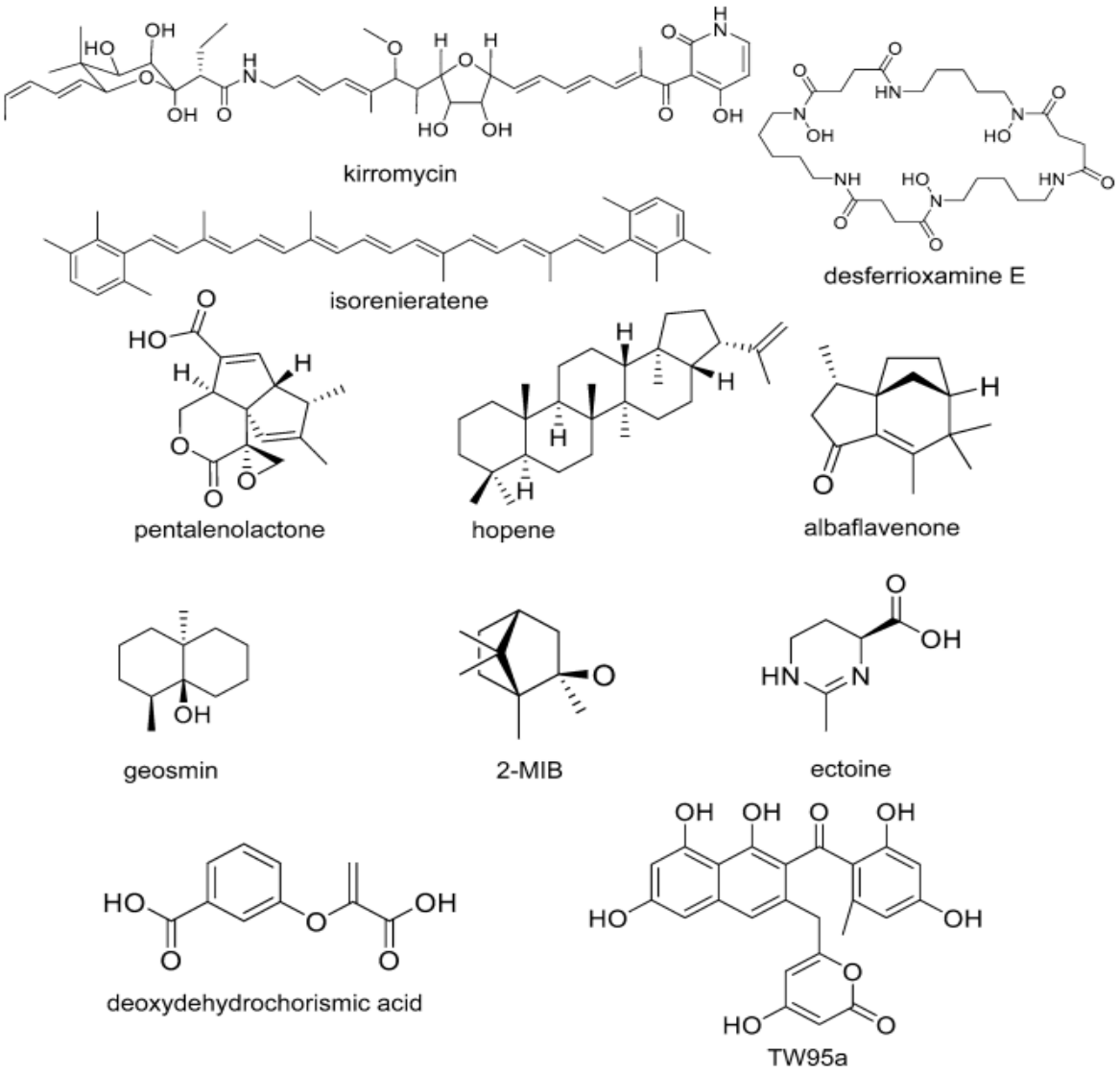


Figure 2. 18. Structures of identified and predicted secondary metabolites encoded in *S. collinus* Tü 365 genome (taken from Iftime *et al.*, 2015).

2.2.28 *Streptomyces albulus* ZPM

S. albulus ZPM was isolated from the soil of Zi-Peng Mountain, west of Hefei, China using the methylene blue screening method. It produces the homopolymer antibiotic, ε-poly-lysine (ε-PL) (Wibowo *et al.*, 2013). The complete genome sequence of *S. albulus* ZPM revealed a single linear chromosome composed of 9 784 577 bp, with the pZPM234 plasmid and an average G+C content of 72.2%. Analysis revealed 44 gene clusters for secondary metabolites

in *S. albulus* ZPM (Wang *et al*., 2015b). See figure 2.19 below for the chemical structure of the antibiotic ε-PL.

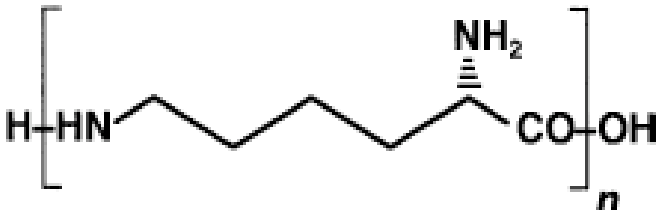


Figure 2. 19. Chemical structure of ε-poly-lysine (taken from Yoshida and Nagasawa, 2003).

2.2.29 *Streptomyces vietnamensis*

S. vietnamensis was isolated from a forest soil sample in Vietnam. It is an aerobic that is Gram-positive and catalase-positive and forms a white aerial mycelium and a reddish-brown substrate mycelium. In a study conducted, no antibacterial activity was evident against *Escherichia coli, Pseudomonas aeruginosa, Bacillus. subtilis, Candida. albicansor* or *Penicillium citrinum* (Zhu *et al*., 2007). However, its production for industrial violet-blue pigment has been explored (Deng *et al*., 2011).

2.2.30 *Streptomyces* sp. Mg1

Streptomyces sp. Mg1 produces the macrolide antibiotic chalcomycin A, which has been found to inhibit growth of *B. subtilis* cells (Barger *et al*., 2012). It causes lysis and degradation of *B. subtilis* and its colonies. Genome sequencing revealed that *Streptomyces* sp. Mg1 has 8.7 Mb genome (Hoefler *et al*., 2013). See Figure 2.20 for the structure of chalcomycin A.

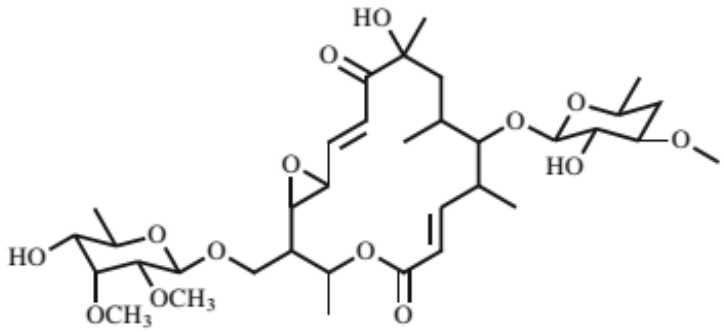


Figure 2. 20. Chemical structure of chalcomycin A (taken from Barger *et al.*, 2012).

2.2.31 *Streptomyces* sp. CNQ-509

This is a marine-derived strain, isolated from a near-shore marine sediment off La Jolla, Califonia in 2001 (Prieto-Davó *et al*., 2008). Compounds such as naphterpin (Gallagher *et al*., 2010), debromomarinone (Pathirana *et al*., 1992), nitropyrrolins (Kwon *et al*., 2010), naphthoquinone (Park and Kwon, 2018) and marinophenazines (Espindola, 2008) have been isolated from this strain. Its complete genome sequence has been described (Rückert *et al*., 2015). See Figure 2.21 for the metabolite structures produced by the strain (Leipoldt *et al*., 2015).

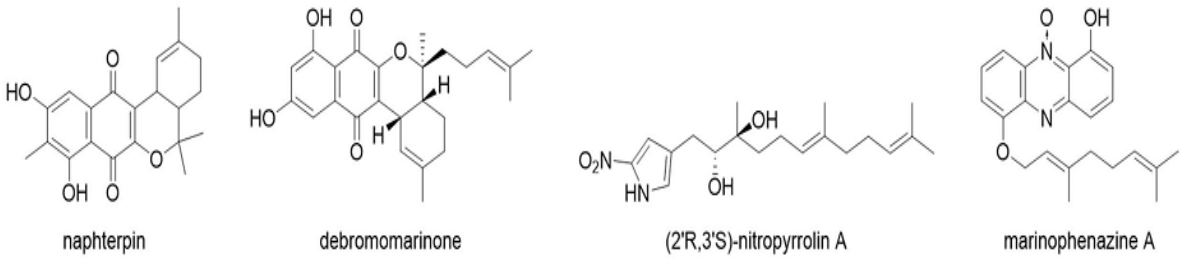


Figure 2. 21. Secondary metabolites produced by *Streptomyces* sp. CNQ 509 (taken from Leipoldt *et al.*, 2015).

This species contains 29 putative gene clusters for the biosynthesis of secondary metabolites, some of them potentially involved in the formation of meroterpenoid molecules.

Streptomyces sp. CNQ-509 is a remarkable producer of various terpenoid compounds (Leipoldt *et al*., 2015).

2.2.32 *Streptomyces* sp. CFMR 7

Streptomyces sp. strain CFMR 7, which naturally degrades rubber (Chia *et al*., 2014), was isolated from a rubber plantation in Penang, Malaysia. Whole genome sequencing and assembly resulted in two contigs with a total genome size of 8.248 Mb (Nanthini *et al*., 2015). It has also been found to degrade latex gloves, latex condoms and latex car tyres (Nanthini and Sudesh, 2017).

2.2.33 *Streptomyces* sp. CdTB01

Streptomyces sp. strain CdTB01 was isolated from soil contaminated with heavy metals. The strain demonstrates tolerance to high concentrations of heavy metals, particularly cadmium. Two contigs with a total genome size of 10.19 Mb were identified in the whole genome sequencing and assembly (single circular chromosome of 9 902 731 bp and linear plasmid of 288 836 bp). Numerous homologous genes known to be involved in heavy metal resistance were also found in the genome (Zhou *et al*., 2016a).

2.2.34 *Streptomyces reticuli*

The bacterium *S. reticuli* is known for its production of an unusual mycelia-associated cellulase (avicelase) (Schlochtermeier *et al*., 1992), which can degrade crystalline cellulose to cellobiose (Schrempf and Walter, 1995). It consists of a linear 8.3 Mb chromosome, a linear 0.8 Mb megaplasmid, a linear 94 kb plasmid and a circular 76 kb plasmid (Wibberg *et al*., 2016).

2.2.35 *Streptomyces* sp. 4F

Streptomyces sp. 4F was isolated from the soil in China. It is one of the fast-growing and moderately thermophilic (growing at both 30°C and 50°C) *Streptomyces* strains (Chen and Qin, 2011). This characteristic has led to its use in antibiotic expressions (Chen and Qin, 2011) and DNA assembly (Chen *et al*., 2013).

2.3 Introduction to cytochrome P450 monooxygenases

Cytochromes P450 monooxygenases (CYPs/P450s) are found throughout all the biological kingdoms of life. They belong to the superfamily of proteins containing a heme cofactor, making them hemoproteins. P450s are also referred to as mixed function oxidases; they can catalyse a variety of enzymatic reactions and transform endogenous chemicals into more polar and/or detoxified derivatives (Bernhardt, 2006, Isin and Guengerich, 2007, Sono *et al*., 1996). They derive their name from a distinctive characteristic of being bound to membranes within a cell (cyto) and containing a heme pigment (chrome and P) that absorbs light at a wavelength of 450 nm when exposed to carbon monoxide (Figure 2.22) (Guengerich *et al*., 2009). The absorption was discovered in 1958 as an unusual spectrum taken from rat liver microsomes with a peak of absorbance at 450 nm (Klingenberg, 1958). In 1962, it was proved that CYP was a terminal oxidase by showing the ability to catalyse the C21 hydroxylation of 17-hydroxyprogesterone in adrenal cortical microsome (Estabrook *et al*., 1963).

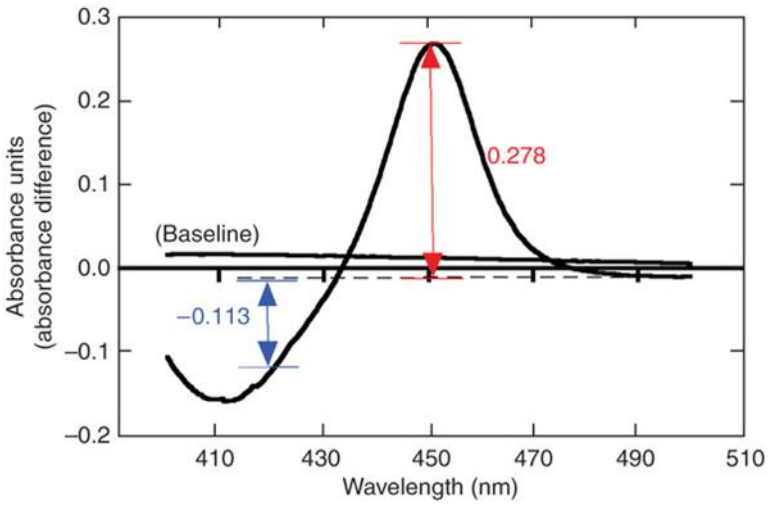


Figure 2. 22. Typical cytochrome P450 monooxygenase ferrous-CO versus ferrous-difference spectrum (taken from Guengerich *et al*., 2009).

Monooxygenase reaction is the most common example of a reaction carried out by a cytochrome P450 enzyme; in the reaction, one atom of oxygen is inserted into the aliphatic position of an organic substrate [RH] and the other atom is reduced to water, as in the reaction below:

$$NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + ROH$$

In these reactions, RH represents an oxidisable drug substrate and ROH is the hydroxylated metabolite.

2.4 Applications of P450s

One of the surprising discoveries about the genomics of the P450 superfamily is the large number of P450s in the bacterial phyla *Actinobacteria* (Parvez *et al*., 2016). It has been suggested that this large number is promising in terms of their properties and potential

applications in the industry (Notonier *et al*., 2016). Most of those identified so far are generally involved in either biotransformation reactions, e.g. *S. griseolus* is involved in the metabolism of sulfonylurea herbicides to less toxic derivatives through its P450 CYP105A1 (O'Keefe *et al*., 1988), or are part of specialised metabolite biosynthetic pathways, e.g. CYP170B1 catalyses production of the albaflavenone in *S. albus* (Moody *et al*., 2012).

In humans they are a major class of biocatalyst involved in the oxidative metabolism of exogenous as well as endogenous compounds, including drugs, xenobiotics, fatty acids, bile acids and steroids. Numerous P450 applications have also been thoroughly reviewed elsewhere (Greule *et al*., 2018, Guengerich, 2002, Urlacher and Eiben, 2006, Zhang *et al*., 2011). Figure 2.23 (Kelly and Kelly, 2013) illustrates areas of application for microbial P450.

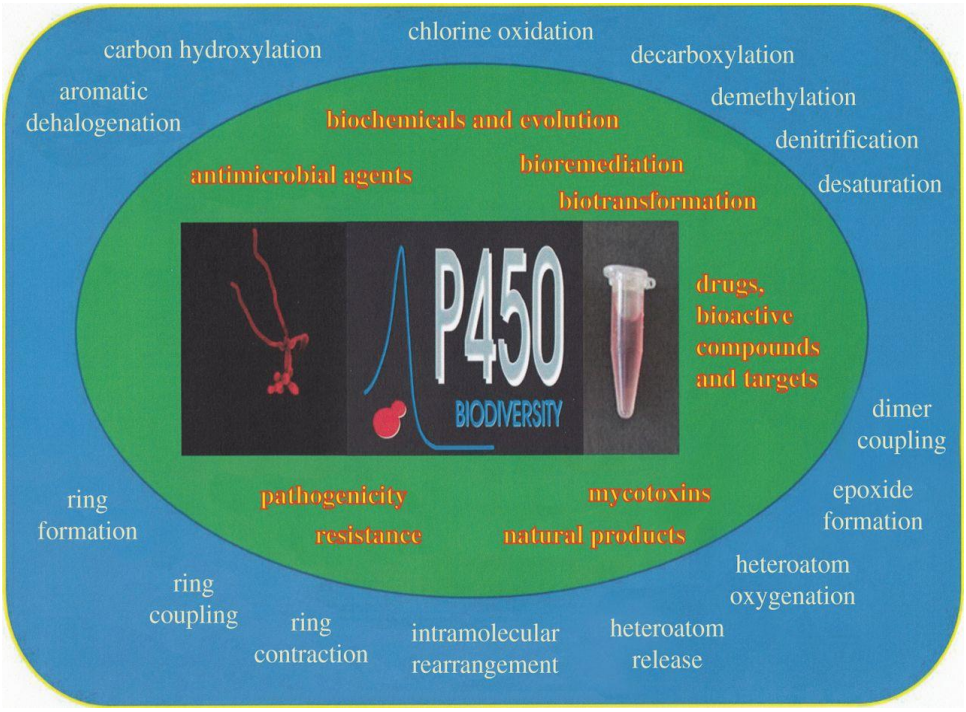


Figure 2. 23. Areas of application for microbial CYPs and reactions associated with them (taken from Kelly and Kelly, 2013).

Many of the bacterial P450s catalyse reactions of environmental or medical significance. Their capability to metabolise thousands of endogenous and exogenous chemicals has led to discovered characteristics that account for their central importance in medicine. Their enzyme family is capable of catalysing the oxidative biotransformation of most drugs and other lipophilic xenobiotics (Guengerich, 2007, Foti *et al*., 2011) and they have been implicated in the biotransformation of most foreign substances, including 70% to 80% of all drugs in clinical use (Zanger and Schwab, 2013). For instance, in the drug discovery and development category, one well-established commercial application of P450 monooxygenases is the biotransformation of steroids to drugs, such as 11β- hydroxylation of reichstein S to hydrocortisone (van Beilen *et al*., 2003). Thus P450s are regarded as the major enzymes involved in drug metabolism, accounting for about 75% of the total metabolism (Guengerich, 2007). Even though there are more than 50 CYP450 enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 enzymes are known to metabolize 90% of drugs (Slaughter and Edwards, 1995, Wilkinson, 2005). P450s can also deactivate drugs directly or such drugs can be deactivated through facilitated excretion from the body. Furthermore, many substances are bio-activated by P450s to form their active compounds (van Beilen *et al*., 2003). Drugs are not the only compounds metabolised by P450s; products of endogenous metabolism, such as bilirubin, are metabolised in the liver by P450s as well (Abu-Bakar *et al*., 2012). P450s are also involved in the conversion of progesterone to cortisone (Peterson *et al*., 1957) and hydroxylation of steroids (Niwa *et al*., 2015, Zehentgruber *et al*., 2010).

Being mostly present in tissues of the body, P450s also play important roles in synthesis and breakdown of hormones, such as steroid hormone synthesis and metabolism (Davies *et al*., 1999, Parker and Schimmer, 1995), cholesterol synthesis (Payne and Hales, 2004), vitamin D metabolism and the beneficial conversion of codeine into morphine (Stefano *et al*., 2012). They

are also involved in the nitration of tryptophan to form thaxtomin A by CYP1048A1 from *S. scabiei* and other *Streptomyces* species (Girvan and Munro, 2016). Another classical example is aspirin, a commonly used non-steroidal anti-inflammatory drug. Aspirin is metabolised by CYP2C8 and CYP2C9 (Preissner *et al*., 2012). Yet at the same time human P450s are the basis for sometimes fatal drug interactions (Guengerich, 2006, Lynch and Price, 2007).

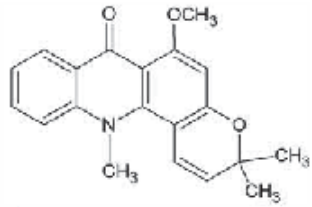
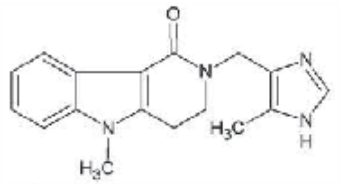
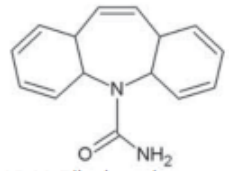
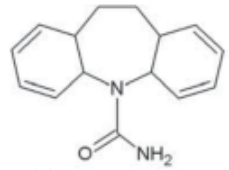
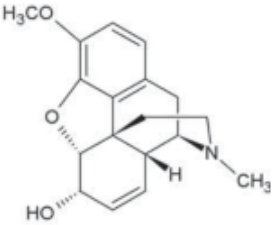
Detection of drugs (clozapine), xenobiotic compounds (styrene) and fatty acids (cholesterol) was made possible by the development of biosensors based on mammalian P450 (CYP1A2, CYP2B4 and CYP11A) models (Paternolli *et al*., 2004). Moreover, biofuel production (Zhang *et al*., 2011, Koch *et al*., 2009) from alkanes or fatty acids has been explored using engineered bacterial P450s, CYP153A6 and OleTJE (a P450 from the CYP152 family) (Matthews *et al*., 2017, Rude *et al*., 2011) to make then valuable industrial compounds. To expand their substrate range for further potential applications, bio-engineering of model bacterial P450s CYP101 and CYP102 has been attempted in several studies (Carmichael and Wong, 2001, Jones *et al*., 2001, Sulistyaningdyah *et al*., 2004), and strategies for P450 recombinant expression are being investigated (Hausjell *et al*., 2018).

While other P450s can render pesticides inactive, some of them are targets for anti-fungals. Still others make key antibiotics such as vancomycin (Cryle *et al*., 2010), antimalarials such as artemisinin (Ro *et al*., 2006) and anti-cancer drugs such as taxol (Jennewein *et al*., 2001). Production of macrolide antibiotic erythromycin and vancomycin by the use of P450eryF (CYP107A1) from *Saccharopolyspora erythraea* (Andersen *et al*., 1993) and P450 OxyA, OxyB and OxyC from *Amycolatopsis orientalis* (Bischoff *et al*., 2005) has been reported. CYP725A1 from yew (*Taxus cuspidate*) (Jennewein *et al*., 2005) and CYP153 from *Mycobacterium sp*. (van Beilen *et al*., 2005) have been used in biosynthesis of the anticancer drugs taxol and perillyl alcohol. Work by Tian *et al*. (2015) demonstrated that P450 107U1 is

required for sporulation and antibiotic production in *S. coelicolor*. Since then research on functional and structural analysis of P450s (Guengerich *et al*., 2016, Sirim *et al*., 2010) and their significance in the drug industry (Manikandan and Nagini, 2018) has continued.

Many laboratories that do not have access to human P450s use *Streptomyces* species to test drugs. *Streptomyces* species are used in drug-testing studies because of functional similarities of human P450 and *Streptomyces* P450s and therefore these methods are frequently used to mimic human drug metabolism studies (Lamb *et al*., 2013). Table 2.2 shows the similar enzymatic reaction catalysed by human and *Streptomyces* species P450s.

Table 2. 2. Selected examples of drugs, the individual human P450 enzymes involved in their metabolism, the specific *Streptomyces* strain used to generate metabolites and the associated P450 reactions observed (taken from Lamb *et al*., 2013).

Drug (pharmacological activity)	Human P450 metabolism (enzyme and oxidative reaction(s) involved) [10]	*Streptomyces* metabolism (strain and observed oxidative reaction(s)) (refs.)
Acronycine (antitumor) 	CYP3A4 3-Methyl, C-9 and C-10 hydroxylation; 6-*O*-demethylation	*Streptomyces spectabilis* 3-Methyl and C-11 hydroxylation [44]
Alosetron (5-HT_3 receptor antagonist) 	CYP2C9 CYP3A4 CYP1A2 3-Methyl, C-9 and C-11 hydroxylation; 6-*O*-demethylation [50]	*Streptomyces griseus* *N*-demethylation; C-4 hydroxylation; *N*-dealkylation; C-2 hydroxylation of imidazole ring [51]
Carbamazepine (antiepileptic) 	CYP3A4 CYP2C8 CYP1A2 CYP3A7 10,11-Epoxidation [42]	*Streptomyces violascens* 10,11-Epoxidation [43]
10,11-Dihydrocarbamazepine (antiepileptic) 	CYP3A4 CYP2C8 CYP1A2 CYP3A7 C-10 hydroxylation followed by oxidation to C-10 keto derivative [42]	*S. violascens* *S. griseus* C-10 hydroxylation [43]
Codeine (analgesic) 	CYP2D6 CYP3A4 CYP3A5 CYP3A7 *N*- and *O*-demethylation [45,46]	*S. griseus* *N*-demethylation; C-14 hydroxylation [48]

Deriving information about an organism through genome mining has appeared to be a promising tool (Du and van Wezel, 2018, Ziemert *et al*., 2016). Predictions of gene functions became possible as genome analysis was done on sequenced strains (Hadjithomas *et al*., 2015).

Mining work has since been done on some species, such as *Streptomyces chattanoogensis* L10 (CGMCC 2644) (Zhou *et al*., 2016b), *Streptomyces collinus* Tü 365 (Iftime *et al*., 2016) and *Streptomyces* sp. YIM 130001 (Schneider *et al*., 2018). Complete gene clusters for *Streptomyces* sp. have so far been identified in the Atlas of Biosynthetic Gene Clusters (Hadjithomas *et al*., 2015). *Streptomyces* species thus show great promise as catalytic tools, even though only a few *Streptomyces* P450s have been functionally and structurally characterised to date (Rudolf *et al*., 2017). P450 are also known for their evolutionary characteristic behaviour. The adaptational changes to P450 and blooms in their linages due to evolution have already been observed in other organisms (Córdova *et al*., 2017, Feyereisen, 2011, Syed and Mashele, 2014) as well as in vertebrates and invertebrates (Kawashima and Satta, 2014). Understanding of their evolutionary mechanisms will assist in revealing important aspects to exploit and consider their metabolites better for future industrial applications (Degtyarenko and Archakov, 1993).

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CHAPTER 3

METHODOLOGY

3.1 *Streptomyces* species and genome databases

Forty-eight *Streptomyces* species of genomes that are available for public use at Integrated Microbial Genomes and Microbiomes from the Joint Genome Institute (https://img.jgi.doe.gov/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa *et al*., 2015) were used in this study. Detailed information on species used in this study, along with their genome database links and genome IDs, are listed in Table 3.1.

3.2 Genome data-mining and identification of P450s

Streptomyces species genomes that are publicly available as listed in Table 3.1 were mined for P450s following the method described elsewhere (Mthethwa *et al*., 2018, Ngwenya *et al*., 2018, Parvez *et al*., 2016). Briefly, for each bacterium, whole proteomes were downloaded and grouped into different protein families using the National Centre for Biotechnology and Information (NCBI) Conserved Domain Database: NCBI Batch Web CD-search tool (Marchler-Bauer *et al*., 2016). The hit proteins grouped under the cytochrome P450 monooxygenases superfamily were selected for further study. The bacterial genome available at Integrated Microbial Genomes and Microbiomes from the Joint Genome Institute was mined for P450s using InterPro code “IPR001128”. The hit protein sequences were downloaded and subjected to the NCBI Batch Web CD-Search Tool (Marchler-Bauer *et al*., 2016). Proteins that grouped under the P450 superfamily were selected for further analysis. The selected proteins were searched for the presence of P450 characteristic motifs such as EXXR and CXG. Proteins having one of the motifs were considered as fragment/pseudo-P450s. For each organism, P450s

identified at KEGG and Integrated Microbial Genomes and Microbiomes were compared. A final total count is presented by deleting the same P450s found in both genome databases.

Table 3. 1. List of *Streptomyces* species used in the study. Species names, codes, the respective genome database links and genome IDs from NCBI were listed in the table.

Species name	Species code	Database link	Genome ID
Streptomyces coelicolor	Sco	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=637000305	NC_003888
Streptomyces avermitilis MA-4680	Sma	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=637000304	BA000030
*Streptomyces griseus*_NBRC_13350	Sgr	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=641522653	AP009493
Streptomyces globisporus	Sgb	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=GeneDetail&page=genePageMainFaa&gene_oid=2668573307	CP013738
Streptomyces scabiei 87.22	Scb	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=646564576	FN554889
Streptomyces sp. Sirex AA-E	Ssx	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2523533511	CP002993
Streptomyces violaceusniger Tu 4113	Svl	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=648276750	CP002994
Streptomyces cattleya NRRL 8057	Sct	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2511231113	FQ859185

Streptomyces cattleya NRRL 8058 = DSM 46488	Scy	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2511231200	CP003219
Streptomyces pratensis/flavogriseus IAF 45	Sfa	http://www.genome.jp/kegg-bin/show_organism?org=sfa	CP002475
Streptomyces bingchenggensis	Sbh	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=646862346	CP002047
Streptomyces hygroscopicus subsp. *jinggangensis* 5008	Shy	http://www.genome.jp/kegg-bin/show_organism?org=shy	CP003275
Streptomyces hygroscopicus subsp. *jinggangensis* TL01	Sho	http://www.genome.jp/kegg-bin/show_organism?org=sho	CP003720
Streptomyces venezuelae	Sve	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2718218231	FR845719
Streptomyces davawensis	Sdv	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2561511188	HE971709
Streptomyces albus J1074	Salb	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2541047081	CP004370
Streptomyces albus DSM 41398	Sals	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2639762818	CP010519
Streptomyces sp. PAMC 26508	Strp	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2561511190	CP003990
Streptomyces fulvissimus	Sfi	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2554235391	CP005080

Streptomyces collinus	Sci	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2554235367	CP006259
Streptomyces rapamycinicus	Src	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2563366593	CP006567
Streptomyces albulus NK660	Salu	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2579778836	CP007574
Streptomyces albus ZPM	Sall	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2639762896	CP006871
Streptomyces lividans	Slv	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2597490034	CP009124
Streptomyces glaucescens	Sgu	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2627854123	CP009438
Streptomyces vietnamensis	Svt	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2627853829	CP010407
Streptomyces sp. 769	Stre	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2627853736	CP003987
Streptomyces cyaneogriseus	Scw	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2648501341	CP010849
Streptomyces lydicus A02	Sld	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2651869762	CP007699

Streptomyces xiamenensis 318	Sxi	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2630968921	CP009922
Streptomyces sp. Mg1	Strm	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2579778547	CP011664
Streptomyces sp. CNQ-509	Strc	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2654587513	CP011492
Streptomyces ambofaciens	samb	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2654588008	CP012382
Streptomyces pristinaespiralis HCCB 10218	Spri	http://www.genome.jp/kegg-bin/show_organism?org=spri	CP011340
Streptomyces sp. CFMR 7	Scz	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2654587638	CP011522
Streptomyces sp. CdTB01	Scx	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2687453384	CP013743
Streptomyces reticuli	Srw	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2687453570	LN997842
Streptomyces sp. 4F	Strf	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2687453197	CP013142
Streptomyces leeuwenhoekii C34(2013)	Sle	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2582581028	LN831790
Streptomyces rubrolavendulae	Srn	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2765235980	CP017316

Streptomyces parvulus	Spav	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2718218336	CP015866
Streptomyces lydicus 103	Slc	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2718217986	CP017157
Streptomyces sp. SAT1	Strt	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2718218381	CP015849
Streptomyces clavuligerus	Sclf	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=647533233	CP016559
Streptomyces griseochromogenes	Sgs	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2684622604	CP016279
Streptomyces sp. S10 (2016)	Stsi	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2687453302	CP015098
Streptomyces lincolnensis	Sls	http://www.genome.jp/kegg-bin/show_organism?org=sls	CP016438
Streptomyces noursei	Snr	https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=TaxonDetail&page=iprGeneList&ext_accession=IPR001128&taxon_oid=2602042089	CP011533

3.3 Assigning P450 family and P450 subfamily to orphan P450s

The above selected P450s were subjected to Basic Local Alignment Search Tool (BLAST) analysis against all named bacterial sequences on the Cytochrome P450 Homepage (Nelson, 2009) to identify the closest named homolog P450. Based on the percentage identity to the named homolog P450, i.e. >40% amino acid identity and >55% amino acid identity, P450s were grouped under the same family and same subfamily (Nelson, 2006). P450s that had less than 40% and 55% amino acid identity to the named homolog P450s were assigned to new P450 families and new P450 subfamilies. Some *Streptomyces* species P450s were annotated and made available at the Cytochrome P450 Homepage (Nelson, 2006). In this case, the same nomenclature for P450s was continued. The *Streptomyces* P450 protein sequences along with their names were available as Dataset 1 at https://www.nature.com/articles/s41598-019-40646-y#Sec19. For comparative analysis the P450 data for mycobacterial species were retrieved from published literature (Parvez *et al*., 2016).

3.4 Construction of the P450s phylogenetic tree

The phylogenetic tree of P450s was constructed as described previously (Chen *et al*., 2014). First, the P450 protein sequences were aligned using the HMMER package 3.1b2 (http://hmmer.org/) by adjusting them to the P450 profile hidden Markov model PF00067 from the Pfam database (http://pfam.xfam.org/) (Finn *et al*., 2015). Then, the phylogenetic tree from P450s alignments was generated by FastTree 2.1.10 using the maximum-likelihood method (http://www.microbesonline.org/fasttree/) (Price *et al*., 2010). Finally, the phylogenetic tree was displayed by iTOL (http://itol.embl.de/upload.cgi) (Letunic and Bork, 2016).

3.5 Analysis of P450 diversity percentage

The percentage contribution of the number of P450 families in the total number of P450s in an organism is considered the P450 diversity percentage (Parvez *et al*., 2016, Sello *et al*., 2015). This formula was employed previously to measure the P450 diversity percentage among species in a genus (Parvez *et al*., 2016, Sello *et al*., 2015). However, in order to compare two genera of a phylum where the number of species will be different, a new formula (shown below) has been formulated to obtain an average P450 diversity percentage per species.

$$\text{P450 diversity percentage} = \frac{100 \text{ x Total number of P450 families}}{\text{Total number of P450s x number of species}}$$

The above formula will nullify the number of species used and will give an accurate P450 diversity percentage comparison between the genera within a phylum; previously this was not employed (Parvez *et al*., 2016, Sello *et al*., 2015). A point to be noted is that this formula is useful only when the number of species shared between the genera is relatively similar. For comparative analysis the P450 diversity percentage data for mycobacterial species were retrieved from published literature (Parvez *et al*., 2016).

3.6 Generation of P450 profile heatmaps

The presence or absence of P450s in *Streptomyces* species was shown with heatmaps generated using P450 family data following the method described elsewhere (Mthethwa *et al*., 2018). Briefly, the data were represented as −3 for family presence (green) and 3 for family absence (red). A tab-delimited file was imported into Multi-experiment Viewer (Mev) (Saeed *et al*., 2003). Hierarchical clustering using a Euclidean distance metric was used to cluster the data.

Forty-eight *Streptomyces* species formed the horizontal axis and CYP family numbers formed the vertical axis.

3.7 Functional analysis of P450s

Considering the large number of P450s identified in this study and the availability of functional data for some P450s (Rudolf *et al*., 2017), a literature survey on the functional analysis of *Streptomyces* species P450s was carried out and used in this study. The functional role of P450s in *Streptomyces* physiology is presented at P450 family level and subfamily level. Furthermore, functional analysis of some *Streptomyces* species P450 was predicted based on the characterised homologous P450s from other organisms.

3.8 References

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Identification of *Streptomyces* P450s

Genome-wide data mining and annotation of P450s in 48 *Streptomyces* species revealed the presence of 1 625 P450s in their genomes (Fig. 4.1 and Table 4.1). Among these 1 625 P450s, all had characteristic glutamic acid and cysteine at the EXXR and CXG motifs, respectively; however, 34 P450s lacked one or both P450 characteristic motifs, EXXR and CXG, owing to short amino acid sequences and were thus regarded as fragment/pseudo-P450s (Table 4.2). The presence of short P450s/pseudo-P450s is common in organisms. Two false positive P450 fragments were identified in *S. ambofaciens* ATCC 23877 and another one in *Streptomyces* sp. CdTB01. These P450 fragments were not included in the final count. The P450 count in the *Streptomyces* species ranged from 16-69 P450s, with an average of 34 P450s (Fig. 4.2A and Table 4.1). Among the *Streptomyces* species selected for the study, *Streptomyces* sp. CNQ-509 and *Streptomyces* sp. 4F have the lowest number of P450s (16 P450s) and *Streptomyces albulus* ZPM the highest number of P450s (69 P450s) in their genomes (Fig. 4.2A). The percentage coverage of P450s in the *Streptomyces* species ranged from 0.2% to 1.1% in (Table 4.1). Comparison of P450s revealed that species belonging to the genera *Streptomyces* and *Mycobacterium* had almost the same patterns in terms of the average number of P450s in their genomes, the highest average percentage contribution of P450s in the genome (≥1%) and highest number of P450s for a species (*S. albulus* ZPM has 69 P450s and *M. rhodesiae* NBB3 has 70 P450s).

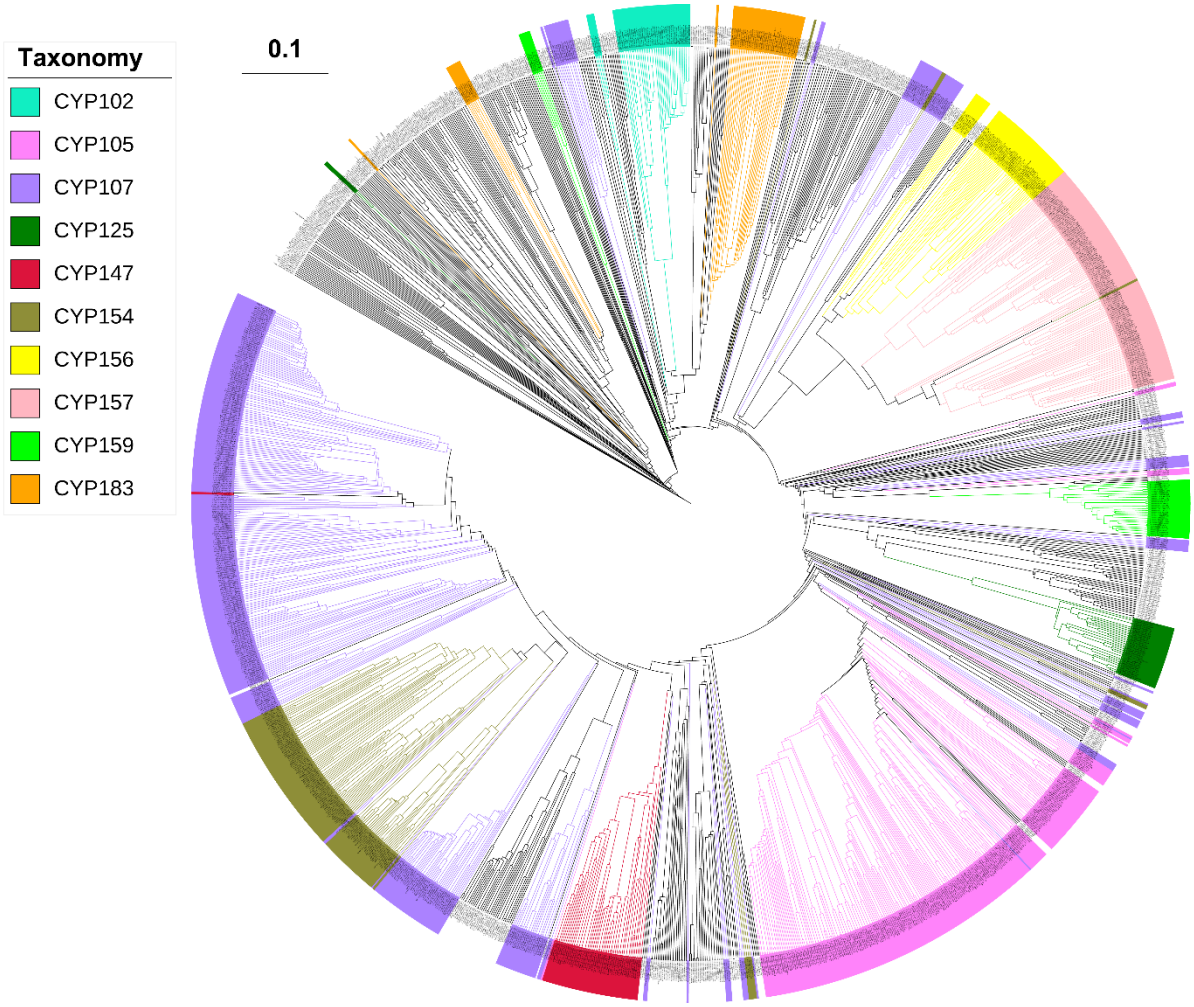

Figure 4. 1. Phylogenetic analysis of *Streptomyces* P450s. P450 families that are dominant in *Streptomyces* species are highlighted in different colours.

Table 4. 1. P450 family and subfamily level comparative analysis of P450s in 48 *Streptomyces* species. Open reading frames (ORFs) for each species were obtained from the genome database listed in Table 3.1. The percentage of P450s was calculated considering ORFs as 100%.

Species name	Species code	No. of P450s	No. of P450 families	No. of P450 subfamilies	ORFs	% of P450s
Streptomyces albulus ZPM	sall	69	27	52	8191	0.8
Streptomyces albulus NK660	salu	64	27	50	8086	0.8
Streptomyces noursei	Snr	64	26	52	8691	0.7
Streptomyces violaceusniger	Svl	51	16	42	8985	0.6
Streptomyces bingchenggensis	Sbh	50	26	44	10022	0.5
Streptomyces rapamycinicus	Src	63	23	56	10002	0.6
Streptomyces cattleya NRRL 8057 = DSM 46488	Scy	42	21	39	7569	0.6
Streptomyces sp. 769	Stre	59	24	49	9553	0.6
Streptomyces hygroscopicus subsp. *jinggangensis* 5008	Shy	38	18	33	9108	0.4
Streptomyces cattleya NRRL 8057 = DSM 46488	Sct	41	20	38	7475	0.5
Streptomyces hygroscopicus subsp. *jinggangensis* TL01	Sho	37	18	33	8878	0.4
Streptomyces avermitilis	sma	53	24	45	7676	0.7
Streptomyces collinus	Sci	34	16	27	7113	0.5
Streptomyces lydicus	Sld	40	19	35	8888	0.5
Streptomyces sp. Mg1	Strm	37	21	36	7117	0.5
Streptomyces leeuwenhoekii	Sle	36	17	34	7005	0.5
Streptomyces pratensis	Sfa	29	16	26	6572	0.4
Streptomyces reticuli	Srw	47	26	43	8090	0.6
Streptomyces griseus	Sgr	28	13	24	7136	0.4
Streptomyces sp. PAMC 26508	Strp	29	16	26	7073	0.4
Streptomyces sp. SirexAA-E	Ssx	24	10	22	6357	0.4
Streptomyces davawensis	Sdv	32	19	30	8616	0.4

Streptomyces cyaneogriseus	Scw	33	16	30	5830	0.6
Streptomyces lincolnensis	Sls	24	15	23	8590	0.3
Streptomyces pristinaespiralis	spri	23	12	18	7352	0.3
Streptomyces venezuelae	Sve	23	16	21	7453	0.3
Streptomyces sp. CFMR 7	Scz	24	13	20	6716	0.4
Streptomyces vietnamensis	Svt	30	20	29	7356	0.4
Streptomyces xiamenensis	Sxi	20	12	19	5484	0.4
Streptomyces coelicolor	Sco	18	10	17	8152	0.2
Streptomyces albus J1074	Salb	18	9	18	5832	0.3
Streptomyces ambofaciens	samb[#]	19	10	18	7793	0.2
Streptomyces lividans	Slv	20	10	18	7360	0.3
Streptomyces scabiei	Scb	30	16	30	8746	0.3
Streptomyces glaucescens	Sgu	18	11	17	6567	0.3
Streptomyces albus DSM 41398	Sals	25	13	24	7330	0.3
Streptomyces fulvissimus	Sfi	19	10	16	6925	0.3
Streptomyces sp. CNQ-509	Strc	16	11	16	6407	0.2
Streptomyces rubrolavendulae	Srn	20	12	19	5425	0.4
Streptomyces clavuligerus	sclf	65	30	58	5981	1.1
Streptomyces griseochromogenes	sgs	46	24	40	9064	0.5
Streptomyces sp. S10(2016)	stsi	20	15	20	7661	0.3
Streptomyces globisporus	sgb	23	13	19	6654	0.3
Streptomyces sp. CdTB01[#]	scx	26	17	25	8743	0.3
Streptomyces parvulus	spav	25	15	25	6714	0.4
Streptomyces lydicus	slc	32	13	29	6872	0.5
Streptomyces sp. SAT1	strt	25	15	22	6110	0.4
Streptomyces sp. 4F	strf	16	11	15	6792	0.2

#, Two and one false positives P450 fragments were identified in *Streptomyces ambofaciens* ATCC 23877 and *Streptomyces* sp. CdTB01, respectively. These P450 fragments were not considered in the final count.

Table 4. 2. *Streptomyces* species pseudo/fragment P450s. P450s were represented with their name following the protein ID (in parenthesis) and species code.

Fragment/pseudo P450s (34 P450s)	**Comment**
CYP1453B-fragment(2712578143)SMA	NO EXXR
CYP2340B2P(2712584497)SMA	Truncated P450
CYP178A3P(2712585028)SMA	No EXXR
CYP105B23(2712586946)SMA	No EXXR
CYP107AM-fragment1(646978401)SBH	No EXXR
CYP107AS-fragment1(2511677887)SCT	No EXXR and CXG
CYP107AS(2511977159)SCY	No EXXR and CXG
CYP125A25-fragment(647544071)SCLF	No EXXR and CXG
CYP161F1P(647544976)SCLF	Short P450 with EXXR and CXG
CYP183U1P-N-term(647548353 SCLF	No EXXR & N-terminal missing
CYP183U1P-C-term(647548354)SCLF	C-terminal missing
CYP155A-fragment2(2649527539)SCW	No EXXR
CYP155A-fragment1(2649527540)SCW	No EXXR
CYP152D-fragment1(2649527873)SCW	No CXG
CYP1047B3P(2685041405)SGS	No CXG
CYP125A32(SHJG_3828)shy	No EXXR and CXG
CYP125A32(SHJGH_3593)sho	No EXXR and CXG
CYP125A31(2562375444)SHO	No EXXR and CXG
CYP107L52P(2652740080)SLD	No EXXR and CXG
CYP107CJ2(2652740805)SLD	No EXXR and CXG
CYP183B(2603775408)SNR	No EXXR and CXG
CYP147B6(2603782400)SNR	No EXXR
CYP154D20Pb(2689319916)SRW	No CXG
CYP154D20Pa(2689319917)SRW	No EXXR and CXG
CYP105AC45P(2628747249)STRE	No EXXR and CXG
CYP105AC43P(2628747250)STRE	No EXXR and CXG
CYP183B4P(2628747539)STRE	No EXXR
CYP154D21P(2628749871)STRE	No EXXR
CYP113D4(2628752396)STRE	No EXXR
CYP105B-fragment(648750691)SVL	No EXXR
CYP107Z26Pa(648758019)SVL	No EXXR
CYP107Z26Pb(648758020)SVL	No EXXR and CXG
CYP1530A3Pb(2688559483)SCX	No EXXR
CYP105Bfragment(2633776877)SXI	No EXXR

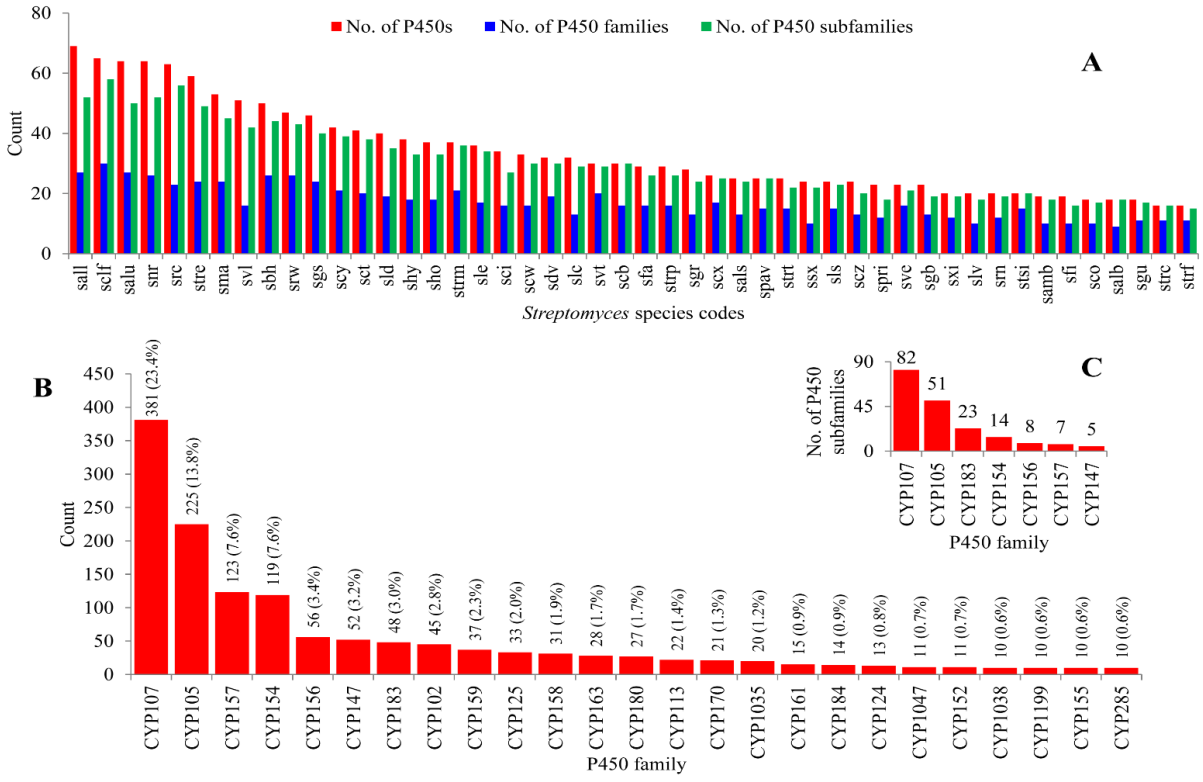

Figure 4. 2. Comparative analysis of P450s in 48 *Streptomyces* species. (A) Comparative analysis of the number of P450s, P450 families and P450 subfamilies in 48 *Streptomyces* species. Each *Streptomyces* species is presented with its code (for details see Table 4.3). (B) P450 family-level comparative analysis in *Streptomyces* species. P450 families that are dominant in *Streptomyces* species are presented in the figure. The numbers next to the family bar indicate the total number of P450s and percentage contribution (parenthesis) by a particular family to the total number of P450s (for details see Table 4.5). (C) P450 subfamily-level comparative analysis among seven dominant P450 families. The numbers next to bars indicate the number of P450 subfamilies in that family.

4.2 *Streptomyces* species have more diverse P450s than mycobacterial species

As per International P450 Nomenclature Committee Rules (Nelson, 2006), all 1 625 P450s identified in 48 *Streptomyces* species were grouped into 144 P450 families and 377 P450 subfamilies (Fig. 4.2 A and Table 4.3). Among the families and subfamilies, 66 new P450 families and 144 new P450 subfamilies were identified in *Streptomyces* species (Table 4.4). Most of the new P450 subfamilies were identified in the P450 families CYP107 (62 new subfamilies) and CYP105 (38 new subfamilies) (Table 4.4). A detailed list of newly identified P450 families and P450 subfamilies in *Streptomyces* species is presented in Table 4.4. An interesting feature observed during phylogenetic analysis of *Streptomyces* P450s is that some P450 family members are not grouped together (Fig. 4.1), despite being annotated according to the rules set by the International P450 Nomenclature Committee (Nelson, 2006), suggesting that sometimes the phylogenetic-based annotation of P450s could be detecting similarity cues beyond a simple percentage identity cut-off. It is especially difficult to assign subfamily membership in large families such as CYP105 and CYP107.

Analysis of P450 family conservation across 48 *Streptomyces* species revealed that among 144 P450 families identified, only two P450 families, the CYP107 and CYP157 P450 families, are conserved across all *Streptomyces* species (Fig. 4.3 and Table 4.3). Certain P450 families, such as CYP105 and CYP156, tend to co-occur in some but not all *Streptomyces* species (top of the heatmap).

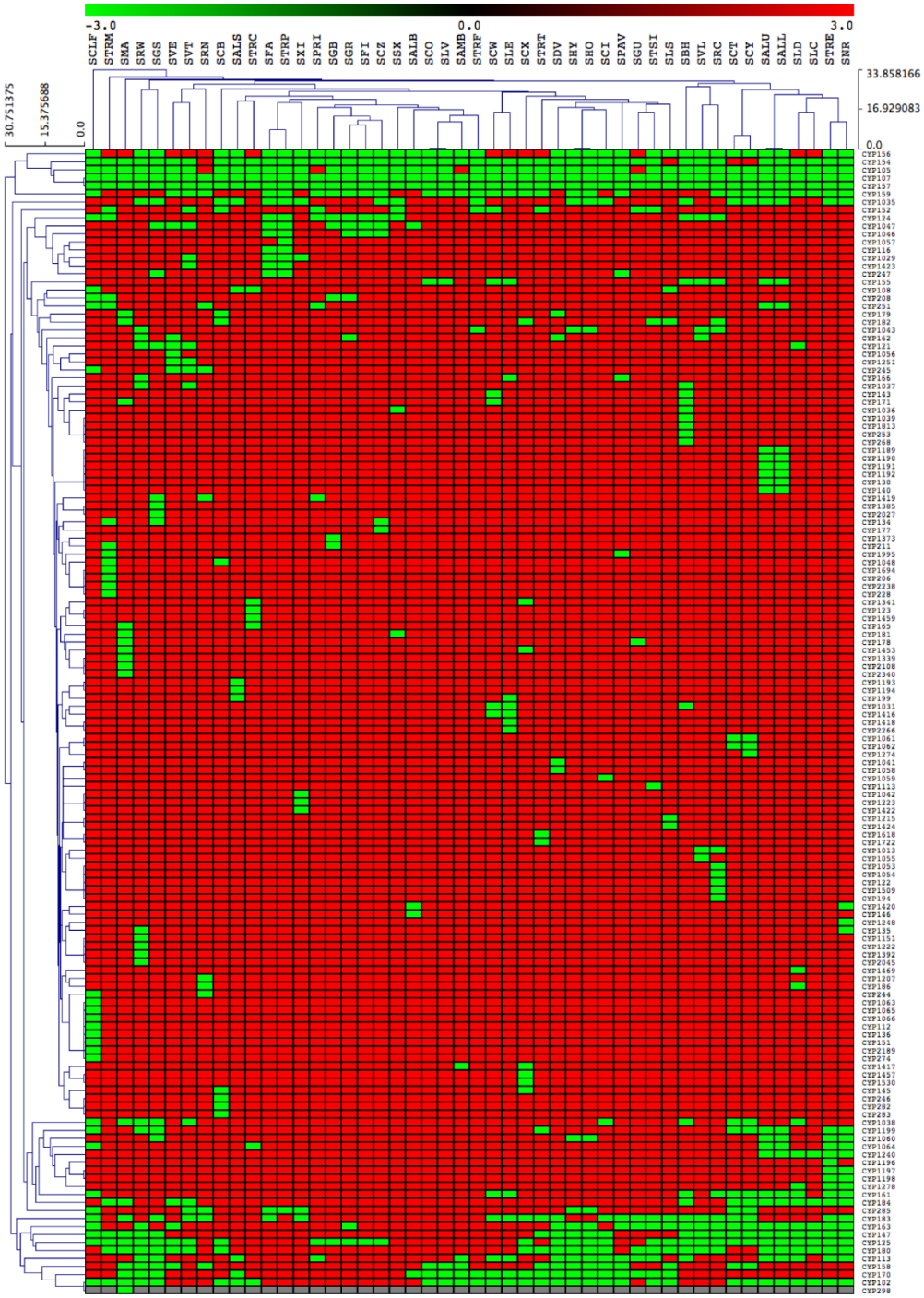

Figure 4. 3. Heatmap of the presence or absence of P450 families in 48 *Streptomyces* species. Forty-eight *Streptomyces* species form the horizontal axis (see Table 3.1 for species codes) and P450 families (right side) and the phylogenetic relationship among P450 families (left side) form the vertical axis. The data have been represented as -3 for family presence (green) and 3

for family absence (red). The data used in the generation of the figure are available as Dataset 2 at https://www.nature.com/articles/s41598-019-40646-y#Sec19.

Table 4. 3. Comparative analysis of P450 families in 48 *Streptomyces* species.

	Streptomyces species codes																																															
P450 family	SCO	SMA	SGR	SGB	SCB	SSX	SVL	SCT	SCY	SFA	SBH	SHY	SHO	SVE	SDV	SALB	SALS	STRP	SFI	SCI	SRC	SALU	SALL	SLV	SGU	SVT	STRE	SCW	SLD	SXI	STRM	STRC	SAMB	SPRI	SCZ	SCX	SRW	STRF	SLE	SRN	SPAV	SLC	STRT	SCLF	SGS	STSI	SLS	SNR
1005								1	1						1						1				1	1								1								1						
1013							1														1																											
1029										1								1								1				1																		
102	1	2			2			1	1			2	2		2		1			1		1	1	1			1	2	1		1	1	1			1	2	1	2		1	2	1	1	4	2	2	1
1031											1																	1											1									
1035					1	1		1	1	1	1	1	1				1	1		1		1	1				1			1					1		1	1							1			1
1036						1					1																																					
1037											1															1											1											
10		1						1	1		1									1						1			1								1							1	1			

38																																																
1039										1																																						
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1047			1	1						1				1		1		1	1							2									1										1			
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1059																				1																												
105	2	7	1	1	2	5	14	11	11	4	2	4	4	2	1	2	4	4	1	5	11	12	11	2		3	20	5	8	4	3	3			1	1	6	2	6		3	9	2	5	2	1	3	15
1060												1	1									1	1				1																		1			1
1061								1	1																																							
1062								1	1																																							
1063																																												1				
1064																						1	1				1					1												1				1
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1066																																												1				
107	3	11	10	6	6	6	13	11	11	4	9	9	9	6	7	5	7	4	7	7	18	15	19	3	5	6	12	7	11	5	11	2	5	6	6	5	9	3	9	5	4	12	5	14	9	2	5	17
108																	2															1												1			1	

1113																																														1		
1112																																												1				
1113											1	1	1		2		1			1		1	1		1		1	1	1				1	2			1		1		1				1	1		1
1151																																					1											
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1190																						1	1																									
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124			1	1		1	1			1	1							1	1		1										1			1	1								1				
12													1												1																						

51																																																
125		1	1	1	1		1	1	1		1	1	2	1	1				1	1	1	1	1			1	1		1		1			1	1	1	1					1		2	1	1	1	1
1274									1																																							
1278																											1		1																			1
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1469																													1																			
146																1																																
147		4					2	1	1		2	3	2		1					2	1	6	5				4		2		1						1					2	1	1	5			5
1509																					1																											
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152					1	2																			1	1		1			1			1				1					1			1		
1530																																				2												
154	2	5	3	2	2	3	2			4	3	3	2	1	2	3	2	4	2	1	2	3	3	4	2	3	3	3	1	1	4	1	4	5	3	2	4	1	3		2	1	2	5	4	2		5
155	1						1				1										1	1	1	1				2											1									
156	2		1	1	3	2	4	1	1	2	5	1	1		2	1	2	2	1	1	4	1	1	2			1			1			1	1	1		1	1			2			1	2	1	1	1
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161								1	1		1										1	2	2				1	1	1										1					2				1
162			1				1							1	1																						1											
163			1				1	1	1		1	1	1	1	1						1	2	5		1		1		1								2				1	1		1		1	1	1
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170	1	1										1	1		1	1	1			1				1	1			1					1			1	1	1	1		1		1		1	1	1	
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181		1				1																																										
182		1			2																1															1										1	2	
183		1					3	2	2	1	4				1					3	3				1	1	1	2		1						1			2	2	1		1	10	1	2	1	1
184		2						1	1		1			1								1	1			1	1		1		1											1						1
186																													1											1								
194																					2																											
1995																															1										1							
199																	1																						1									
2027																																													2			
2045																																					1											
206																															1																	
208			1	1																											1													1				
2108		1																																														
211				1																											1																	
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89																																														
2238																														1																
2266																																				1										
228																														1																
2340		1																																												
244																																					1				1					
245													1												1												1				1					
246					1																																								
247										1								1																					1				1			
251																						1	1							1				1					2				1			
253											2																																		
268											1																																		
274																																										2				
282					1																																								
283					1																																								

285								1	1	1		1	1					1								1				1										1				1				
298		1																																														

Table 4. 4. New P450 families and subfamilies identified in *Streptomyces* species.

New families		Total number
CYP1035-CYP1039; CYP1047; CYP1048; CYP1053-CYP1059; CYP1060-CYP1066; CYP1189-CYP1194; CYP1196-CYP1199; CYP1251; CYP1274; CYP1416-CYP1420; CYP1422-CYP1424; CYP154; CYP155-CYP159; CYP161; CYP1618; CYP170; CYP171; CYP178-CYP184; CYP2045; CYP208; CYP2108; CYP211; CYP2189; CYP2238; CYP246; CYP282; CYP283		66
New subfamilies (144)		
P450 family	Total number of new subfamilies	Name of the subfamily
CYP102	2	B, G
CYP105	38	AT, AU, AV, AW, AX, AY, AZ, B, BA, BB, BC, BD, BE, BF, BG, BH, BQ, BR, BS, BT, BU, BV, BW, D, DB, DC, DD, DE, DF, DG, H, M, N, P, Q, R, U, Z
CYP107	62	AD, AK, AL, AM, BK, BW, BY, BZ, CD-CH, CJ, CK-CN, CP-CW, DU, DW, EA-EH, EJ, EK-EN, EP-ER, KW-KZ, LA-LG, P, T-Y
CYP112	1	B
CYP113	4	G, J, Y, Z
CYP1222	1	D
CYP1223	1	B
CYP123	1	D
CYP1240	1	B
CYP135	2	D, G
CYP136	2	E, F
CYP143	1	C
CYP145	3	B, C, D
CYP147	2	B, K
CYP152	1	D
CYP166	1	C
CYP186	1	D
CYP251	3	E, F, G
CYP1005	1	B
CYP1278	1	B
CYP1339	1	B
CYP1341	1	E

CYP162	1	C
CYP163	4	C, D, G, H,
CYP1995	2	B, C
CYP199	1	R
CYP274	2	B, C
CYP285	2	B, D
CYP298	1	B

The P450 family number ranged from nine to 30; *S. clavuligerus* and *S. albus* J1074 have the highest and lowest number of P450 families, respectively (Fig. 4.2A and Table 4.1). P450 subfamilies ranged from 15 to 58, with *Streptomyces* sp. 4F and *S. clavuligerus* having the lowest and highest number of P450 subfamilies (Fig. 4.2A and Table 4.1). Analysis of P450 families revealed that 25 P450 families were highly populated and contributed 85% of all the P450s identified in 48 *Streptomyces* species (Fig. 4.2B and Table 4.5). This indicates that these P450 families play an important role in *Streptomyces* species' physiology and are thus maintained in high numbers. It is noteworthy that P450s possibly provide luxury functions, as most of the bacteria lack P450s. This phenomenon of maintaining the highest number of certain P450 family members is not new and has been observed in microorganisms such as fungi (Jawallapersand *et al*., 2014, Kgosiemang *et al*., 2014, Qhanya *et al*., 2015, Syed *et al*., 2014). However, unlike fungal species where only specific subfamilies are populated for a dominant P450 family (Jawallapersand *et al*., 2014, Kgosiemang *et al*., 2014, Qhanya *et al*., 2015, Syed *et al*., 2014), *Streptomyces* species' dominant P450 family analysis revealed high diversity in terms of the number of subfamilies (Fig. 4.2C): the CYP107 family has 82 subfamilies, followed by CYP105 with 51 subfamilies, CYP183 with 23 subfamilies, CYP154 with 14 subfamilies, CYP156 with eight subfamilies, CYP157 with seven subfamilies and CYP147 with five subfamilies (Fig. 4.2C).

Table 4. 5. Comparative analysis of P450 family members in *Streptomyces* species. The percentage contribution of each P450 family is calculated as percentage contribution to the total number of P450s (1 625 P450s) identified in 48 *Streptomyces* species.

P450 family	Number of P450s	% contribution to total number of P450s
CYP107	381	23.4
CYP105	225	13.8
CYP157	123	7.6
CYP154	119	7.3
CYP156	56	3.4
CYP147	52	3.2
CYP183	48	3.0
CYP102	45	2.8
CYP159	37	2.3
CYP125	33	2.0
CYP158	31	1.9
CYP163	28	1.7
CYP180	27	1.7
CYP113	22	1.4
CYP170	21	1.3
CYP1035	20	1.2
CYP161	15	0.9
CYP184	14	0.9
CYP124	13	0.8
CYP1047	11	0.7
CYP152	11	0.7
CYP1038	10	0.6
CYP1199	10	0.6
CYP155	10	0.6
CYP285	10	0.6
CYP1005	8	0.5
CYP182	8	0.5
CYP1060	7	0.4
CYP251	7	0.4
CYP1043	6	0.4
CYP1064	6	0.4
CYP1240	6	0.4
CYP1046	5	0.3
CYP108	5	0.3

CYP121	5	0.3
CYP162	5	0.3
CYP1029	4	0.2
CYP1189	4	0.2
CYP145	4	0.2
CYP165	4	0.2
CYP166	4	0.2
CYP179	4	0.2
CYP208	4	0.2
CYP245	4	0.2
CYP247	4	0.2
CYP1031	3	0.2
CYP1037	3	0.2
CYP1278	3	0.2
CYP134	3	0.2
CYP1419	3	0.2
CYP1423	3	0.2
CYP171	3	0.2
CYP178	3	0.2
CYP1013	2	0.1
CYP1036	2	0.1
CYP1048	2	0.1
CYP1061	2	0.1
CYP1062	2	0.1
CYP116	2	0.1
CYP1190	2	0.1
CYP1191	2	0.1
CYP1192	2	0.1
CYP1197	2	0.1
CYP1198	2	0.1
CYP1251	2	0.1
CYP130	2	0.1
CYP1341	2	0.1
CYP135	2	0.1
CYP136	2	0.1
CYP140	2	0.1
CYP1416	2	0.1
CYP1417	2	0.1
CYP1420	2	0.1
CYP143	2	0.1
CYP1453	2	0.1

CYP1530	2	0.1
CYP1618	2	0.1
CYP181	2	0.1
CYP186	2	0.1
CYP194	2	0.1
CYP1995	2	0.1
CYP199	2	0.1
CYP2027	2	0.1
CYP211	2	0.1
CYP244	2	0.1
CYP253	2	0.1
CYP274	2	0.1
CYP1039	1	0.1
CYP1041	1	0.1
CYP1042	1	0.1
CYP1053	1	0.1
CYP1054	1	0.1
CYP1055	1	0.1
CYP1056	1	0.1
CYP1057	1	0.1
CYP1058	1	0.1
CYP1059	1	0.1
CYP1063	1	0.1
CYP1065	1	0.1
CYP1066	1	0.1
CYP1113	1	0.1
CYP112	1	0.1
CYP1151	1	0.1
CYP1193	1	0.1
CYP1194	1	0.1
CYP1196	1	0.1
CYP1207	1	0.1
CYP1215	1	0.1
CYP1222	1	0.1
CYP1223	1	0.1
CYP122	1	0.1
CYP123	1	0.1
CYP1248	1	0.1
CYP1274	1	0.1
CYP1339	1	0.1
CYP1373	1	0.1

CYP1385	1	0.1
CYP1392	1	0.1
CYP1418	1	0.1
CYP1422	1	0.1
CYP1424	1	0.1
CYP1457	1	0.1
CYP1459	1	0.1
CYP1469	1	0.1
CYP146	1	0.1
CYP1509	1	0.1
CYP151	1	0.1
CYP1694	1	0.1
CYP1722	1	0.1
CYP177	1	0.1
CYP1813	1	0.1
CYP2045	1	0.1
CYP206	1	0.1
CYP2108	1	0.1
CYP2189	1	0.1
CYP2238	1	0.1
CYP2266	1	0.1
CYP228	1	0.1
CYP2340	1	0.1
CYP246	1	0.1
CYP268	1	0.1
CYP282	1	0.1
CYP283	1	0.1
CYP298	1	0.1

Comparative analysis of P450 family dynamics between the genera *Streptomyces* and *Mycobacterium* revealed the presence of the highest number of P450 families and P450 subfamilies in *Streptomyces* species (Fig. 4.4). *Streptomyces* species have 144 P450 families and 377 P450 subfamilies compared to 77 P450 families and 132 P450 subfamilies identified in mycobacterial species (Fig. 4.4). *Streptomyces* species also have the highest number of new P450 families (66) and new P450 subfamilies (144) in their genomes compared to mycobacterial species (Fig. 4.4).

Interestingly, only two P450 families (CYP107 and CYP157) conserved in *Streptomyces* compared to mycobacterial species where 10 P450 families, namely CYP51, CYP123, CYP125, CYP130, CYP135, CYP136, CYP138, CYP140, CYP144 and CYP1128, were conserved (Parvez *et al*., 2016).

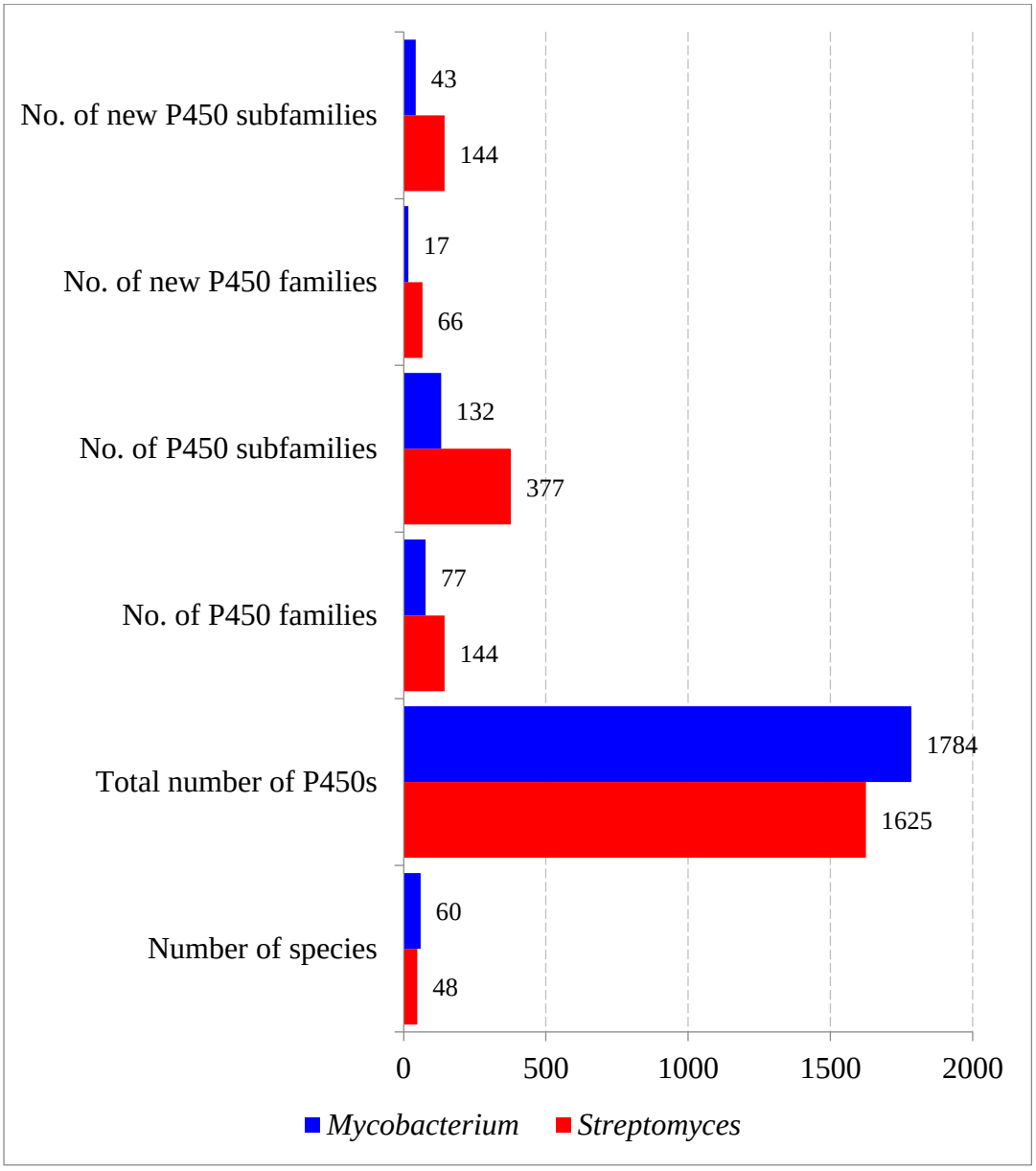

Figure 4. 4. Comparative analysis of key features of P450s between the genera *Streptomyces* and *Mycobacterium*. Y-axis indicates the count for each of the key features.

4.3 *Streptomyces* and *Mycobacterium* species show contrasting P450 profiles

Comparative analysis of P450 profiles between the genera *Streptomyces* and *Mycobacterium* revealed that species belonging to these genera have different P450 profiles with few similarities (Fig. 4.5). Despite both genera belonging to the same phylum, *Actinobacteria*, only 21 P450 families were found to be common and quite a large number of P450 families were found to be unique to *Streptomyces* (123 P450 families) and *Mycobacterium* (56 P450 families) (Fig. 4.5). In the 21 P450 families commonly found between the two genera, an interesting feature was observed in terms of the number of member P450s (Fig. 4.6). A significant difference in the number of member P450s in the commonly shared P450 families was observed between *Streptomyces* and *Mycobacterium* (Fig. 4.6). The P450 families CYP102, CYP105, CYP107, CYP147, CYP161 and CYP183 were highly populated in *Streptomyces* species, whereas the P450 families CYP108, CYP121, CYP123-CYP125, CYP130, CYP135, CYP136, CYP140, CYP143 and CYP268 were highly populated in mycobacterial species (Fig. 4.6).

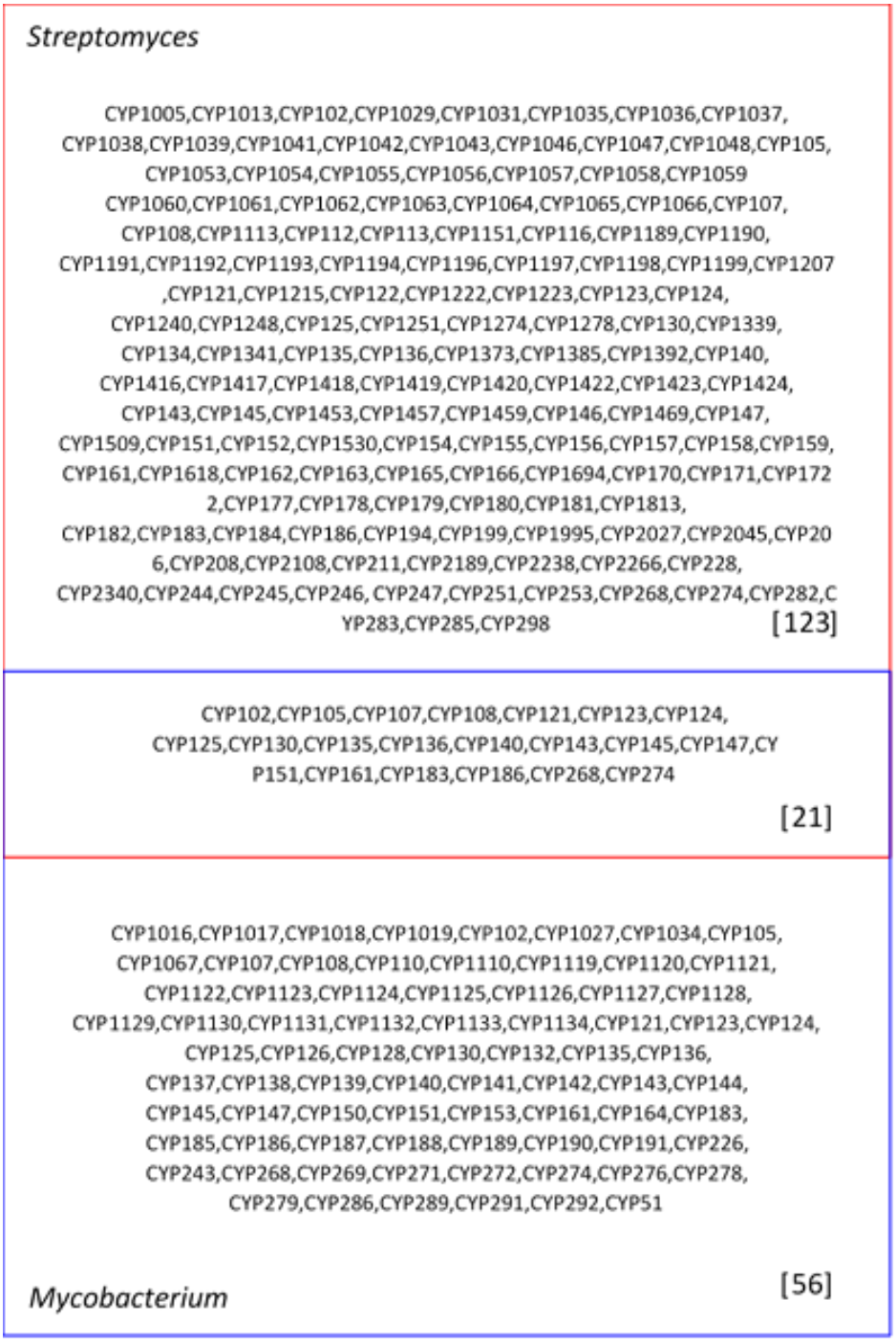

Figure 4. 5. Comparative analysis of P450 families between *Streptomyces* and *Mycobacterium*. The numbers in parenthesis indicate the number of P450 families that are common and those that are unique to each genus.

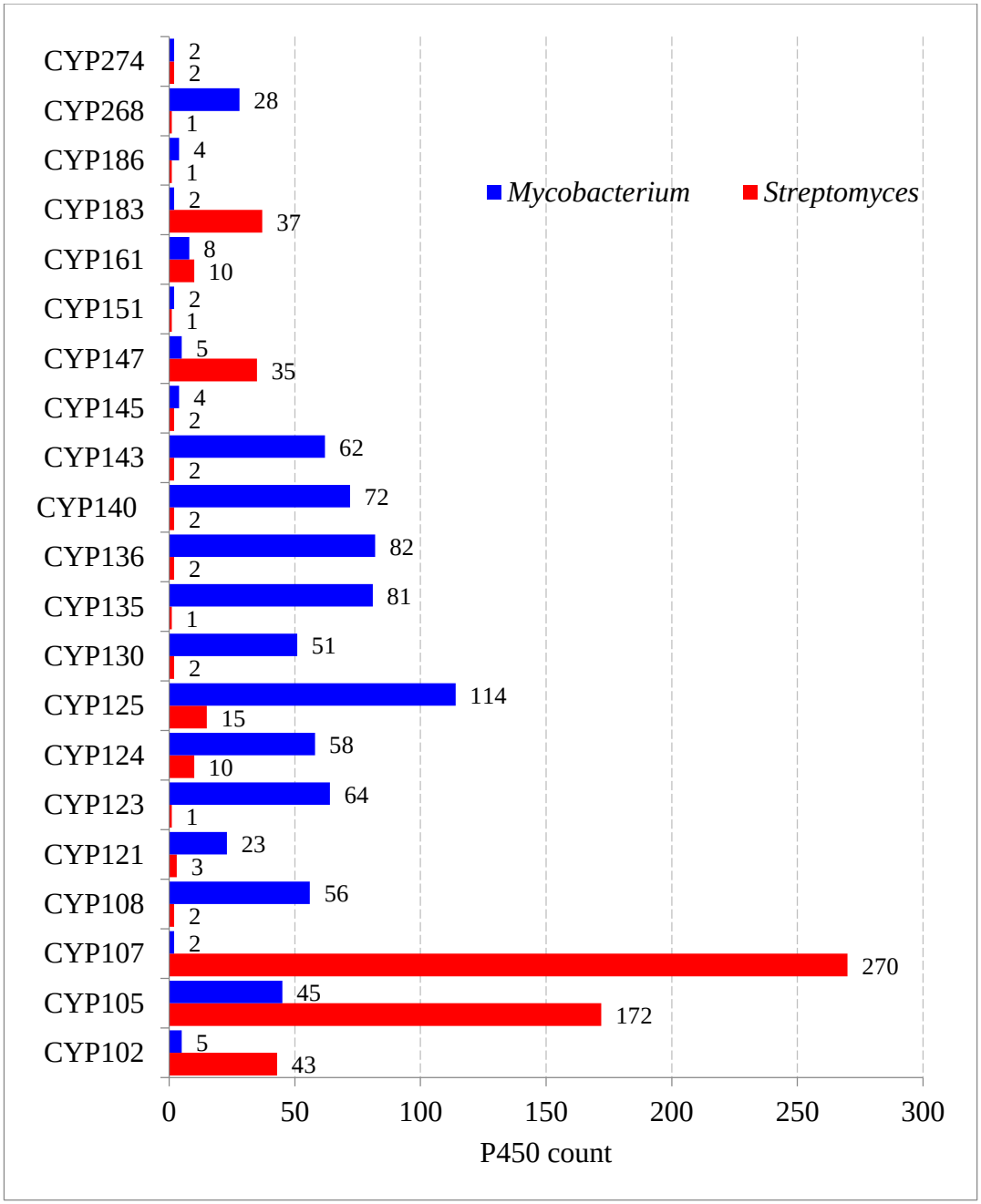

Figure 4. 6. Comparative analysis of member P450s in P450 families common between the genera *Streptomyces* and *Mycobacterium*. The numbers next to bars represent the number of P450s in the P450 family.

Differences were also observed in the number of dominant P450 families in the two genera (Fig. 4.7A and B). Only seven P450 families, namely CYP107, CYP105, CYP157, CYP154, CYP156, CYP147 and CYP183, contributed 62% of all P450s in *Streptomyces* species, whereas 15 P450 families, namely CYP125, CYP189, CYP150, CYP136, CYP135, CYP138, CYP140, CYP123, CYP143, CYP142, CYP144, CYP124, CYP108, CYP51 and CYP187, contributed 60% of all P450s in *Mycobacterium* (Fig. 4.7A and B). An interesting feature was that the percentage contribution of families was highest in *Streptomyces*, i.e. 23.4% by CYP107 and 13.8% by CYP105, compared to *Mycobacterium* P450 families, where the highest contribution was 6.4% by CYP125 (Fig. 4.7A and B). Furthermore, differences in P450 profiles between the two genera were observed in terms of type of dominant P450 families (Fig. 4.7A and B). A comparison of the dominant P450 families between the two genera revealed that none of the dominant P450 families was common between them (Figures 4.5 and 4.7 A and B).

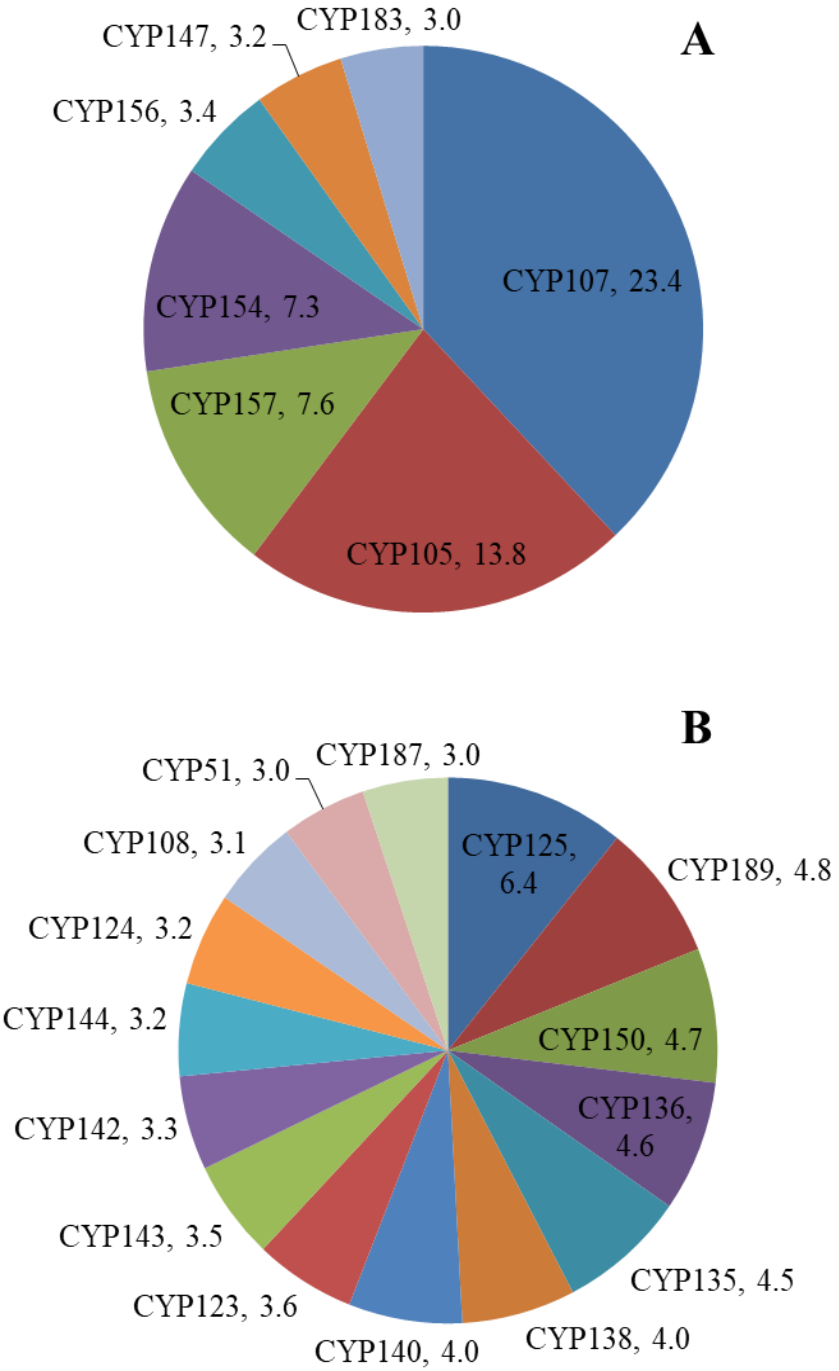

Figure 4. 7. Comparative analysis of dominant P450 families between the genera *Streptomyces* (A) and *Mycobacterium* (B). P450 families that are dominant in a genus are presented along with their

names and percentage contribution to the total number of P450s in a genus. A detailed analysis of the percentage contribution of each P450 family in *Streptomyces* species is presented in Table 4.5.

4.4 Predicted functions of *Streptomyces* P450s

Functional analysis of P450s based on characterised homolog P450s from other microorganisms and P450s from *Streptomyces* species (Rudolf *et al*., 2017) revealed that most of the *Streptomyces* P450s were involved in secondary metabolite production (Table 4.6). This strongly supports the concept that these *Streptomyces* P450s play a key role in the production of chemically diverse secondary metabolites, as a large number of P450 families were found in 48 *Streptomyces* species. Detailed analysis of P450 functions according to general functions and specific functions at P450 family and P450 subfamily level is presented in Tables 4.6 and Tables 4.7, respectively. The P450 family CYP180 was found to be part of a gene cluster that produces geosmin (Lamb *et al*., 2003). Among the P450 families involved in secondary metabolite production, 88 P450 families are uniquely present in *Streptomyces* species compared to mycobacterial species (Fig. 4.5 and Tables 4.6 and 47). P450 families, namely CYP105, CYP107, CYP161, and CYP183, which are highly populated in *Streptomyces* species compared to mycobacterial species (Fig. 4.7), were found to be involved in secondary metabolite production (Tables 4.6 and 4.7). This strongly suggests that these P450 families have been populated in *Streptomyces* species owing to their importance and necessity in secondary metabolite production. Therefore, the diversity among these P450 families, judged by the large number of P450 subfamilies, presumably serves to increase the resultant chemical diversity further across different *Streptomyces* species. It is well established that P450s are one of the key enzymes contributing to the diversity of secondary metabolites in organisms (Greule *et al*., 2018). In contrast to the P450 families highly populated in *Streptomyces* species, P450 families that are highly populated

in mycobacterial species such as CYP125, CYP124, CYP108, CYP140 and CYP268 (Fig. 4.7B) are involved in steroid (cholesterol) and hydrocarbon (lipids, alkenes, long chain acetate and ketone) hydroxylation (Fig. 4.7B and Table 4.6), suggesting that these P450 families possibly help mycobacterial species to assimilate the host compounds. It is noteworthy that results from this study revealed that some of these P450 families are rarely (CYP125 and CYP140) or never (CYP268) part of secondary metabolite BGCs in mycobacterial species (Tables 4.6 and 4.7).

Table 4. 6. Functional analysis of P450s. Functional analysis of P450s in Table 4.6 is presented at family level. Functional analysis of specific family members of the *Streptomyces* species is presented in Table 4.7.

P450 family	General function	References
Functional analysis of *Streptomyces* P450s		
CYP102	Fatty acid hydroxylation	(Lamb *et al*., 2010)
CYP105	Biotransformation or degradation of xenobiotics, and biosynthesis of secondary metabolites	(Li *et al*., 2013, Moody and Loveridge, 2014)
CYP107	Biosynthesis of secondary metabolites	(Sherman *et al*., 2006)
CYP113	Biosynthesis of secondary metabolites	(Zhang *et al*., 2013)
CYP122	Metabolism of terpenoids and polyketides	(Chen *et al*., 2012)
CYP158	Biosynthesis of secondary metabolites (flaviolin]	(Zhao *et al*., 2012a, Zhao *et al*., 2007)

CYP154	Production of secondary metabolites and hydroxylation of steroids	(Cheng *et al*., 2010, Podust *et al*., 2004, Podust *et al*., 2003)
CYP170	Biosynthesis of secondary metabolites	(Moody *et al*., 2012, Takamatsu *et al*., 2011, Zhao *et al*., 2009)
CYP1048 & CYP264	Production of phytotoxins	(Healy *et al*., 2000, Yu *et al*., 2013)
CYP161	Biosynthesis of secondary metabolites	(Kelly *et al*., 2010)
CYP151 & CYP248	Biosynthesis of secondary metabolites	(Zocher *et al*., 2011)
CYP163	Biosynthesis of secondary metabolites	(Haslinger *et al*., 2014)
CYP129 & CYP131	Biosynthesis of secondary metabolites	(Dickens *et al*., 1997, Jaffrezou *et al*., 1996, Walczak *et al*., 1999)
CYP162	Biosynthesis of secondary metabolites	(Xie *et al*., 2007)
CYP245 & CYP244	Biosynthesis of secondary metabolites	(Huang, 2003, Molnár *et al*., 1996)
CYP183	Biosynthesis of secondary metabolites	(Quaderer *et al*., 2006)
Functional analysis of P450s from other microorganisms		
CYP121	Synthesis of mycocyclosin, a natural product	(Belin *et al*., 2009)
CYP125	Cholesterol oxidation	(McLean *et al*., 2009, Ouellet *et al*., 2010)
CYP124	Cholesterol and lipids hydroxylation	(Johnston *et al*., 2009)
CYP152	Alkene production	(Belcher *et al*., 2014)

CYP108	Terpineol hydroxylation	(Hasemann *et al*., 1994)
CYP116	Degradation of toxic compounds (herbicides and alkyl aryl ethers)	(Çelik *et al*., 2006, Warman *et al*., 2012)
CYP140	Mycolactone toxin synthesis	(Mve-Obiang *et al*., 2005)
CYP165& CYP146	Secondary metabolite production (vancomycin biosynthesis)	(Cryle *et al*., 2010, Pylypenko *et al*., 2003)
CYP199	Oxidation of benzoic acid derivatives	(Bell *et al*., 2008, Bell *et al*., 2012)
CYP268	Hydroxylation of long chain branched acetate and ketone	(Child *et al*., 2018)

Table 4. 7. Functional analysis of P450s. Functional analysis of P450s was presented at P450 subfamily level with respect to *Streptomyces* species.

Species name	CYP P450	Function	References
Streptomyces coelicolor A3(2)	CYP170A1	Catalyses the oxidation of epi-isozizaene to an epimeric mix of 5-albaflavenol	(Zhao *et al*., 2009)
	CYP102B1	Fatty acid hydroxylase	(Lamb *et al*., 2010)
	CYP105N1	Oxidase in coelibactin siderophore biosynthesis Monooxygenase involved in coelibactin synthesis	(Lim *et al*., 2012, Zhao *et al*., 2012a)
	CYP107U1	Putative steroid oxidase with role in sporulation and antibiotic synthesis	(Tian *et al*., 2013)
	CYP154A1	Di-pentaenone cyclisation Involved in polyketide metabolism	(Podust *et al*., 2004, Cheng *et al*., 2010)

	CPY158A1 CYP158A2	C–C coupling in flaviolin polymerisation	(Zhao *et al*., 2007, Zhao *et al*., 2012b)
	CYP170A1	Two-step allylic oxidation of epi-isozizaene to albaflavenone in albaflavenone biosynthesis	(Zhao *et al*., 2009)
	CYP154C1	12- and 14-carbon macrolactone monooxygenase e.g., narbomycin hydroxylase	(Podust *et al*., 2003)
Streptomyces avermitilis	CYP102D1 C1-CYP105D6 C26-CYP105P1	Fatty acid hydroxylase	(Choi *et al*., 2012, Xu *et al*., 2010)
	CYP107W1	Oligomycin	(Han *et al*., 2015)
	CYP105D6	C1-hydroxylation of filipin	(Xu *et al*., 2010)
	CYP105D7	Filipin hydroxylase 1-deoxypentalenic acid hydroxylase	(Takamatsu *et al*., 2011)
	CYP105P1	Filipin hydroxylation	(Xu *et al*., 2010)
	CYP170A2	Two-step allylic oxidation of epi-isozizaene to albaflavenone	(Takamatsu *et al*., 2011)
	CYP171A1	C6 and C8a avermectin algycone hydroxylation	(Lamb *et al*., 2011)
	CYP183A	Pentalene hydroxylase	(Quaderer *et al*., 2006)
Streptomyces scabiei	CYP1048A1	Direct nitration of L-tryptophan with NO, O2, redox partners, and NADPH Plays a novel catalytic role in the biosynthesis of a cyclic dipeptide phytotoxin Involved in the production of the plant toxin thaxtomin, responsible for potato common scab	(Challis *et al*., 2012, Lawrence *et al*., 1990, Yu *et al*., 2013)
	CYP246A1	Thaxtomin phenylalanyl di-hydroxylase in thaxtomin A biosynthesis	(Healy *et al*., 2002)

Streptomyces griseolus	CYP105A1	Catalyses highly selective oxidations of diterpenoids Vitamin D3 hydroxylase involved in the conversion of vitamin D3 to its active form 1α,25-hydroxy vitamin D3	(Hayashi *et al.*, 2008, Janocha *et al.*, 2013, Sugimoto, 2008)
	CYP154C3	Catalyses monooxygenation reactions of a range of steroids	(Makino *et al.*, 2014)
Streptomyces venezuelae	CYP107L1	Involved in ring decoration of macrolide antibiotics Catalyses regioselective C-12 hydroxylation of narbomycin (the final step of pikromycin biosynthesis) 12- and 14- carbon macrolactone, e.g narbomycin and YC-17 hydroxylation	(Sherman *et al.*, 2006)
Streptomyces natalensis	CYP161A2 (PimD)	4,5-Desepoxypimaricin epoxidase in pimaricin biosynthesis	(Kelly *et al.*, 2010)
Streptomyces thioluteus	CYP151A (AurH)	Oxidation and ring formation to convert deoxyaureothin to aureothin	(Zocher *et al.*, 2011)
	CYP248A1	Aureothin synthase	(Zocher *et al.*, 2011)
Streptomyces himastatinicus ATCC 53653	CYP107B (HmtN)	γ-Hydroxylation of an unusual pipera-zic acid (Pip) motif in himastatin biosynthesis	(Zhang *et al.*, 2013)
Streptomyces himastatinicus ATCC 53653	HmtT	Regio- and stereospecific C2/C3 epoxidation of L-tryptophan indole ring and subsequent cyclisation forming hexahydropyrroloindole in himastatin biosynthesis	(Zhang *et al.*, 2013)
Streptomyces sp. Acta 2897	CYP163B3 (P450 Sky)	Three successive β-hydroxylations of separate PCP-bound L-amino acid precursors in skyllamycin biosynthesis	(Haslinger *et al.*, 2014, Uhlmann *et al.*, 2013)
Streptomyces nodosus	CYP161A3 (AmphL) CYP105H4 (AmphN)	Polyketide oxidative tailoring reactions	(Agarwal, 2006, Caffrey *et al.*, 2001)

Streptomyces thermotolerans	CYP107C1	C12-C13 epoxidation of carbomycin B to make carbomycin C	(Ashy *et al*., 1980)
Streptomyces bikiniensis	ChmH1	C20 methyl macrolide hydroxylation	(Ward *et al*., 2004)
Streptomyces clavuligerus	CYP105M1 (orf10)	Possible clavaminic acid derivative epoxidase	(Reading and Cole, 1977)
Streptomyces sp. strain C5	CYP129A2 (dox A) CYP131A2 (dnrQ)	C10,C13, C14 anthracycline glycine DNR precursor hydroxylations and likely aglycone core oxidation	(Dickens *et al*., 1997, Jaffrezou *et al*., 1996, Walczak *et al*., 1999)
Streptomyces graminofaciens	GfsF	C8-9 macrolide epoxidation then C10 hydroxylation	(Kataoka *et al*., 2000, Kudo *et al*., 2010)
Streptomyces tsukubaensis	CYP122A4 (FkbD)	Four-electron C-9 FK506 precursor oxidation	(Chen *et al*., 2012)
Streptomyces pulveraceus	FosK	C18 fostriecin hydroxylation	(Kong *et al*., 2013, Liu *et al*., 2013)
Streptomyces himastatinicus	CYP107B (HmtN) and HmtT and HmtS	Piperazic acid (Pip) motif ^{y}-hydroxylation and C2/C3L-tryptophan epoxidation cyclisation to hexahydropyrroloindole and biaryl aromatic coupling of depsipeptide monomers	(LEET *et al*., 1996, Ma *et al*., 2011, Zheng *et al*., 2013)
Streptomyces tendae	CYP162A1	Histidine β-hydroxyation to form nikkomycins X and I	(Lauer *et al*., 2001, Xie *et al*., 2007)
Streptomyces spheroids	CYP163A1 (NovI)	PCP-loaded tyrosine β-hydroxylation	(Chen and Walsh, 2001, Steffensky *et al*., 2000)
Streptomyces peucetius	CYP105F2	Oleandomycin tailoring hydroxylation	(Rodriguez *et al*., 1995, Shrestha *et al*., 2008)
	CYP107A1	Catalyses the H2O2-mediated dealkylation of 7-ethoxycoumarin	(Niraula *et al*., 2011)

Streptomyces hygroscopicus	CYP107G1 (rapN) CYP122A2 (rapJ) CYP122A3	C9, C26, C27 and C32 rapamycin macrolactone hydroxylation	(Huang, 2003, Molnár *et al*., 1996)
Streptomyces sp. tp-a0274	CYP245A1 (StaP) and CYP244 A1 (StaN)	Aryl-aryl coupling of chromopyrrolic acid and C-N linkage of staurosporine aglycone	(Huang, 2003, Molnár *et al*., 1996)
Streptomyces griseochromogenes	TauI/TmcR	C5 tautomycetin oxygenation	D. Kim *et al*., 2012; F. Wang *et al*., 2012
Streptomyces fradiae	CYP105L1 (TylH1,orf7), CYP113B1 (TylI), CYP154B1	Likely C23 methyl lactone ring oxidase (CYP105L1) and C20 methyl O-mycaminosyl-tylactone hydroxylation (CYP113B1)	(Fouces *et al*., 1999, Merson-Davies and Cundiiffe, 1994)
Streptomyces sp. 307-9	TamI	C10 oxidation of tirandamycin C to E, then C11-12 epoxidation C18 hydroxylation	(Carlson *et al*., 2010, Carlson *et al*., 2011)
Streptomyces albus	CYP170B1	Produces albaflavenone from *epi*-isozizaene	(Moody *et al*., 2012)

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CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVES

Adaptation is key for the survival of an organism. Organisms adapt to different ecological niches by changing their gene pool and thus changing their physiology to make them suitable for survival in the new environment. The effect of ecological niches or lifestyle on P450s' evolution in organisms such as animals, plants, fungi and oomycetes has been observed. In this study, for the first time, the influence of lifestyle on the evolution of P450s in a bacterial population has been presented. Ample evidence of the impact of lifestyle on shaping the P450 profile in species belonging to the genera *Streptomyces* and *Mycobacterium* is identified in the study. It is clear that different lifestyles influenced the P450 profiles in *Streptomyces* and *Mycobacterium*, hence the differences observed between the two genera in terms of number of P450s, P450 family and subfamily diversity, type of dominant and unique P450 families and differences in number of P450s in common P450 families. Furthermore, functional analysis of P450s suggests that in *Streptomyces*, P450s are destined for secondary metabolite production, whereas in *Mycobacterium* they are destined for utilisation of host lipids or synthesis of novel lipids. Future works involves assessing the physiological function of P450s in order to identify their role, if any, in the adaptation of *Streptomyces* species or mycobacterial species to different ecological niches.