The University of South Bohemia in České Budějovice Faculty of Science

Searching for the polyenes in *Streptomyces* associated with bark beetles

Master thesis

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Annotation

This thesis was focused on investigating unexplored sources, such as bark beetles, to find new potential producers of polyene antifungal compounds with low cytotoxicity. *Streptomyces* strains were isolated, identified, and their bioactivity and spectrum of fungal inhibition were evaluated. Additionally, data mining from polyene databases was conducted to search for specific gene markers involved in production of polyenes.

Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

Linz, 13.12. 2023

Sara Khaled Youssef Sayed

Sara sayed

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Abbreviations

ACP	Acyl Carrier Protein			
AMR	Antimicrobial resistance			
Asp	Aspartic acid			
AT	Acyltransferase			
BCCO	Biology Centre Collection Organisms			
BGC	Biosynthetic Gene Cluster			
BLAST	Basic Local Alignment Search Tool			
CAAL	Candida albicans			
CYP 450	Cytochrome P450			
DH	Dehydratase			
DOBISCUIT	Database of Biosynthesis clusters Curated and Integrated			
DSM	Difco Sporulation Medium			
ER	Enol Reductase			
GC Content	Guanine-Cytosine content			
His	Histidine			
KR	Ketoreductase			
KS	Ketosynthase			
MBS	McBeth Scale medium			
MiBIG	Minimum Information about a Biosynthetic Gene cluster			
NCBI	National Center for Biotechnology Information			
PBS	Phosphate Buffered Saline			
PKS	Polyketide synthase			
PSAE	Pseudomonas aeruginosa			
R2A	Reasoner's 2A agar			
rDNA	Ribosomal deoxyribonucleic acid			
rRNA	Ribosomal ribonucleic acid			
Ser	Serine			
STAU	Staphylococcus aureus			
TE I	Thioesterase type I			

Abstract

Actinomycetes are filamentous gram-positive bacteria that are characterized by having a high GC content in their genomes. They form symbiotic relationships with various organisms and produce secondary metabolites crucial for interactions with hosts. Moreover, they serve as an important source for development of new clinical drugs as antifungal agents. Polyenes are the 2nd most frequently used antifungals. Most polyenes that have been identified so far are produced by streptomycetes. However, they have limitations, such as drug resistance, low bioavailability after oral administration, and high cytotoxicity.

The main objectives of the thesis were to explore new niches, particularly bark beetles to search for novel putative producers of polyenes or other antifungals with low cytotoxicity and to search for specific gene markers involved in the production of polyenes.

First, isolation, purification, characterization, and identification of the *Streptomyces* strains were done. Then the inhibitory activity against different indicator strains were evaluated. Promising strains were tested for inhibitory activity against fungi obtained from different environments. Second, the amino acid and nucleotide sequences of the polyene biosynthetic gene clusters of *Streptomyces* sp. strains were downloaded from databases. Phylogenetic analyses of CYP 450 and TE type I were performed to determine conservative regions and to identify the possibility of the usage of these enzymes and their coding regions as gene markers for polyene biosynthesis. The amino acid sequences of the conserved region of TE type I for polyenes were translated back to nucleotide sequences and used for designing the primers and tested them against *Streptomyces* strains using *in-silico* PCR.

The results of morphological characterization showed that some isolates were *Streptomyces*-like strains, while other isolates did not resemble actinomycetes. These results were confirmed by 16S rRNA identification. Moreover, the bioassay results indicated that most *Streptomyces* strains exhibited inhibitory activity against *Candida albicans* and inhibited β-hemolysis of *Staphylococcus aureus*. The four most promising isolates showed a weak inhibitory effect against fungal strains obtained from different areas. The analysis of cytochrome P450 of the polyene BGCs revealed the presence of two distinct groups of CYP 450 enzymes, each group demonstrates regiospecific oxidation. However, some polyene BGCs possess no cytochrome P450. Therefore, CYP 450 is not specific to polyenes and cannot be used as a gene marker. Moreover, the results of thioesterase type I gene analysis showed the presence of some domains, which are unique and specific to polyenes, but lack a high level of conservation to design specific PCR primers.

1. Introduction

Actinomycetes are aerobic filamentous gram-positive bacteria that are characterized by having high GC content in their genomes [1]. The most common and important genera are *Actinomyces*, *Corynebacterium, Frankia, Gardnerella, Mycobacterium, Nocardia, Propionibacterium and Streptomyces* [2]. Actinomycetes are found free in soil, freshwater, and marine environments. Moreover, they are living in symbiosis with fungi, plants, insects as well as in the gastrointestinal tract of animals and humans [3]. Natural products produced by actinomycetes play a key role in the interaction with their hosts [4].

1.1.Actinomycetes associated with arthropods.

The majority of the interactions between actinomycetes and their hosts are beneficial as actinomycetes are not only producing natural products that enable their hosts to defend themselves, their offspring and their food source against pathogens or pests, but also, they produce enzymes that break down complex natural polymers like lignocellulose into simple compounds that can serve as source of nutrients and energy for the host [4].

One of the most well-studied examples of a highly integrated symbiosis is the association between leaf-cutting ants, their mutualistic fungal cultivar, and actinomycetes [5]. Leaf-cutting genera of ants as *Atta* and *Acromyrmex* provide the mutualistic fungus *Leucoagaricus gongylophorus* with fresh leaf materials needed for their growth, producing swollen tips of fungal hyphae called gonglydia that serve as a nutrient source for the ants. Actinomycete strain of *Pseudonocardia* is hosted on specific regions of ant's cuticle for the protection of the fungal garden from potential invasion by harmful pathogens by producing antibiotics as dentigerumycin, nystatin P1 and selvamicin [4].

Similar to ants, fungi and actinomyces tripartite symbiosis, fungus-growing termites are associated with *Streptomyces*. They feed on plant material and provide their plant-containing faecal matter to their fungal cultures known as *Termitomyces* producing gonglydia that collected by termites for nourishment [5]. Although the host-associated *Streptomyces* species are producing natural products as microtermolides A and B that show inhibitory activity against the competitor fungus *Pseudoxylaria*, it is unclear if they protect the termites or their fungus against infection [4].

Moreover, different genera of female digger wasps as *Philanthus*, *Trachypus* and *Philanthinus* are cultivating *Candidatus Streptomyces philanthi* in their antennal glands to protect their offspring against fungal infections [4]. In addition to that endophytic strain of *Streptomyces*

bacteria protects strawberry plants from gray mold pathogens and protects pollinating bees against insect pathogens [6].

Furthermore, bark beetles (subfamily of beetles Scolytinae) cultivate ambrosia fungi in a specific storage compartment known as mycangium to cover the nutritional needs for themselves and their developing larvae [4], [7]. They are also surrounded by *Streptomyces* in their mycangia and subcortical galleries, which produce antifungal antibiotics with the potential to protect themselves against competitor fungi [8].

1.2. Streptomyces

Streptomyces is the largest genus of *Actinobacteria* that belong to the family Streptomycetaceae and order Actinomycetales [9]. It contains 1,184 species and 73 subspecies that are identified (http://www.bacterio.net/streptomyces.html, accessed on 13th October 2023). *Streptomyces* has received the attention of researchers due to its significant role in soil ecology, its diverse phylogenetic distribution, and its production of valuable secondary metabolites [10]. It produces a wide range of bioactive compounds, including antibiotics, enzymes, anti-hypertensives, immunosuppressants and antitumor agents, making them significant in both medical and industrial applications [11]. Two-thirds of all known antibiotics are produced by *Actinobacteria*, of which the majority are produced by *Streptomyces* (Figure 1) [10].



Figure 1: Antibiotics produced by Actinobacteria [3].

1.2.1. Genomic features and morphology of *Streptomyces*

They are aerobic filamentous gram-positive bacteria. Their genomes typically exhibit a GC content ranging from 68 to 78 mol% [12]. Streptomycetes have some of the largest genomes

compared to other bacterial species. Their genome sizes typically range from 8.7 Mbp to 11.9 Mbp [13]. The number of protein-coding sequences (CDSs) ranges between 5,361 and 11,170 [14]. The number of total biosynthetic gene clusters (BGCs) in streptomycetes genomes varies from 18 (e.g., *Streptomyces* sp. CLI2905 and *Streptomyces tendae* 139) to 53 (e.g., *Streptomyces hygroscopicus* XM201 and *Streptomyces sp.* YIM 121038), with an average of 32 [14]. Biosynthetic gene clusters (BGCs) are groups of organized genes that work together to produce secondary metabolites. Each BGC is responsible for synthesizing one or more similar compounds, which may differ mainly in terms of their strength and/or specificity of biological activities [15]. One *Streptomyces* strain can produce several secondary metabolites as it can possess several biosynthetic gene clusters (BGCs), so called one strain many compounds (OSMAC) approach [16], for example *Streptomyces* sp. SD85 (Figure 2) [17].



Figure 2: Circular representation of Streptomyces sp. SD85 chromosome [17].

The life cycle of the streptomyces is a complex process that composed of many stages, particularly when it is growing on solid surfaces such as agar or soil particles (Figure 3). Firstly, free spores are released into the environment. Then germination of the spores takes place, forming germ tubes that grow by branching into substrate mycelium. *Streptomyces* forms a long chain of spores after chromosome segregation and septation of the aerial mycelium [1], [12]. Substrate mycelium is responsible for absorption of nutrients [1], while aerial mycelium is responsible for productive spores [10].



Figure 3: Schematic diagram of the Life cycle of Streptomyces [12].

1.2.2. Secondary metabolites produced by *Streptomyces* and their ecological importance

Secondary metabolites are small organic molecules that are mainly classified into volatile and soluble compounds. Geosmin is one of the volatile compounds, which is produced by *Streptomyces* and responsible for the earthy smell of the soil after rain [18].

Although secondary metabolites are not essential for bacterial growth, they have important ecological roles (Figure 4). Soil bacteria including *Streptomyces* produce antimicrobial compounds that have different bioactivities based on their concentration. At inhibitory concentrations, they can serve as microbial warfare to inhibit the growth of the other microbial competitors that are growing in the same niche. However, if they are produced at subinhibitory concentrations, they act as signaling molecules that have different cellular functions as cell development, biofilm formation, inducing cell motility, and nutrient utilization. Secondary metabolites with antimicrobial properties have the potential to stimulate sporulation and provide protection against predators by knocking their genes that are involved in antibiotic production, thus decreasing the growth rate on prey cells. As mentioned above, secondary metabolites as antibiotics also play a significant role in protecting hosts against infections [18].



Figure 4: Schematic diagram for the ecological importance of bacterial secondary metabolites [15].

Polyenes antibiotics are one of the secondary metabolites that have antifungal activity [1]. The majority of polyenes identified so far are produced by soil filamentous actinomycetes of the *Streptomyces* genus [19].

1.3. Polyene antibiotics

1.3.1. History of polyenes as antifungal Drugs

Nystatin is the first polyene macrolide antifungal antibiotic. It was discovered in 1950 by Hazen and Brown and named fungicidin. Since then, over 90 different members of this group have been identified and more are being discovered each year. Moreover, Amphotericin B was discovered in the 1950s and was available on the market in 1957. It is considered the standard treatment for severe invasive fungal infections [20].

1.3.2. Polyenes structures

Polyenes are compounds that have polyketide chains with 3-8 conjugated double bonds and can have either linear or cyclic structures (macrolactam and macrolactone) [19]. They can be classified based on the number of conjugated double bonds into trienes, tetraenes, pentaenes, hexaenes, and heptaenes [20]. The main structural characteristics of polyenes include the presence of a hydrophobic polyene region combined with a hydrophilic polyol portion, which is often glycosylated [19].

Linear polyenes polyketides (LPPs) are one of the crucial members of the polyene family [21]. Typical LPPs such as mediomycin A, mediomycin B and clethramycin show potent antifungal activity. These three polyenes are produced by streptomycetes, and they have similar structures: the same polyene scaffold with the same numbers of polyols in the same positions. However, they differ by the moieties linked to the central structure: clethramycin possesses guanidine moiety instead of amino group and mediomycin B has no sulfate moiety (Figure 5) [22].



Figure 5:Structures of clethramycin and mediomycins [19].

Macrolactam polyenes are a group of macrolactam family that contain a conjugated carbon skeleton and a nitrogen-containing moiety that forms a ring via lactamization. Macrolactam polyenes can be further classified into two subgroups based on the different β-amino acid starter units (Figure 6). Both β -amino acid starter units are synthesized from L-glutamate. The first subgroup uses 3-methylaspartate (3-meAsp) as the starter unit, while the second subgroup utilizes 3-aminobutyrate [17].



Figure 6: Structures of polyene macrolactams that are classified based on the β -amino acid starter unit.

Most of these polyene macrolactams are produced by *Streptomyces* genus, except for micromonolactam and macrotermycin A, which are produced by different *Actinobacteria Micromonospora* and *Amycolatopsis* strains, respectively [17].

Polyene macrolide antibiotics are strong antifungal secondary metabolites that are commonly used in human therapy nowadays [23]. They typically consist of a large ring made up of 20-40 carbon atoms, linked to a deoxysugar called mycosamine via a β -glycosidic bond and exocyclic

carboxy group (Figure 7) [23], [20]. They have four to eight conjugated carbon double bonds that constitute a part of their macrolide ring structure and a series of hydroxyl groups in positions opposite to double bonds [20] that form a ring via lactonization [19].



Figure 7: Common structure of polyene macrolide antibiotics [17].

1.3.3. Mechanism of action

The activity of the polyene antibiotics is closely linked to their structure, as their amphipathic structure enables them to penetrate the cytoplasmic membranes of different organisms [19]. The polyene chain is oriented towards the lipid environment, while the polyol chain faces the interior of the pores (Figure 8) [24]. This interaction can result in either irreversible destruction of the membrane or transient and reversible channel formation [19].



Figure 8: Schematic diagram for the orientation of nystatin in the cytoplasmic membrane forming transmembrane channel [22].

1.3.4. Biosynthesis of polyenes

Polyenes biosynthesis is a complex process involving multifunctional enzymes known as type I polyketide synthases (PKSs). Type I PKS are organized into modules, which consist of several domains (Figure 9). Polyenes are synthesized through three main steps: loading of the starting molecule, chain extension and termination of the final product. Many enzymatic reactions with different enzymes are involved in the process such as acyltransferase (AT), which catalyzes the attachment of the substrate (e.g., acetyl or malonyl) to the acyl carrier protein (ACP), and

ketosynthase (KS), which catalyzes the condensation of substrates attached in ACP followed by reducing of the keto ester catalyzed by ketoreductase (KR). Moreover, dehydratase (DH) catalyzes the dehydration of the compound resulting in formation of carbon – carbon double bond and releasing of water molecule and enol reductase (ER) catalyzes the reduction of the double bond in the molecule [25].



Figure 9: Type I PKS structure with 3 modules and 15 domains [25].

Thioesterase Type I (TE) is associated with ACP at the C-terminus of the last module of PKS. It catalyzes polyketide chain release by intramolecular cyclization or direct hydrolysis that results in the release of a linear product [26]. The catalytic mechanism of TE type I involves the utilization of a catalytic triad consisting of Aspartic acid (Asp), Histidine (His), and Serine (Ser). The mechanism for macrocyclization, which involves an intramolecular nucleophile, proceeds as follows: First, a linear intermediate from Acyl Carrier Protein (ACP) undergoes transesterification to bind to the catalytic serine of TE. Then, the product is offloaded through an intramolecular nucleophilic attack (Figure 10) [27].



Figure 10: The mechanism of macrocyclization catalyzed by amphotericin B-thioesterases (AMB TE) and nystatin (NYS) TE [27].

Moreover, the mechanism for producing linear polyene, which involves an intermolecular nucleophile, follows a similar process: The linear intermediate from ACP undergoes transesterification to bind to the catalytic serine of TE. Then, the linear polyene is directly hydrolyzed and released via intermolecular nucleophilic attack (Figure 11) [28].



Figure 11: The catalytic mechanism for releasing linear polyenes.

The resulting polyketide product undergoes further modifications by one or two steps of oxidation and glycosylation via cytochrome P450 and glycosyltransferase respectively (Figure 12) [29]. Those enzymes are known as tailoring enzymes.

Oxidation is a common modification at which hydroxyl groups are introduced into the polyene backbone. This step is typically catalyzed by cytochrome P450 enzymes (CYP), which exhibit regiospecificity in hydroxylation reactions [30]. For CYP450s to carry out their enzymatic reaction, two electrons are required. Most of the electrons they receive come from ferredoxins that are soluble iron-sulfur (Fe-S) cluster proteins [31]. In a previous study, CYP 450 was used as a gene marker to isolate potential polyene-producing actinomycetes. This involved conducting a PCR-based genome screening, utilizing the specificity of CYP 450 for polyenes [30].

Glycosylation is a process at which amino sugar moieties are attached to specific sites on the polyene structure. Most polyene antibiotics are glycosylated with D-mycosamine (3,6-dideoxy-3-aminomannose). The process of N-glycosylation of D-mycosamine in polyenes results in the production of antibiotics that have reduced toxicity and exhibit similar activity to the original compound. However, these glycosylated antibiotics possess increased water solubility, leading to improved bioavailability [32].



Figure 12: Schematic diagram for nystatin biosynthesis in *Streptomyces noursei* ATCC 11455, NysN and NysL (Cytochromes P450) are involved in oxidation, while NysDI is involved in glycosylation of nystatin [21].

1.3.5. Drawbacks of the polyenes in antibiotic therapy

Although polyene antifungals are effective in fungal infection treatment, they have several drawbacks that limit their use. These antibiotics are light-sensitive and some of them have high cytotoxicity as they exhibit high affinity to human cholesterol due to the similarity between human and fungal cell membranes. The ergosterol of the fungal cell membrane has an extra methyl group and 2 double bonds. (Figure 13) [33].



Figure 13: The structure of ergosterol and cholesterol with highlighted structural differences.

Moreover, polyene antibiotics have poor absorption when taken orally. They are also susceptible to the development of drug resistance. Antimicrobial resistance (AMR) threatens effective prevention and treatment of not only bacterial infections, but also fungal infections as they develop resistance against antimicrobial drugs that were previously effective in eliminating or controlling them [34].

Immunocompromised individuals, such as those undergoing chemotherapy or organ transplantation, are particularly vulnerable to fungal infections as candidiasis caused by *Candida* species and pulmonary aspergillosis caused by *Aspergillus* fungi. *Candida auris* is an example of an emerging multidrug-resistant pathogen that can affect various parts of the body, including the bloodstream, leading to systemic infections that can be fatal if not promptly treated. Furthermore, *Aspergillus fumigatus* is resistant to azole antifungal drugs and can lead to severe respiratory complications and even death if left untreated [35].

These examples highlighted the critical importance of effective prevention and treatment strategies for fungal infections, thereby the discovery of novel antifungals becomes a top priority for researchers.

2. Research objective

The primary objective of this thesis focused on investigating unexplored niches, particularly bark beetles, to search for novel putative producers of polyenes or other antifungals with low cytotoxicity. This involved isolating and identifying *Streptomyces* sp. strains, known for their potential in producing secondary metabolites with antimicrobial properties. These strains were then further characterized to evaluate their bioactivity and spectrum of fungal inhibition.

Another aspect of the research was to test the hypothesis whether streptomycetes inhibitory activities are specific to fungi present in the same niche or if they are random.

Moreover, data mining from polyene databases was carried out to search for a specific gene marker that is essential for the synthesis of polyenes. This was accomplished by a deep understanding of the biosynthetic gene clusters (BGCs) of the polyenes produced by *Streptomyces* sp. and evolutionary related actinomycetes. The first hypothesis was that Cytochrome P450 could be used as a specific gene marker for polyene production. The main aim of this work was to evaluate the matching of the primer pair that was designed in a previous study with CYP 450 sequences of a more robust dataset of polyenes. Furthermore, an alternative hypothesis was formulated after conducting an extensive data mining to search for a specific gene marker. The hypothesis was that thioesterase type I is a good gene marker for the identification of polyene-producing actinomycetes, specifically streptomycetes.

3. Materials and Methods

3.1. Field sampling

Branches and twigs of infested trees of different species were collected from Kunratický les forest, in Prague, Czech Republic. 19 samples of adults, larvae, and detritus (taken separately) were extracted from tree material and stored in Eppendorf tubes at 4°C until processing. The tubes contained either adults or larva and detritus. Each sample was labeled with the specific tree ID from where it was collected and the material type. Samples that contained adult beetles are labeled with tree ID+A, while the samples that contain larvae and detritus are labeled with tree ID+D. The number and the code of the samples are listed in Table 1.

Sample	Sample	Tree ID	Bark beetle ID	Material type
number	code			
S1	PA1Da	Picea abies	Cryphalus abietis	Galleries with larvae and
			pityographus	detritus
S2	QR3Da	Quercus	Scolytus intricatus	Galleries with larvae and
		robur		detritus.
S 3	PA2D	Picea abies	Cryphalus abietis	Galleries with larvae and
				detritus.
S4	QR1D	Quercus	Scolytus intricatus	Galleries with larvae and
		robur		detritus
S 5	QR3Db	Quercus	Scolytus intricatus	Galleries with larvae and
		robur		detritus
S6	PA1Db	Picea abies	Cryphalus abietis	Galleries with larvae and
			pityographus	detritus
S7	TC1D	Tilia cordata	Enrnoporus tiliae	Galleries with larvae and
				detritus.
S8	PA1A	Picea abies	Cryphalus abietis	Adults.
			pityographus	
S9	AA2A	Abies alba	Cryphalus piceae	Adults
S10	A A 2D	Abias alba	Cmmhalus piacaa	Calleries with larves and
510	AA2D	Ables alba	Cryphalus picede	datritus
S 11	DS1D	Dinus	Pityonkthorus nityogranhus	Galleries with larvae and
511	1310	silvestris	1 liyophinorus pilyographus	detritus
S12	PS1A	Pinus	Pityonhthorus nityographus	Adults
012	1517	silvestris		Adults.
S13	PS2D	Pinus	Pityophthorus pityographus	Galleries with larvae and
510	1020	silvestris		detritus.
S14	AA1D	Abies alba	Cryphalus piceae	Galleries with larvae and
				detritus.
S15	FE1D	Fraxinus	Hylesinus varius	Galleries with larvae and
		excelsior	(=Leperisinus varius, L.	detritus.
			fraxini)	

Table 1: The number and the code of the collected samples.

S16	QR2D	Quercus	Scolytus intricatus	Galleries with larvae and
		robur		detritus
S17	PA1Dc	Picea abies	Cryphalus abietis	Galleries with larvae and
			pityographus	detritus
S18	TC1D	Tilia cordata	Enrnoporus tiliae	Galleries with larvae and
			_	detritus.
S19	QR3Dc	Quercus	Scolytus intricatus	Galleries with larvae and
		robur		detritus

3.2. Isolation buffer and media preparation

3.2.1. Isolation buffer preparation

100 mL of 1% Phosphate Buffered Saline (PBS) solution with Tween 80 (Carl Roth Gmbh & Co.

KG, Germany) was prepared as follow:

Component	Volume
Distilled water	80 mL
Sodium chloride	0.8 g
Potassium chloride	0.02
Sodium phosphate	0.144 g
Potassium phosphate	0.0245
Tween 80	10 μL

Tween 80 was added after adjusting the pH to 7.4. Then the total volume was adjusted to 100 mL using a measuring cylinder. The buffer was sterilized by autoclaving for 20 minutes at 121 °C.

3.2.2. Isolation media preparation

Sterile McBeth Scale (MBS), Reasoner's 2A agar (R2A), Krainsky's asparagine (ATCC 236), Humic acid vitamin (HV) and Colloidal Chitin solid media were used for the isolation of bacterial strains. The composition of each medium is described as follow:

Table 3: Composition of MBS solid medium [37].

Component	Amount (g/L)
Starch	10.0
CaCO ₃	3.0
K ₂ HPO ₄	1.0

(NH ₄) ₂ SO ₄	2.0
MgSO _{4.} 7H ₂ O	1.0
NaCl	1.0
Agar	25.0
Reverse osmosis water	Up to 1.0 L

The R2A solid agar medium contained 18.12 g L^{-1} of R2A agar (HiMedia Laboratories, Germany).

Table 4: Composition of R2A solid agar medium [38].

Component	Amount (g/L)
Casein acid hydrolysate	0.500
Yeast extract	0.500
Proteose peptone	0.500
Dextrose	0.500
Starch	0.500
Dipotassium phosphate	0.300
Magnesium sulphate	0.024
Sodium pyruvate	0.300
Agar	15.00

Table 5: Composition of ATCC 236 solid medium [39].

Component	Amount (g/L)
Glucose	11.0
K ₂ HPO ₄	0.5
L-Asparagine	0.5
Agar	20.0
Reverse osmosis water	Up to 1.0 L

Component	Amount (g/L)
Humic acid	1.0 *
Na ₂ HPO ₄	0.5
KCl	1.71
MgSO ₄ .7H ₂ O	0.05
FeSO ₄ ·7H ₂ O	0.01
CaCO ₃	0.02
B-vitamins	**
Cycloheximide	0.05
Agar	18.0
Reverse osmosis water	Up to 1.0 L

Table 6: Composition of Humic vitamin agar medium [40].

* Humic acid was dissolved in 10 ml of 0.2 N NaOH.

** 0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxin-HC1, inositol, Ca-pantothenate, paminobenzoic acid, and 0.25 mg of biotin.

Table 7: Co	omposition	of Collo	idal chitin	solid me	dium [8].
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Component	Amount (g/L)
Colloidal chitin	4.0
K ₂ HPO ₄	0.77
MgSO ₄	0.244
KHPO ₄	0.37
FeSO ₄ ·7H ₂ O	0.001
MnCl ₂ ·4H ₂ O	0.001
ZnSO ₄ ·7H ₂ O	0.001
Agar	20.0
Reverse osmosis water	Up to 1.0 L

10 ml of stock solution containing 0.1 g of trace elements (FeSO₄·7H₂O, g MnCl₂·4H₂O and ZnSO₄·7H₂O) was prepared. Then 1 mL of the stock solution was added to 1 L of media resulting in a final concentration of 0.001 g/L of trace elements.

The pH of the media was adjusted to 7.0 ± 0.2 using HCl or NaOH. The media were subsequently sterilized through autoclaving for 20 minutes at 121 °C and approximately 20 mL were poured

into each sterile Petri dish in a laminar flow box (TelStar, Biostar). Then cycloheximide was added to inhibit fungal contamination (100 μ g/mL) and nalidixic acid was added to inhibit fast growing bacteria (50 μ g/mL) as follow:

	Isolation of	Isolation of	Isolation of
	samples 1 and 2.	samples 3 and 4.	samples 5-19.
Media used for	MBS, ATCC, R2A	MBS, ATCC, R2A	ATCC and Colloidal
isolation	and HV agar.	and HV agar	Chitin media.
Antibiotics added	Cycloheximide to all	Cycloheximide to all	Cycloheximide to
to the media	isolation media.	isolation media.	both media.
		Nalidixic acid to	Nalidixic acid to
		ATCC and R2A.	ATCC.

	Table 8	8:	Summary	table	for t	he	antibiotics	added	to	the	isolation	media.
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3.2.3. Media used for purification and cultivation of streptomycetes.

Moreover, M2 solid medium was used for purification of *Streptomyces*-like isolates and refreshing of the bacterial strains from glycerol stocks.

Table 9: Composition of M2 solid medium [37].

Component	Amount (g/L)
Malt extract	10.0
Yeast extract	4.0
Glucose	4.0
Agar	20.0
Reverse osmosis water	Up to 1.0 L

Oatmeal Agar medium was used for cultivation of streptomycetes before testing their antifungal activity.

Table 10:	Composition	of Oatmeal A	gar medium [37].
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Component	Amount (g/L)
Oatmeal	20.0
Agar	18.0
FeSO ₄ . 7H ₂ O	0.001
MnCl ₂ .4 H ₂ O	0.001
ZnSO4·7H2O	0.001

Reverse osmosis water	Up to 1.0 L

10 ml of stock solution containing 0.1 g of trace elements (FeSO₄·7H₂O, g MnCl₂·4H₂O and ZnSO₄·7H₂O) was prepared. Then 1 mL of the stock solution was added to 1 L of media resulting in a final concentration of 0.001 g/L of trace elements.

Nutrient agar was used for testing the *Sterptomyces*-like isolates against *Bacillus subtilis*. It contained 31 g/L of nutrient agar (HiMedia Laboratories, Germany) to which an additional 5 g agar was added.

The pH of the media was adjusted to 7.0 ± 0.2 using HCl or NaOH. The media were subsequently sterilized through autoclaving for 20 minutes at 121 °C and approximately 20 mL was poured into each sterile Petri dish in a laminar flow box (TelStar, Biostar).

3.2.4. Media for the cultivation of bioindicator microorganisms

Muller-Hinton agar with sheep blood (5%) (KHM agars) were purchased from Dulab, s.r.o. (Suché Vrbné, Czech Republic). It was used for the cultivation of bioindicator microorganisms such as *Pseudomonas aeruginosa* (PSAE), *Staphylococcus aureus* (STAU) and *Candida albicans* (CAAL).

3.2.5. Media used for cultivation of fungi (micromycetes) and the antifungal test.

Potato dextrose agar (PDA) was prepared for cultivation of filamentous fungi. It contained 24 g/L of the potato dextrose broth (HiMedia Laboratories, Germany) to which 13 g of agar were added. Moreover, malt extract agar was used for the antifungal test.

Component	Amount (g/L)
Malt extract	20.0
Peptone	4.0
Agar	18.0
Reserve osmosis water	Up to 1.0 L

Table 11: Composition of malt extract solid medium [37].

The pH of Potato dextrose agar was adjusted to 5.6 ± 0.2 , while the pH of the malt extract solid medium was adjusted to 7 ± 0.2 . The media were subsequently sterilized through autoclaving for 20 minutes at 121 °C and poured into sterile Petri dishes in a laminar flow box (TelStar, Biostar).

3.3. Bacterial isolation and purification of the promising strains

The volume of 1% PBS solution with Tween-80 added to the samples and the number of ten folds of serial dilutions that prepared for each sample were adjusted based on the amount of the material collected.

Firstly, 500 μ L or 1000 μ L of 1% PBS solution with tween 80 was added to the samples. Then the samples containing larvae and detritus were homogenized using manual glass homogenizer and mixed well by vortex while the samples containing adult bark beetles were homogenized using ultrasonic for 2 minutes and mixed by vortex. Two to three tenfold serial dilutions of the samples were prepared using 1% PBS solution with Tween-80 as depicted in figure 14.



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The first two samples, PA1Da (S1) and QR3Da (S2) were used for pilot testing of the isolation procedure and dilutions. Dilutions were prepared for sample S1 at 10x, 100x, and 1000x, while dilutions for sample S2 were prepared at 100x and 1000x. Then 100 μ l of the diluted were inoculated onto MBS, ATCC, R2A and HV agar media that contain cycloheximide (3 plates for each medium and concentration).

Then the conditions were slightly modified for the isolation of samples PA2D (S3) and QR1D (S4) to improve the growth of actinomycetes. Nalidixic acid was added to R2A and ATCC media and the samples were preheated (dry heating) at 70 °C for 15 minutes before adding the buffer to destroy the fast-growing bacteria, while streptomyces withstand high temperature.

 $100\,\mu$ l of the diluted samples were inoculated onto the same four media (3 plates for each medium and concentration).

The conditions were further modified for the isolation of the other 15 samples (S5-S19) to optimize the growth of actinomycetes, specifically *Streptomyces* sp. Colloidal chitin medium was

used to enhance the growth of *Streptomyces* sp., as it grows at a slower rate compared to other bacteria. Nalidixic acid was added to ATCC 236 medium, and the 15 samples were preheated (dry heating) at 70 °C for 20 minutes before adding the buffer. 100 μ l of the diluted samples were inoculated onto ATCC 236 and colloidal chitin media (3 plates for each medium and concentration).

Then the samples were spread evenly over the surface using sterile L-shaped hockey sticks. The samples were incubated at 28 °C and the growth of the colonies were checked regularly for 6 weeks.

Furthermore, all *Streptomyces*-like colonies from each plate were isolated on M2 medium. Then they were transferred again to M2 medium for further purification (Figure 15). Glycerol stocks of the promising isolates were prepared by adding a full loop of biomass to 300 μ L of 50% glycerol and 700 μ L of Luria-Bertani (LB) broth. Glycerol serves as a cryopreservant, so the glycerol stocks of bacteria can be stored stably at -20°C for short-term and at -80°C for longterm.





3.4. Morphological characterization of the isolates

Morphology has been an important characteristic to identify *Streptomyces* like isolates [36]. The isolates were inoculated onto M2 agar. Various morphological observations, including color of aerial and substrate mycelium, and pigment production, have been used to identify streptomycetes.

Light microscopy was used for the observation of the filamentous type of growth to check the purity of the isolate and compare between the structure of filamentous spore chain of *Streptomyces* versus the non-filamentous spore of non-actinomycetes strains. One drop of LB submerse culture of each strain was added to the microscopic slide, covered with the coverslip, and observed under the microscope using objective lens (40x magnification).

3.5. Genotyping of the promising strains

3.5.1. DNA extraction

The DNA of 12 *Streptomyces*-like strains were extracted for identification of the strains. Firstly, Luria-Bertani (LB) broth was prepared as follow:

Component	Amount (g/L)
Tryptone	10.0
Yeast extract	5.0
NaCl	5.0
Reverse osmosis water	Up to 1.0 L

Table 12: Composition of LB broth [41].

20 mL of the medium was poured into small Erlenmeyer flasks using a measuring cylinder. The flasks were covered with stoppers and foil. Then the medium was sterilized using autoclave for 45 minutes. Submerse cultivation of the samples was done by adding a full loop of biomass into LB broth. The samples were incubated for three days at 28 °C on a shaker. After that, 100 μ L of each sample was inoculated on M2 medium to ensure that there is no contamination. 40 mg of the biomass of each sample was harvested from the culture by centrifugation. Then the DNA of the samples were extracted using the protocol provided by NucleoSpin Microbial DNA kits (Macherey-Nagel GmbH, Germany). The concentration and purity of the DNA of each isolate was measured by Nanodrop One spectrophotometer (Thermo Scientific). The most frequently used method to determine the purity of DNA is by comparing the absorbance of DNA at 260 nm (A₂₆₀) to that of proteins at 280 nm (A₂₈₀). Additionally, the A₂₆₀/A₂₃₀ ratio was measured to identify the presence of impurities coming from extraction solutions like Trizol, phenol, guanidine hydrochloride, and guanidine thiocyanate.

3.5.2. Polymerase chain reactions (PCR) and gel electrophoresis BOX-PCR Fingerprinting

BOX-PCR is a fast and inexpensive technique that is used for fingerprinting analysis of morphologically similar strains (S6, S10 and S18) by amplifying repetitive element regions (boxA) in the bacterial genome [37].

Firstly, the master mix for 12 reactions was prepared as follow:

Master mix components	Concentration	Final concentration	1x (μL)	Total (μL)
MilliQ water			6.8	81.6
2x LA Hot Start Master Mix (Top-Bio)	2x	1x	12.5	150
DMSO	100%	1%	2.5	30
BSA	5 mg/ml	0,04 mg/ml	0.2	2.4
BoxA1-primer				
5'-CTACGGCAAGGCGACGCTGA				
CG-3'	(0,3 ug/ul)	0,012 ug/ul	1.0	12.0

Table 13: Master mix components for BOX-PCR [42].

Then 23 μ L of the master mix and 2 μ L of the purified DNA of the samples (200 ng/ μ L) and positive control were added to each Eppendorf microtubes. For the DNA samples with a concentration higher than 200 ng/ μ L, only 1 μ L of the purified DNA and 1 μ L of MilliQ water were added. Negative control was prepared by adding 2 μ L of MilliQ water instead of DNA to ensure that no contaminated nucleic acid has been added to the master mix or samples during sample processing and positive control was used to test the efficiency of the polymerase chain reaction. The thermal cycling was 95°C for 7 min, followed by 30 cycles of 90 °C for 30 s, 53°C for 1 min, and 65°C for 8 min, with a final extension step of 65°C for 15 min.

Moreover, PCR products were separated by gel electrophoresis using 1% of agarose gel. It was prepared by mixing 1.2 g of agarose powder and 120 mL of 1X Tris-acetate-EDTA (TAE) buffer in an Erlenmeyer flask. The mixture was swirled and heated using the microwave till boiling. The solution was then slowly poured into the gel mold after being slightly cooled with tap water. Then the comb was added, and the gel was allowed to cool for 20 minutes. The gel was placed into the gel rig and enough 1X TAE was added to cover the top of the gel. 5 μ L of Gene ladder (GeneRuler 1 kb, Thermo Fisher scientific), the samples, positive and negative controls were loaded on the gel. Then the gel was set to run at 100V and 400 mA for 50 minutes. Then the gel was stained by immersing it in a 1X TAE bath supplemented with ethidium bromide for 20 minutes and observed under UV302.

• 16S rDNA PCR

The prokaryotic 16S rRNA gene is 1550 base pairs (bp) long. It is used for amplification of 16S rDNA of the promising strains to send them for sequencing.

Firstly, the master mix for 17 reactions was prepared as follow:

Master mix components	Concentration	Final concentration	1 x (μL)	Total (µL)
MilliQ water			10.0	170.0
pAf				
(5`-AGAGTTTGATCCTGGCTCAG-3`)	10 uM		0.75	12.75
pHr				
(5`-AAGGAGGTGATCCAGCCGCA-3`)	10 uM		0.75	12.75
2X Fast Start Master (Roche)	5000 U/ml	30 U/ml	12.5	212.5

Table 14: Master mix components for 16S rDNA PCR [43].

Then 24 μ L of the master mix and 1 μ L of the purified DNA of the 12 samples and positive control. For the negative control 1 μ L of MilliQ water was added. The thermocycler protocol that used for amplifying of 11 *Streptomyces*-like strains was 95°C for 3 min, followed by 34 cycles of 94 °C for 1 min, 66°C for 30 s, and 72°C for 1 min 30 s, with a final extension step of 72°C for 5 min. The same protocol was used for amplifying one strain (non-*Streptomyces*), except the annealing temperature was 61°C rather than 66°C.

Furthermore, gel electrophoresis was carried out to ensure the presence of the PCR products. Firstly, 1 μ L loading dye (6X) was added to 5 μ L of the samples. Then 6 μ L of this mixture and 5 μ L of the gene ladder were loaded on 1% agarose gel. The running conditions, staining and observation steps were kept the same. Amplified DNA fragment was bi-directionally sequenced using Sanger technology by SEQme, s. r. o. (Dobříš, Czech Republic). Then the closest relatives of the strains were determined, along with their accession and strain numbers.

3.5.3. Identification of the strains using 16S rRNA

The sequences of the 12 samples were analyzed and manually edited by evaluation of the chromatograms. Then the forward and reverse reads of the sequences were assembled using Geneious software version R8.1.9. and a FASTA file of the consensus sequence was created. Then FASTA files were uploaded onto the National Center for Biotechnology Information (NCBI) platform and analyzed by nucleotide BLAST (blastn) against rRNA/ITS databases of 16S rRNA sequences (Bacteria and Archaea) to search for closely related species.
3.6. Screening of isolates for antimicrobial activity

3.6.1. Bioassay against indicator microorganisms

6 different *Streptomyces* sp. that were previously obtained from bark beetles from Papua New Guinea and Morocco (BCCO strains) [14] and 14 *Streptomyces*-like isolates were tested against different pathogens as *Pseudomonas aeruginosa* (Gram-negative bacteria), *Staphylococcus aureus* (Gram-positive bacteria) and *Candida albicans* (yeast) to determine the inhibitory effect of the secondary metabolites produced by the strains. The strains were refreshed from glycerol using M2 media and incubated for 2 weeks at 28 °C. The streptomycetes. were inoculated in lines on the blood agar and cultivated for 48 hours. Then lines of the indicator strains were inoculated perpendicularly to the samples. The size of the inhibitory zone of the pathogens and the hemolytic zone of the samples as well as the pathogens were measured after three days. Moreover, the sporulation of streptomycetes was evaluated, with a scoring that is ranging from 0 to 3. A score of 3 indicated full sporulation, while a score of 0 indicated that the strains were not sporulating.

Then the bioassay was repeated at different incubation periods of the promising isolates (S6/6, S10/3, S10/25, and S18/15), in order to determine the optimum duration required for the isolates to produce secondary metabolites. In this bioassay, the inhibitory activity of the promising isolates was tested against *Staphylococcus aureus* and *Candida albicans*, as the previous bioassay had shown their susceptibility to inhibition. The *Streptomyces* strains were inoculated in vertical lines and incubated for different time intervals (2 days to 6 days). Then lines of *Staphylococcus aureus* and *Candida albicans* were inoculated on the same plate perpendicularly to the samples and the results were evaluated after three days.

Moreover, to test if the inhibitory effect is due to the secondary metabolite produced by the sample only or it is a synergistic effect, the bioassay was repeated at which *Staphylococcus aureus* and *Candida albicans* were inoculated separately after 3-6 days of incubation of the *Streptomyces* strains.

3.6.2. Bioassay against *Bacillus subtilis*

Bacillus subtilis is a gram-positive bacterium which is not pathogenic to humans. Nine *Streptomyces* like isolates were tested against bacillus subtilis. Firstly, the isolates have grown for 2 weeks at 28 °C. Then 100 μ L of bacillus subtilis Difco Sporulation Medium (DSM) was inoculated onto nutrient agar and spread well over the surface using sterile L-shaped hockey sticks. The plates were kept for 20 minutes to dry. Then, two 11 mm-diameter agar discs containing the cultivated *Streptomyces* like isolates (S) were transferred to the nutrient agar

containing *Bacillus subtilis* with a distance 3 cm between them as depicted in Figure 16. The plates were kept overnight at 37 °C.



Figure 16: Schematic diagram for the Cultivation method of the Isolates with Bacillus subtilis.

3.6.3. Antifungal activity test

To determine whether the inhibitory activities by *Streptomyces* strains are specific to fungi present in the same niche or if they are random, four promising isolates (S6/6, S10/3, S10/25, and S18/15) and three previously obtained streptomycetes (BCCO 10_1099, 10_1104 and 10_1106) isolated from different environment (Morocco and Papua New Guinea) were tested against four fungi collected from various locations.

Strain code	Strain number	Strain Identification	Countries
CCF 3535	-	Graphium asporum	Czech Republic
CCF 3546	-	Ophiostoma piceae	Czech Republic
CCF 4450	-	Ophiostoma minus	Poland
CCF 6041	-	Akanthomyces muscarium	Hungary
BCCO 10_1099	B1	Streptomyces violascens (closest	Papua New Guinea
		relative)	
BCCO 10_1104	B2	Streptomyces albolongus (closest	Papua New Guinea
		relative)	
BCCO 10_1106	B3	Streptomyces fumigatiscleroticus,	Morrocco
		spiralis (closest relative)	
S6/6	B4	Streptomyces-like strain	Czech Republic
S10/3	B5	Streptomyces-like strain	Czech Republic

Table 15: Bacterial and fungal strains used for the antifungal activity test.

S10/25	B6	Streptomyces-like strain	Czech Republic
S18/15	B7	Streptomyces-like strain	Czech Republic

Firstly, the selected Bacteria (B) and Fungi (F) were cultivated on oatmeal agar and potato dextrose agar (PDA), respectively. They were incubated at 28 °C for 10 days. Then 11 mmdiameter agar discs with grown Fungus/Bacterium were transferred to the malt extract agar in duplicates for each strain combination. The distance between the discs was approximately 3 cm. Moreover, an agar disc with each fungus was transferred to a separate malt extract plate as a control (Figure 17). The plates were incubated at 28°C for one month. The results - size of the inhibition zone - were recorded after 3 weeks for fast growing fungal strains and 4 weeks for slow growing ones.



Figure 17: Transferring of discs of the selected bacteria and fungi to malt extract agar.

3.7. Genome Screening of antifungal polyenic secondary metabolites.

3.7.1. Data mining from polyene databases

Since polyenes have a common structure essential for their functions, they have common genes which may act as gene markers (Figure 18). Data mining from polyene databases such as the National Center for Biotechnology Information (NCBI), Minimum Information about a Biosynthetic Gene cluster (MIBiG) and Database of Biosynthesis clusters Curated and Integrated (DOBISCUIT), was carried out to identify a specific gene marker required for the production of polyenes.



Figure 18: Illustration for the biosynthetic gene cluster of the polyenes with common genes highlighted.

• Cytochrome P450 as a gene marker

Based on a previous study, where a primer pair was specifically designed for cytochrome P450 and utilized for PCR-based genome screening to isolate potential polyene-producing actinomycetes strains [30].

Firstly, nucleotide and amino acid sequences of polyene BGCs were downloaded from publicly available databases such as DOBISCUIT (Accessed in October 2022) [38] and MIBiG (Accessed in October 2022) [39]. Then a FASTA file was created for the amino acid sequence of CYP 450 of 6 polyenes. Multiple sequence alignment of CYP 450 using the graphical interface of Geneious version 2022.0.2 (Biomatters Ltd., Auckland, New Zealand) was done using Muscle (Version 3.5). The aligned sequences were edited manually by removing the gaps and used for phylogenetic tree construction using Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0.13 [40]. Maximum likelihood statistical method and 100 bootstrap replications were selected.

• Thioesterase type I as a gene marker

After conducting an extensive data mining process to search for another gene marker, the alternative hypothesis was that thioesterase type I could serve as an effective gene marker for identifying polyene-producing actinomycetes, particularly streptomycetes. It was based on a previous literature that highlighted the presence of a distinct group of PKS-TE domains responsible for producing polyene macrolides. These domains exhibited relatively low sequence

similarity compared to TEs of other non-polyene macrolides such as Erythromycin and Pikromycin [29].

Firstly, nucleotide sequences of polyene biosynthetic gene clusters were downloaded from JDB, NCBI and MIBiG. Then FASTA file was created for nucleotide sequences of the last module of type I polyketide synthase that associated with thioesterase type I, which included 26 polyenes and 2 non-polyenes (Pikromycin and Erythromycin). The sequences were checked that they are starting with start codon and have stop codon at the end. Then the sequences of the last module were uploaded on AliView software (Alignment Viewer and Editor). The sequences were translated into amino acid sequences using bacterial genetic code and translation frame 1. Then multiple sequence alignment of the last module of polyketide synthase using AliView was performed using Muscle and selecting the option "Realign everything as Translated Amino Acids". The aligned amino acid sequences were checked, edited manually and the gaps were removed. Then the amino acid sequences of ACP-TE were annotated using antiSMASH bacterial version [41] and extracted from the whole module. The results of the alignment of amino acid sequences of the thioesterase (TE) gene were compared to the results provided by Zhou, Yucong, et al. [29]. The Conserved region of TE for the polyenes and non-polyenes was extracted and the large gaps that occupied only by non-polyenes were removed. Then the Amino acid sequences were translated back to nucleotide sequences and used for designing the forward and reverse primers.

3.7.2. Designing of primer pairs

Ten primer pairs were designed on the consensus sequences using Geneious software version R8.1.9 (Biomatters Ltd., Auckland, New Zealand) by Clicking the Primers button and selecting Design New Primers. The parameters were adjusted as shown in Figure 19 for optimization of the primer design. The optimum product size was adjusted to 500 bp. Then the best option for the primer pairs was selected based on the % GC content, primer melting temperature (Tm), Hairpin Tm and Self-Dimer Tm and used for designing degenerate primer pairs. Moreover, it is important that the sequence of the primers have guanine (G) or cytosine (C) within the last 5 nucleotides at the 3' end as they form 3 hydrogen bonds thus better stability. This is known as GC clamp [42].

Primer	DNA Probe				
		Size Min:	12 ≑ Optimal:	18 🜩 Max:	27 🜩
		Tm Min:	50 ≑ Optimal:	60 🜩 Max:	65 🜩
		%GC Min:	40 ≑ Optimal:	60 🜩 Max:	80 🚖
		Product Tm Min:	0 🜩 Optimal:	0 🜩 Max:	0
		Max Tm Difference:	5 🜩		GC Clamp: 1
		Max Dimer Tm:	55 ≑		Max Poly-X: 3 🛨
		Max 3' Stability:	9 🌲		

Figure 19: Optimization of the primer's properties.

Moreover, the degenerate primer pair was designed manually using Geneious software version 8.0.4 (Biomatters Ltd., Auckland, New Zealand) by following the procedure that mentioned on Geneious website [43]. Firstly, the file with the aligned sequences was selected. Then the consensus bottom was selected, and the threshold was set to 50 % at which the bases matching was at least 50% of the sequences. The highlighting of the sequences was adjusted as depicted in Figure 20 for easy observation of the differences.



Figure 20: Screenshot illustrating the settings for designing degenerate primers.

The regions of the best option out of the 10 primer pairs that were designed before were selected on the consensus sequence in the alignment. For the forward primer, the region was selected from left to right while the region of the reverse primer was selected from right to left. The Add Annotation button was selected and the calculated characteristics of the primer, including the Tm range based on the degeneracy were checked. Then the name of the primer was entered, and the primer was added to the consensus as an annotation by clicking Ok.

3.7.3. In silico PCR

In silico PCR, also known as virtual PCR, is a computational method used to simulate the polymerase chain reaction (PCR) process. An online tool called "*In silico* PCR amplification" (<u>http://insilico.ehu.es/PCR/index.php?mo=Streptomyces</u>, accessed in October 2023) was used for testing the efficiency and specificity of the designed primer pair [44]. The primer pair was uploaded and tested against all available *Streptomyces* strains allowing up to 2 mismatches, but in 1 nucleotide in 3' end. The maximum lengths of the bands were adjusted to 600 nucleotides (Figure 21).

		Input primers in fasta format								
Primer 1 ¹	5'-	-3' <u>C</u>								
Primer 2 ¹	5'-	-3' <u>C</u>								
Migroorganism										
APPLY TO ALL Streptomyces										
 Include plasmids (if available) Allow 2 mismatches, but in 1 										
Maximum	lengt	th of bands								
600		nucleotides								
¹ Degenerated nucleotides are allowed; A+T+G+C must be 10 or more.										
		Info								

In silico PCR amplification

Figure 21: Screenshot illustrating the settings for testing the primer pair.

4. Results

4.1. Bacterial isolation and purification

The isolation and purification of *Streptomyces*-like strains from samples that contain either adults or larva and detritus, yielded a total of 44 isolates. The results of the isolation process for the first four samples, PA1Da (S1), QR3Da (S2), PA2Da (S3) and QR1Da (S4) indicated the presence of a few promising colonies (*Streptomyces*-like) alongside many non-actinomycetes colonies. Specifically, no promising colonies were isolated from S1 or S3 while four promising isolates were obtained from S2, and one promising isolate was obtained from S4. Moreover, the results of the modified procedure for the isolation of S5-S19 revealed the presence of many promising colonies as different actinomycetes-like strains, specifically many *Streptomyces* strains were isolated. A comprehensive overview of the results is provided in Table 16.

Sample number	Sample code	Code of isolate	No. promising isolates (<i>Streptomyces</i> -like)	Are there some different types?	Glycerol stocks	Isolation media
S1	PA1Da	-	Х	-	-	-
S2	QR3Da	\$2/1 \$2/2 \$2/3 \$2/5	4	No	4	ATCC (10 ⁻³ ,10 ⁻⁴)
S 3	PA2Da	-	Х	-	-	-
S4	QR1Da	S4/1	1	No	1	MBS (10 ⁻⁴)
S 5	QR3Db1	-	Х	-	-	
S6	PA1Db	S6/6	1	No	1	Chitin (10 ⁻¹)
S7	TC1D	-	Х	-	-	-
S8	PA1 Adult	-	Х	-	-	-
S9	AA2 Adult	-	X	-	-	-
S10	AA2D	\$10/1-20 \$10/25 \$10/34	22	No	22	Chitin and ATCC (10 ⁻¹)
S11	PS1D	-	Х	-	-	-
S12	PS1 Adult	S12/3	1	No	1	ATCC (10 ⁻¹)
S13	PS2D	-	Х	-	-	
S14	AA1D	S14/1	1	-	1	ATCC (10 ⁻²)
S15	FE1D	-	Х	-	-	-
S 16	QR2D	S16/2	2	No	2	Chitin (10 ⁻¹)
S17	PA1D	-	Х	-	-	-
S18	TC2D	(S18/1- S18/5)	12	yes	12	Chitin (10^{-1}) and ATCC (10^{-3})

Table 16: Summary table for the isolation and purification of the *Streptomyces*-like strains.

		S18/8 S18/9 S18/11 (S18/13- S18/15)				
		S18/17				
S19	QR3DB2	-	Х	-	-	-

4.2. Morphological characterization of the isolates

Based on the morphological characteristics, such as filamentous growth and formation of aerial mycelium (Figure 22), it was confirmed that samples S2, S6, S10, and some of the S18 isolates were *Streptomyces*-like strains. Isolate S16/2 was identified as an actinomyces-like strain. However, S4/1, S12/3, S14/1, S18/8 and S18/9 isolates exhibited red colonies with atypical morphology, which did not resemble actinomycetes (table 17). Furthermore, it was observed that the streptomyces-like isolates obtained from S2 were replicates of one strain, while the isolates obtained from S18 were replicates of a different strain. Most of S10 isolates had the same color of substrate mycelium, whereas only two isolates had different colors. Moreover, samples S6, S10 and S18 showed similar morphological characteristics.

Sample	Sample code	Code of	Color of	Color of	Pigment
number		isolate	aerial	substrate	produced
			mycelium	mycelium	
			(AM)	(SM)	
S2	QR3Da	S2/1	White (Wa)	Pale yellow	Yes
		S2/2		(Co4a)	
		S2/3		A DESCRIPTION OF	
		\$2/5			
			1		
S4	QR1Da	S4/1	Red	Red	No
S6	PA1Db	S6/6	White-light	Yellowish	Yes
			gray (Wa	brown	
			GY2ih)	(Coo2m)	

Table 17: The appearance of the aerial mycelium and Reverse side of plate showing substrate mycelium of the promising isolates.

S10	AA2D	S10/1 S10/2 S10/4-19 S10/25 S10/34	Gray (GY2fe)	Ochre (Coo4r)	Yes
		\$10/3 \$10/20	Gray (GY2fe)	Greenish brown (Co4s)	Yes
S12	PS1 Adult	S12/3	Red	Red	No
S14 S16	AA1D OR2D	S14/1 S16/1	Red Pale orange	Red Pale orange	No No
		S16/2			

S18	(S18/1-S18/5) S18/11 (S18/13- S18/15) S18/17	White-light gray (Wa GY2ih)	Brown (Coo3r-Co3r)	Yes
	S18/8 S18/9	Red	Red	No



S18/17 Isolate

S4/1 Isolate

Figure 22: The spore chains morphology of *Streptomyces sp.*(S18/17) versus the spores of non-actinomyces strain (S4/1) observed using light microscopy.

4.3. Genotyping of the promising strains

4.3.1. DNA extraction

The concentration and purity of the DNA of each isolate was measured spectrophotometrically to make sure that the DNA extraction of the isolates has taken place successfully and to adjust the concentration of the DNA used for the PCR as too high concentration may lead to inhibition of the DNA amplification.

The results showed that the A₂₆₀/A₂₈₀ ratio of the DNA samples ranged from 1.87 to 1.97 all samples had A₂₆₀/A₂₃₀ ratio around 2, except for isolates S10/25 and S18/5, which had relatively low absorbance at around 1.2 (Table 18).

Code of isolate	Concentration of	Absorbance	Absorbance		
	DNA (ng/µL)	(A260/A280)	(A260/A230)		
(82/1	104.3	1.97	2.23		
S6/6	377.0	1.93	2.09		
S16/2	288.7	1.93	2.12		
S10/3	232.8	1.92	1.87		
S10/15	174.3	1.92	1.95		
S10/20	115.7	1.91	1.62		
S10/25	122.3	1.90	1.21		
S18/1	137.2	1.90	1.66		
S18/5	62.8	1.87	1.21		
S18/8	202.6	1,90	1.96		
S18/15	245.4	1.92	1.95		
S18/17	104.5	1.89	1.73		

Table 18: The concentration and the absorbance of DNA samples.

4.3.2. Polymerase chain reactions and gel electrophoresis

• BOX-PCR for morphologically similar strains

Gel electrophoresis was used to represent the BOX-PCR results of morphologically similar isolates as the banding patterns can be analyzed to determine the genetic relatedness and diversity among the isolates. Each lane on the gel corresponds to a different isolate. Figure 23 shows that the banding patterns of amplified DNA fragments of the isolates are identical. PCR amplification was confirmed by successfully amplifying the DNA of the positive control. Moreover, no bands were observed for the negative control, indicating the absence of contamination in the master mix.

Ladder	1	2	3	4	5	6	7	8	9	10	11	Well number	Code of isolate
				-								1	S6/6
												2	S10/3
												3	S10/15
												4	S10/20
												5	S10/25
18. IS												6	S18/1
=												7	S18/5
- Second	•											8	S18/15
												9	S18/17
-												10	Negative control
												11	Postitive control

Figure 23: The amplified DNA fragments of the morphologically similar isolates visualized on a 1% agarose gel.

• 16S rDNA PCR

Gel electrophoresis was performed to confirm the successful amplification of 16S rDNA of the samples before sending them for sequencing. As depicted in Figure 24, the gel image demonstrates the presence of the bands at 1550 base pairs (bp), indicating that the amplification of the 16S rDNA has taken place successfully.

Ladder 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Well number	Code of isolate
AND THE REAL				ling - 1			T.					and in		Seal of	unicol all	1	S2/1
																2	S6/6
																3	S16/2
																4	S10/3
-																5	S10/15
-																6	S10/20
-			100						8							7	S10/25
			121													8	S18/1
	Ξ.	1						83								9	S18/5
																10	S18/15
100																11	S18/17
																12	Positive control
																13	Negative control
																14	S18/8
																15	Positive control
																16	Negative control

Figure 24: The amplified 16S rDNA fragments are visualized on a 1% agarose gel.

4.3.3. Identification of the strains using 16S rRNA

The sequences of the bacterial isolates were analyzed by nucleotide BLAST (blastn) to search for closely related species and the results are represented in Table 19. The table includes the code of isolate, the closest matching species identified by blastn, the percentage of sequence similarity, the accession number, and the E-value. The top matches for each isolate are listed, providing valuable information for the identification of the bacterial isolates.

Code of	Closest Matching	Identity %	Accession	E-value	type of isolate
Isolate	Species		number		
S2/1	Streptomyces flavogriseus strain CBS 101.34 Or Streptomyces flavovirens_strain NBRC 3716	99.86% 99.86%	NR_028988.1 NR_112509.1	0.00	Filamentous Actinobacteria
S6/6	Streptomyces flavogriseus strain CBS 101.34 Or Streptomyces flavovirens strain NBRC 3716	99.65% 99.65%	NR_028988.1 NR_112509.1	0.00	Filamentous Actinobacteria
S16/2	<i>Gordonia sputi</i> strain 3884 Or <i>Gordonia</i> <i>aichiensis</i> strain F9028	99.79% 99.64%	NR_037031.1 NR_037030.1	0.00	Non- filamentous Actinobacteria
S10/3	Streptomyces flavogriseus strain CBS 101.34 Or Streptomyces flavovirens strain NBRC 3716	99.65% 99.65%	NR_028988.1 NR_112509.1	0.00	Filamentous Actinobacteria
S10/15	Streptomyces flavogriseus strain CBS 101.34	99.72%	NR_028988.1	0.00	Filamentous Actinobacteria

Table 19:Results of blastn analysis for identification of bacterial isolates.

	Or				
	Streptomyces flavovirens strain NBRC 3716	99.72%	NR_112509.1	0.00	
S10/20	Streptomyces	99.79%	NR_028988.1	0.00	Filamentous
	flavogriseus strain CBS 101.34 Or Streptomyces	00.70%	NR_112509.1	0.00	Actinobacteria
	NBRC 3716	99.79%			
S10/25	Streptomyces flavogriseus strain CBS 101.34 Or	99.93%	NR_028988.1	0.00	Filamentous Actinobacteria
	Streptomyces flavovirens strain NBRC 3716	99.93%	NR_112509.1	0.00	
S18/1	<i>Streptomyces</i> <i>flavogriseus</i> strain CBS 101.34 Or	99.71%	NR_028988.1	0.00	Filamentous Actinobacteria
	Streptomyces flavovirens strain NBRC 3716	99.71%	NR_112509.1	0.00	
S18/5	Streptomyces flavogriseus strain CBS 101.34 Or Streptomyces	99.72%	NR_028988.1	0.00	Filamentous Actinobacteria
	<i>flavovirens</i> strain NBRC 3716_	99.72%	NR_112509.1	0.00	
S18/15	Streptomyces flavogriseus strain CBS 101.34 Or Streptomyces	99.86%	NR_028988.1	0.00	Filamentous Actinobacteria
	<i>flavovirens</i> strain NBRC 3716	99.86%	NR_112509.1	0.00	
S18/17	<i>Streptomyces</i> <i>flavogriseus</i> strain CBS 101.34 Or	99.91%	NR_028988.1	0.00	Filamentous Actinobacteria
	Streptomyces flavovirens strain NBRC 3716	99.91%	NR_112509.1	0.00	

S18/8	Methylobacterium tardum strain RB677	99.64%	NR_041443.1	0.00	Non- <i>Actinobacteria</i> (Proteobacteria)

Based on the results of the blastn analysis, it can be observed that most of the bacterial isolates have the same closest matching *Streptomyces* species, but with different percentages of identity. Out of all the isolates, only two do not belong to the *Streptomyces* species. Isolate S16/2 is classified as a member of the *Gordonia* genus, which is gram-positive *Actinobacteria*, and isolate S18/8 is identified as a gram-negative Proteobacteria known as *Methylobacterium tardum*.

4.4. Screening of isolates for antimicrobial activity

To assess the potential for bioactive compound production, the six BCCO strains and the confirmed *Streptomyces* strains isolated in this study were screened for antimicrobial activity against different bacteria and fungi.

4.4.1. Bioassay against Indicator microorganisms

The sporulation of the strains, hemolytic zone size and pathogen inhibitory zone size were recorded for each sample tested in the bioassay and represented in table 20.

Code of Isolate	Sporulation	Area of hemolytic zone (mm)	Size of inhibitory zone of β-hemolysis of <i>Staphylococcus</i> <i>aureus</i> (mm) Promising isolate (Vertical Line) <i>Staphylococcus</i> <i>aureus</i> (Horizontal Line)	Size of inhibitory zone of <i>Candida albicans</i> . (mm) Promising isolate (Vertical Line) <i>Candida albicans</i> (Horizontal Line)
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Table 20:Results of the bioassays of *Streptomyces* isolates and BCCO strains after 72 hours.

BCCO 10_1092	1	5		Weak partial inhibition
BCCO 10_1093	3	3	3	2
BCCO 10_1095	3	4	3	4
BCCO 10_1099	2	3	3	10
BCCO 10_1102	0	0	0	0
BCCO 10_1105	0	0	0	0
S2/1	0	0	0	0
S2/2	0	0	0	0

0				
S2/3	0	0	0	0
S2/5	0	0	0	0
S6/6	3	2	3 Stoph. Au	
S10/3	3	2		
S10/15	3	1	1	Partial inhibition
S10/20	3	1	1	Partial inhibition
S10/25	3	1		1
S18/15	3	2	3	1
S18/1	3	1	2	Partial inhibition

S18/5	3	1	2	Partial inhibition
S18/17	3	1	2	Partial inhibition

Based on the results of the bioassay, it was observed that all strains exhibited a hemolytic zone except BCCO 10_1102, BCCO 10_1105 and S2 isolates. The size of the hemolytic zone varied among the isolates, with isolate BCCO 10_1092 showing the largest zone (5 mm).

Moreover, the results showed that although all strains are not affecting the growth of *Staphylococcus aureus*, some of them exhibited inhibitory activity against β -hemolysis of *Staphylococcus aureus*.

Furthermore, none of the tested isolates exhibited any inhibitory effects against *Pseudomonas aeruginosa* (gram-negative bacteria). The results also show that all strains, except BCCO 10_1102, BCCO 10_1105, and S2 isolates, have inhibitory activity against *Candida albicans*. It was observed that the isolates with a hemolytic zone also exhibited an inhibitory effect against *Candida albicans*, whereas BCCO 10_1102, BCCO 10_1105, and S2 isolates also exhibited an inhibitory effect against *Candida albicans*, whereas BCCO 10_1102, BCCO 10_1105, and S2 isolates did not have a hemolytic zone or inhibitory effect.

The most promising isolates (S6/6, S10/3, S10/25, and S18/15) were selected, and the bioassay was repeated at different incubation periods of the isolates to determine the optimum time required for the potential isolates to produce secondary metabolites.

Table 21: Table 21: Results of the bioassays of the potential Isolates after 72 hours. The strains
were incubated for 3-6 days before adding Staphylococcus aureus and Candida albicans (same
plate).

Code of Isolate	Incubation period of the isolates	Size of hemolytic zone (mm)	Promising isolate (Vertical Line) <i>Staphylococcus aureus</i> (Horizontal Line)	Size of inhibitory zone of β- hemolysis of Staphyloco ccus aureus (mm)	Promising isolate (Vertical Line) Candida albicans (Horizontal Line)	Size of the inhibitory zone of CAAL. (mm)
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S6/6	2 days	1	STAU	2	Weak partial inhibition
S6/6	3 days	2	Staph. Au	4	1
S6/6	4 days	3	S TAU-	9	Partial inhibition
S6/6	5 days	3	STAU	12	12
S6/6	6 days	4	570-	Complete inhibition	Complete inhibition
S10/3	2 days	4	STAU	2	Partial inhibition
S10/3	3 days	4	StoPh-AN	1	1
S10/3	4 days	5	Stoph-AU	23	Complete inhibition

S10/3	5 days	5	STRE	13		20
S10/3	6 days	6	E.	Complete inhibition	Storg	Complete inhibition
S10/25	2 days	1	Ship. Au	0		0
S10/25	3 days	2	Str. Berrie	0		0
S10/25	4 days	3	STAU	6		Partial inhibition
S10/25	5 days	3	STRU	6		Partial inhibition
S10/25	6 days	3	STAU	23		Complete inhibition

S18/15	2 days	2	Staft. Au	0		Partial inhibition (Weak inhibition)
S18/15	3 days	2	Stept. AU	0		Partial inhibition
S18/15	4 days	3	H I	6		Partial inhibition
S18/15	5 days	3	STR	11		Partial inhibition
S18/15	6 days	3	STAU	Complete inhibition	SIBJIS	Complete inhibition

The results showed that the isolates have a significant inhibitory effect against *Candida albicans* and inhibition of the β -hemolysis of *Staphylococcus aureus* after 4 days of incubation and the highest inhibitory effect was observed on day 6.

To test if the inhibitory effect is due to the secondary metabolite produced by the *Streptomyces* strains only or it is a synergistic effect, the bioassay will be repeated at which both pathogens (CAAL & STAU) will be inoculated separately against S10/3 after 3-6 days of incubation and

STAU only will be inoculated against the other isolates (S6/6, S10/25 & S18/15) after 3-6 days of incubation.

Table 22: Results of the bioassays of the potential Isolates after 72 hours. The strains were inoculated on blood agar and incubated for 3-6 days before adding *Staphylococcus aureus* and *Candida albicans* (different plates).

Code of Isolate	Incubation period of the isolates	Size of hemolytic zone(mm)	Promising isolate (Vertical Line) <i>Staphylococcus aureus</i> (Horizontal Line)	Size of inhibitory zone of β-hemolysis of STAU. (mm)	Promising isolate (Vertical Line) <i>Candida albicans</i> (Horizontal Line)	Size of inhibitory zone of CAAL (mm)
S6/6	3 days	2	STAU	1		Weak partial inhibition
S6/6	4 days	3	STAU	6		Partial inhibition
S6/6	5 days	4	STAU	12		4
S6/6	6 days	5		20		16
S10/3	3 days	4	STAU	4		Weak Partial inhibition

S10/3	4 days	5	STAU	16	CHAL	19 (Partial inhibition)
S10/3	5 days	5	STAU	18		21
S10/3	6 days	6	STAU	Complete Inhibition		23
S10/25	3 days	3		1		Weak partial inhibition
S10/25	4 days	3		6		1
S10/25	5 days	4	STAD	Complete inhibition		5
S10/25	6 days	4	STAU	10		15
S18/15	3 days	2	STAU	1		Weak partial inhibition

S18/15	4 days	3	STAU	6	1
S18/15	5 days	3	STAU	12	3
S18/15	6 days	4	STAU	Complete inhibition	15

The results demonstrated that the inhibitory zone of *Candida albicans* is significantly larger when both *Streptomyces* and *Staphylococcus aureus* were present on the same plate, compared to when only *Streptomyces* was inoculated.

4.4.2. Bioassay against Bacillus subtilis

The results showed that none of the tested isolates exhibited any inhibitory effects against *Bacillus subtilis*, as evidenced by the absence of a halo zone (Table 23).

Table 23: Results of	of inhibitory	activity	against	Bacillus	subtilis.
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Code of Isolate		Code of Isolate	
Control	Contro Biss 6.	S12/3	512.13 Ja-1-, Jun 23



4.4.3. Antifungal activity test

The inhibition zone of fungal strain CCF 4450 was measured and recorded after three weeks, while the inhibition zones of the other fungal strains were measured after four weeks. This difference in measurement time was due to the relatively faster growth rate of CCF 4450 compared to the other fungal strains.

Code of fungal strain	Strain Identification	Strains B1, B2 and B3	Strains B4 and B5	Strains B6 and B7
CCF 4450	Ophiostoma minus	Inhibition zone B1: 2 mm B2: 13 mm B3: 0 mm	Inhibition zone B4: 1mm B5: 3 mm	Inhibition zone B6: 1mm B7: 2 mm
CCF 6041	Akanthomyces muscarium	Inhibition zone B1: 3 mm B2: 6 mm B3: 2 mm	Inhibition zone B4: 2 mm B5: 2 mm	Inhibition zone B6: 2 mm B7: 2 mm
CCF 3535	Graphium asporum	Inhibition zone B1: 6 mm B2: 8 mm B3: 0 mm	Inhibition zone B4: 2 mm B5: 2 mm	Inhibition zone B6: 2 mm B7: 2 mm
CCF 3546	Ophiostoma piceae	Inhibition zone B1: 3 mm B2: 7 mm B3: 0 mm	Inhibition zone B4: 6 mm B5: 2 mm	Inhibition zone B6: 2 mm B7: 2 mm

Table 24: Results of the inhibitory activity against fungi obtained from different areas.

The results show that BCCO 10_1099 (B1) and BCCO 10_1104 (B2) strains, which were previously collected from Papua New Guinea, demonstrated moderate and strong inhibitory effects, respectively, against the four fungal strains.

The BCCO 10_1106 (B3) strain that was collected from Morocco, exhibited a weak inhibitory effect against fungal strain (CCF 6041), while it has no inhibitory activity against the other three fungal strains.

Moreover, the four isolates S6/6 (B4), S10/3 (B5), S10/25 (B6) and S18/15 (B7) that were collected from the Czech Republic, have a weak inhibitory effect against all fungal strains.

4.5. Genome Screening of antifungal polyenic secondary metabolites.

4.5.1. Data mining from polyene databases

Robust dataset of 26 linear and cyclic polyenes (macrolactone and macrolactam compounds) was created and used for the analysis of cytochrome P450 (CYP 450) and thioesterase type I (TE I) genes. They are listed in groups based on their structures together with bacterial strain, classification, database, and biological activity (Table 25).

Table 25: List of polyene secondary metabolites produced by *Streptomyces sp.* and phylogenetically close actinomycetes that were used in this study.

Compound	Bacterial strain	Classification	Biological activity	Database	Used for analysis of CYP 450	Used for analysis of TE
Macrolactone						
Amphotericin	Streptomyces nodosus-ATCC 14899	Heptaenes	Antifungal	JDB	\checkmark	\checkmark
Candicidin	Streptomyces sp. FR-008	Heptaenes	Antifungal	Mibig	\checkmark	\checkmark
Filipin	Streptomyces avermitilis MA- 4680	Pentaenes	Antifungal and low anti- bacterial activity	Mibig	\checkmark	~
Nystatin	Streptomyces noursei ATCC 11455	Tetraenes	Antifungal	JDB	√	\checkmark
Pimaricin	Streptomyces Natalensis ATCC-27448	Tetraenes	Antifungal and anti- protozoal	JDB	√	\checkmark
Rimocidin	Streptomyces diastaticus 108	Tetraenes	Antifungal	JDB	√	
Reedsmycins	<i>Streptomyces</i> Sp. OUC6819	Pentaenes	Antifungal	Mibig		\checkmark
Eurocidin-D	Streptomyces eurocidicus ATCC 27428	Pentaenes	Antifungal	Mibig		\checkmark
Lucensomycin	Streptomyces achromogenes_ NBRC-14001 Streptomyces cyanogenus	Tetraenes	Antifungal and antiviral activity.	Mibig		\checkmark

		1			1	
Tetramycin	Streptomyces hygrospinosus CGMCC 4.1123	Tetraenes	Antifungal	Mibig		\checkmark
	Pseudonocardia					\checkmark
NPP	<i>-sp.</i> AL041005-10	Tetraenes	Antifungal	NCBI		
	Streptomyces					\checkmark
pentamycin	sp. \$816	Pentaenes	Antifungal	NCBI		
Macrolactam						
Auroramycin	Streptomyces filamentosus strain NRRL	Pentaenes	Antifungal, Anti- MRSA	Mibig NCBI		\checkmark
	15998					
ML-449	Streptomyces sp. MP39-85	Tetraenes	Anti- bacterial, Cytotoxicity	Mibig		\checkmark
BE-14106	Streptomyces sp. DSM-21069	Tetraenes	Anti- bacterial, Cytotoxicity	Mibig		\checkmark
Sipanmycin	Streptomyces sp. CS149	Pentaenes	Cytotoxicity	Mibig		\checkmark
Hitachimycin	Streptomyces scabrisporus CMB-0406	Triene	Anti- protozoal antifungal	Mibig		\checkmark
Heronamide_C	Streptomyces sp. CMB-0406	Tetraenes	No anti- bacterial activity and cytotoxicity	Mibig		\checkmark
_	Streptomyces	Pentaenes		<u> </u>		\checkmark
Sceliphrolactam	sp.SD85		Antifungal	NCBI		•
Bombyxamycin A	Streptomyces sp.SD53	Pentaenes	Anti- bacterial, cytotoxicity	NCBI		\checkmark
cremimycin	Streptomyces sp. MJ635-86F5	Triene	Anti- bacterial (G+), cytotoxicity	NCBI		\checkmark
Linear						
	Streptomyces					\checkmark
Linearmycin A	sp. Mg1		Antifungal	Mibig		
• •	Streptomyces		¥	Ŭ		\checkmark
Mediomycin A	blastmyceticus		Antifungal	Mibig		-
	Streptomyces			<u> </u>		\checkmark
Neomediomycin	sp. RK95-74		Antifungal	Mibig		-

4.5.2 Analysis of Cytochrome P450

Amino acid sequences of cytochrome P450 from 6 polyene biosynthetic gene clusters (BGCs) were used for multiple sequence alignment as depicted in Figure 25. The majority of those polyene BGCs contain two cytochrome P450 genes, with the exception of Candicidin and Rimocidin BGCs.

		10	20	1	30	40	50	0	0 ;	70	80	90	100	110	120	130	140
Amphotericin-8 CYP450 S.nodos(AmphLV1-394					WVNPTPP	PSLEDAAP	SVLRLSPL	LRELQMRA	PUTKIRTPA	DEGWLVTR	AELKOLLH	DERLARAHA	P-ANAPRY	VKSPLMDLL	10 - DVEAARA	AHAELRTLL	POFSARRY
Amphotericin-8_CYP450_S.nodos(AmphN)/1-398			M	TAETEMT	TFAPGCP	VAFPLERP	GRPFPPPE	YADYRAGE	GLVRSELPA	SIGPVWLVTR	EDVRTVLT	DPRISADPSI	R <mark>PGFP</mark> RARR	TGGAPSQSEI	GWEVALDPP	EHDRFRKTL	IPEFTVRKV
Candicidin_CYP450_S.spFR-00/1-393				MT	TSPGPTV	VDFPRRTP	REPLPLSQ	YAEHRKON	GLVQTHLPN	9 RP I WLVTRI	HEDVRAVL T	HPRISANPDI	NEGFPNVGE	TMGVPKQEQI	PGWFVGLDSP	EHDRFRKVL	IPEFTVRRV
Filipin_CYP450_S.avemitilio(pteC)/1-393				M	PEPTADA	PTVPKARS	CPFLPPDG	IADIRAAA	PVTRATFTS	G HEA <mark>WLVT</mark> G	YEEVRALLR	DSSFSVQVPI	HALHTODOV	VTQKPGR	GSLLWODEP	EHTSDRKLL	AKEFTVRRM
Filipin_CYP450_S.avemitilis(pteD)/1-402				- MTETEII	RLTGSPA	PSFPQDRT	CPYOPPKA	YEERRGES	PLTQVTLFD	S RPANLITG	AEGRALLV	DPRLSSDWG	H P D F P V V V R	RTEDRGGLAF	LIGVDOP	VHARORRML	I P S F G M K R M
Nystatin_A1_CYP450_S.nodosus(NysL)/1-393	*******				MSTPTAP	PSLKAEVP	PVLRLSPL	LRELQSRA	PVCKVRTPA	BDEGWLVTR	TELKOLLH	DDRLARAHA	DP - ANAPRY	VHNPFLDLLV	D-DFDLART	LHAEMRSLF	TPOFSARRV
Nystatin_A1_GYP450_S.nodosus(NysNy1-397			· · · · · M	STEADAR	TAAPUCP	VAFPLERP	GRPFPPPE	TATYRGGA	GLVRSELPS	GPVWLVTR	EDVRAVLT	DPRISADPSI	KPGFPKAGR	TGGAPSQYEV	GWEVAMDPP	ENGRERKT	IPEFTVRKV
Piearioin_CVP450_S.natalensis/PieGV1-396 Piearioin_CVP450_S.natalensis/PieGV1-398				TYTOPAAI	PETDPPA	VDEPORKP	GVPEPPPD	YADYRDRK	GLVI SOL SD	DEANLVIR	ALLKULLH	DERIGRINPI	PP-PSAAUT	VKSPFEDLE	SDADAESGRR	CHARTNELL	I PLF SARKV
Rimocidin CYP450 S.diastaticu/1-420	MTHTEPAA	PATCPVT	GATAGAT	DTTDSTG	HGTDPLV	VDFPLRAP	GIPFPPPE	YADYRDKK	GLVLSHLPD	G KRUM UTR	FOURAULT	NPAISSNPDI	HPGEPNVGE	TIGVPRODOLI	OWFVGMDSP	EHORFREAL	PESTURRU
							and the second de	1			C. C		a contraction of the		CHI COMPOL		
	150	J .	160	170		180	190	200	210	220	2	230	240	250	260	270	280
Amphotericin-B_CYP450_S.nodos(AmphL)/1-394	LNMMPMVE	GIAEQIL	NGFAAQE	OPADLRGI	NFSLPYS	LTVLCALI	GIPLOEDG	QLLAVLG -	- EMATLNDA	SVARSOAK	FOLLTO	AGRKRAEPGI	DVISRUCE	T-VPEDERIG	PLAASLEFAG	LDSVATHVD	LOVVLETOY
Amphotericin-8_CYP450_S.nodos(AmphN)/1-398	RELEPAID	QIVDERI	DALLAAG	NSADLIA	DFALSVP	SLVISDLL	GVPKADRD	FFEAKTKV	LVTLSS-TD	QRDEASKA		IQIKGRRPG	DLISRLLO	AGTMNROELS	VSMLLLIAG	HETTANNIG	LOVVQLLTN
Candicidin_CYP450_S.spFR-00/1-393	RELRPAIE	RTVDERI	DAML AGG	NTADLVNI	FALPVP	SLVISALL	G V P SAD R D	FFESRTRT	LVAIRTSTD	ERAEATRO				TOKLSDEELS	VILLEIAG	HETTANNIG	LOVVTLLSH
Filipin_CYP450_S.avemitilis/pteCJ/1-393	QALRPNIC	RIVDEHL	DAIEARG	G P V D L V K	FANAVP	SMVISDLF	GVPVERRA	EFQDIAEN	RVDQDAAAT	AAG MR	LGGLLYOL	VQERRANPG	DULISALIT	TGVVDDMFLM	NAAGTLLIAA	HDTTACMIG	LOTALLLDS
Filipin_CYP450_S.avemitilis/pteDy1-402	NAIRPRLO	SLVDRLL	DDMLAKG	PGADLVS/	AFALPVP	SVALCELL	<mark>g v p</mark> ygdhd	FFEECSRV	GAATSAEAD	A AFGELYTY	HGLVGRK	AEPEDGLL	ELLAROLE	EGOLDHDEVVI	ALVLLVAG	HETTVNALA	LGALTLIGH
Nystatin_A1_CYP450_S.nodosus(NysL)/1-393	MDLTPRVE	ALAEGVL	AHFVAQG	PPADLHN	DFSLPFS	LSVLCALI	GVPAEEQG	KLIAALT-	- KLGELDDP	A RVQEGQDE	FGLLSG	ARRKRITPE	DOVISELCL	K-VPSDERIG	PIASGLLFAG	LDSVASHID	LGTVLFIQH
Nystatin_A1_CYP450_S.nodosus(NysN)/1-397	RELRPVIO	QIVDERI	DAMLAAG	TSADLVE	SFALPVP	SLVISSLL	G V P K V D R D	FFEDRTRV	LVRLSS - TD	ERDKATQA	LLRYLGRL	IQIKORRPGI	DULISELIA	AGTLSROELS	VAML LL I AG	HETTANNIG	LOVVQLLTN
Pimaricin_CYP450_S.natalensis(PimD)/1-396	LEMQPKVE	EAADTLL	DAFIAQG	PPGDLHG	ELTVPFA	LTVLCEVI	G V P P Q R R A	ELTTLLA-	-GIAKLDDR	GAVRACDD	FGYVAGL	VEHKRAEPG	DISREND	G-ELTEDRVA	HLAMG <mark>LL</mark> F <mark>AG</mark>	LDSVASIMD	NOVVLLAAH
Pimaricin_CYP450_S.natalensis(PimG)/1-398	RAMKPATE	RTVDAQL	DAML AAG	NTADLVA	DFALPIP	SLVISALL	G V P P A D R E	FFESRTRV	LVSLRSSTD	D RMAAAKD	LLRYINRL	VEIKQKWGGI	DLITRLLA	TGALAPHEMS	OVLME <mark>LE</mark> I AG	HETTANNIA	LOVVILLAN
Rimocidin_CYP450_S.diastaticu/1-420	RAMKPAIE	RTVDAQL	DAMLAAG	NTADLVA	DETLPIP	SLVISALL	GVPPADRE	FFESRTRV	LVSFRAYSD	DRLAAGKD	LMRYINRL	IEIKKNWGGI	DIVTRULA	TGAIGAHEMS	GVLML <mark>LL</mark> IAG	HETTANNIA	LGVVTLLKN
	290	300	3	10	320	330	340	35	0 36	0	370	380	390	400	410	4	20
Anabolasina CVR/50 Sandar/Anabi V1.394	OI VEALAD	EKINDSG	COL BAA	VACCASI	EVETOR	FLADUTER		TINNENEA	EDDADI COLI	SPARMIT	FORMAN	INAPLARU	MENTANTO	THE ROLL	ASSUEELO	TSGOL NG	TREPUTE
Anobatedala 2 CV24E0 CandeallashE0/1309	WIGOOD	C.L.M.		CUARINCE	RVAUERU	ELCONT IN	ACCA INDI I	ARABUDAD	Chuscoup	e case uu	CO VO VHO	LOONLYRY	CUCIAVOT		AVEVEELO	VYAAUI CA	
Amprovencin-6_017400_3.nodospenprinty 1-308	WIGDDR.			OUNDERST	RVAVEDV	CLASS TO	COLVELL	AAAAAA TE	VED COULT NO	TCODUUS	COVENING		ENELANDY		AVECAOLAY	VACING A	
Canalalain_C17400_3.pb_FR-0013-393	Rewigook		VEELCHLH	SVADMVAL	HVMVDUV	ELABUL R	KOEGIVPLL	ROANHUIE	APOUTHATNE	TEALAY		LUGREVAY	ENEININ		AVECUULA	TATUGILI	CHELF SAM
Filipin_CYP450_S.avernitilispteCy1-393	QUALLRED	PSLVGNA	VEELLRYL	TIGOFOGE	RVATRDV	ELGOVRIA	KGEQVVAHV	LAADFOPA	VEEPERFOI	REAPHLA	TO TO ANOT	TOUCLANT	ELQIVEEI	LINKE FOLKI	ANAVEELKI	REQMYFTO	VHELPVIN
Filipin_CYP450_S.avemitilis(pteD)/1-402	BOIDVLLRD	PGAVSGV	VEELLRFT	SVSDHIVR	- MAKED I	EVGGAT K	AGDAVLVSI	TLMNRDAK	AYENPOILDA	RR NARHHVO	FOHOLHO	LOQNLARA	ELEIALGG	LEARIPGER	AVPLDEVP	KAGHDAQG	PIELPVVW
Nystatin_A1_CYP450_S.nodoaus(NysL)/1-393	QLAAALAD	EKLMRGA	VEEILRSA	KAG - SVLP	RYATADV	PIGDVTR	AGOLVLLDF	TLVNFDRT	VFDEPELFDI	R APNPHLI	FOHOMNH	IGAP APV	NERTAYTE	LFTRLPGLR	VRPVEELR	LSGQLSAG	LTELPVIN
Nyatatin_A1_CYP450_S.nodoaua(NyaN)/1-397	WIGDDR		VEELLRYY	SVADLVAF	RVAVEDV	ELGOQLER	AGEGIVPLI	AAANHDAT	AFAAPSEFDR	R SARSHVA	FOYOVHO	LOQNLVRE	EMDIAYRT	LFARIPSLT	AVPVEELPI	KYDGVLF	LHELPVTW
Pinaricin_CYP450_S.natalenzis(PinD)/1-396	QRAAALAD	PDVMARA	VEEVLETA	RAGGVLPP	RYASEDN	FGGVTR	AGDLVLFOL	GLPNFDER	AFTGPEEFDA	TPNPHL1	FGHG IWH	IGAP LARL	ELRTMFTK	LFTRLPELRE	ELPVEOLRI	L KEGQL SG	FAELRVVW
Pimaricin CYP450 S.natalenzis/PimGV1-398	WIGDDR	A	VEETLRFH	SVADLVSL	RVAVODV	ELAGOLIK	AGEGIVPLV	AAANHDEN	AFECPHAFDE	SARHAVA	FOYOVHO	LOONLVR	EMEVAYRK	LFERIPALE	AVPTOGLO	KYDGVLYD	LNELPVRW
Rimonidia CVP450 S diastation/1-420	WIGDER		FETLREN	SVADLVSI	RVAVEDU	FLAGON	AREGIVELV	AAANHOEE	FACEHALDE	SARGHUA	FOYOVHOL	LOONLYEY	EMEVAYRK	LECRIPALR	OVPEDGEN	KYDGVLY	HELPVRM
tamonaui fatt. 266 Statamaneat 1.468,	Autowerg.		and the second second	ALUELAS	THE REAL	CORRECT OF	AN A REAL	DOUBLE E	STORE OF SEC	a strain a strain			States a subscription			-	

Figure 25: Multiple sequence alignment of the amino acid sequences of cytochrome P450. The highly conserved amino acid residues were highlighted in dark blue, and the sequences used for designed primers in a previous study are indicated with black boxes.

The results demonstrate the presence of the regions where the amino acid residues are highly conserved across the CYP sequences of the six-polyene BGCs. Based on the alignment, a phylogenetic tree was constructed to determine the similarity of the sequences among the CYPs from different BGCs (Figure 26).



Figure 26: Phylogenetic tree of CYP450 gene extracted from BGCs coding for the 6 different polyenes.

This analysis showed that cytochrome P450 genes encoded by the polyene clusters can be classified into two distinct similarity groups within the constructed tree. As shown in Figure 26, the first group consists of PimG, AmphN, NysN, PteD, RimG and fscP whereas the second group includes PimD, AmphL, PteC and NysL.

Upon searching for more polyene biosynthetic gene clusters (BGCs) for further analysis of cytochrome P450 (CYP) sequences, it has been discovered that certain polyenes, such as Reedsmycin (a cyclic polyene), and linear polyenes like Linearmycin-A, Mediomycin-A, and Neomediomycin-B, do not possess the genes encoding for cytochrome P450 as well as ferredoxin.

Therefore, the alternative hypothesis was formulated, and the dataset was expanded by including an additional 20 polyene and 2 non-polyene biosynthetic gene clusters (BGCs).

4.5.3 Analysis of Thioesterase type I

Since thioesterase type I is associated with the acyl carrier protein (ACP) of the last module of type I polyketide synthase, the nucleotide sequence of the whole module was downloaded. The analysis of the nucleotide sequences of the last module of type I polyketide synthase that associated with thioesterase type I revealed that there is only one starting codon and one stop codon, which means that the genes within the module cannot be synthesized individually but rather as a whole module. The nucleotide sequences of the last module of 26 polyenes and 2 non-polyenes were translated and the amino acid sequences were used for multiple sequence alignment. Then the aligned amino acid sequences of thioesterase type I (TE I) and acyl carrier protein (ACP) were extracted.

Then the hypothesis was tested by analyzing the sequence similarity of the gene responsible for encoding TE I within the dataset. The results of the multiple sequence alignment of thioesterase gene were compared to the results provided by Zhou, Yucong, et al [29].

	10	20	30	40	
Pimaricin_ACP-TE-S.chattanoogensis_L-10(ScnS4)/1-363	тр	V <mark>V</mark> RTEV <mark>AAVL</mark>	GHASAKNVDARR	EFYELGFDS	LTS
Amphotericin B ACP-TE-S.nodosus ATCC-14899(AmphKV1-373	LRTLDTAGREKLLTE	L <mark>V</mark> V G F T <mark>A</mark> G L L	GHADPAAVDPER	GFLELGFDS	LVS
Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(NysK)/1-361	L I D	L <mark>v</mark> vgyt <mark>a</mark> gl <mark>l</mark>	GHPDPTAVDPER	GFLELGFDS	LVS
Candicidin_ACP-TE_S.spFR-008f/scFy1-376	E	T <mark>V</mark> VAC <mark>AA</mark> AL <mark>L</mark>	GHTDTSEIDPDR	DFLELGFDS	LIG
Filipin ACP-TE S.avermitilis MA-4680(pteA5)/1-371	E	H <mark>V</mark> RAQSAVVL	GHASAEDVLPTA	HFLELGFDS	LTA
Pimaricin ACP-TE S.natalensis ATCC-27448(Pim S4)/1-364	MTD'	V <mark>V</mark> RTE <mark>AA</mark> AVL	GHASSQNVDARR	EFYELGFDS	LTS
Reedsmycins ACP-TE S.Sp OUC6819(rdmJ)/1-366	EDERFQRILR	L V RETVAQL L	GYPDVDSVTADR	GFVELGLDS	LAT
Eurocidin D ACP-TE S.eurocidicus ATCC-27428/1-373	LREAPEADRDRRIVD	M <mark>v</mark> rae <mark>aa</mark> gvl	GHASADAVEAHR	DFFELGFDS	LTS
Lucensomycin_ACP-TE_S.achromogenes_NBRC-14001(LucE)/1-372	LSGLSAADQETVLGE	LITDYAAAVL	GHADASDMDPER	DFLEAGFDS	LMS
Lucensomycin ACP-TE.S.cyanogenus(LcmE)/1-372	LSGLSAADQHTVLCE	LIVDY <mark>aa</mark> avl	GHADASGMDPER	DFLEAGFDS	LMS
Tetramycin B ACP-TE S.hygrospinosus CGMCC-4.1123(TetrE)/1-360	D	L <mark>V</mark> RTE <mark>AA</mark> AVL	GHASPQGIETQR	EFLELGFDS	LTS
Pentamycin_ACP-TE_S.sp.S816/1-385	LARLEPAEREEKLLE	HIRSQ S <mark>a</mark> vvl	GHESAEDVLPTA	HFLELGFDS	LTA
Selvamicin_ACP-TE_Pseudonocardia_sp.HH130630-07/1-376	LRGLDADAREELLRE	L <mark>V</mark> ISRA <mark>A</mark> SVL	GHTDTTAIDPRQ	EFLSLGFDS	LVA
NPP_A1_ACP-TE_Pseudonocardia_sp.AL041005-10(CPPK)/1-376	- HRLDAAGREALLVE	L <mark>V</mark> TGYT <mark>a</mark> gl <mark>l</mark>	GHEDAAAVDPER	GFLELGFDS	LVA
Auroramycin_ACP-TE_S.roseosporus_NRRL-15998/1-371	LAGLTAEQLDKAMEE	LVLEH <mark>a</mark> gal <mark>l</mark>	GYGDNETIDPER	HFLESGFDS	LTA
BE-14106_ACP-TE_S.sp.DSM-21069(becG)/1-371	LAAMPAQQRERALSD	LVLSL <mark>AA</mark> SV <mark>L</mark>	GHADAEAVDPSR	DFLESGFDS	LTA
Bombyxamycin_A_ACP-TE_S.sp.SD53(bomP1)/1-377	- AQADEAGQEALLME	LVLDR <mark>AA</mark> TL <mark>L</mark>	GYSSADALDAQR	DFLESGFDS	LSA
cremimycin_ACP-TE_S.sp.MJ635-86F5(cmiP6)/1-358	H	LVLAE <mark>AA</mark> AL <mark>L</mark>	GHRGPEDVDADR	GFLESGFDS	LTA
Heronamide_C_ACP-TE_S.sp.CMB-0406(hm G)/1-371	LAAMPADQRERALSD	L <mark>V</mark> LSLAASVL	GHSGPEAVDPAR	DFLESGFDS	LTA
Hitachimycin_ACP-TE_S.scabrisporus(hitP3)/1-381	LAGLSETEQQRETRT	L <mark>V</mark> LAH <mark>AA</mark> QVL	GYDDPAAVDADR	TFLESGFDS	LGA
ML-449_ACP-TE_S.sp /1-371	LAAMPADQRERALSD	L <mark>V</mark> LSLAASVL	GHSGPEAVDPAR	DFLESGFDS	LTA
Sceliphrolactam_ACP-TE_S.sp.SD85(SceT)/1-373	LAAAGPAAQEELLTG	L <mark>v r a r t a</mark> a v l	GHAGPEEIDPES	G F L E A G M D S	VSA
Sipanmycin_ACP-TE_S.sp.CS149(Sip-P5)/1-371	LAGLTAEQLDKAMEE	LVLEH <mark>a</mark> gal <mark>l</mark>	GYGDNETIDPER	HFLESGFDS	LTA
Linearmycin_A_ACP-TE_S.sp.Mg1(InyHI)/1-369	LSGLPEEEQERVLLE	V <mark>V</mark> RTT <mark>AA</mark> AVL	AYPS <mark>P</mark> D <mark>AV</mark> GESQ	QFLELGLDS	LTA
Mediomycin_A_ACP-TE_S.blastmyceticus(med 9)/1-356	E	L <mark>v r tq aa</mark> v v L	<mark>g</mark> fadadaigvg <mark>r</mark>	GFLE <mark>M</mark> GFDS	LTA
Neomediomycin_A_ACP-TE_S.sp.RK95-74(nmd9)/1-366	LAGLDEAEREKVLLD	L <mark>v</mark> rshaavvl	<mark>G</mark> FEDPEAVGATR	GFLELGFDS	LTS
Pikromycin_ACP-TE_S.venezuelae(pikAIV,Non-polyene)/1-379	RSAVLA	M <mark>v</mark> mrq <mark>aa</mark> svl	RCDSPEEVPVDR	PLREIGFDS	LTA
Erythromycin_ACP-TE_Saccharopolyspora_erythraea(Non-polyene)/1-363	APAREM TSQELLE	FTHSHV <mark>a</mark> ai <mark>l</mark>	GHSSPDAVGQDQ	PFTELGFDS	LTA

			60	70		80
Pimaricin ACP-TE-S chattanooriensis 1-10/Scn S4V1-363	VEL	RNRL	STUTO	HLSATVVE	SKTPTD	
Amphotericin B ACP-TE-S.nodosus ATCC-14899(AmphKV1-373	VSL	RNQL	GELLG	LRLPTSVVF	DSKTPVKL	ARHLNEELGDL
Nvstatin A1 ACP-TE S.nodosus ATCC-11455/NvsKV1-361	VGL	RNQL	AEILG	LRLPSSIVE	DSKSPVKL	ARWLHQELANG
Candicidin ACP-TE S.sp. FR-008/fscFV1-376	IEL	RRKL	SEITG	LQLPASIVY	DSGSPNGL	TAWLRTELGAQ
Filipin ACP-TE S.avermitilis MA-4680(pteA5)/1-371	IDL	RRRL	SAATG	LRLPSGLAF	DHPTPARL	AKHLLTRLQST
Pimaricin ACP-TE S.natalensis ATCC-27448(PimS4)/1-364	VEL	RNRL	STVTG	LRLSATVVF	DSKNPTDL	AARLYADLAAQ
Reedsmycins_ACP-TE_S.Sp_OUC6819(rdmJ)/1-366	VEL	RNAL	AKTTT	VRLTAKLIF	ERNTPDLL	ARYLHEELLAD
Eurocidin_D_ACP-TE_S.eurocidicus_ATCC-27428/1-373	VEL	RNRI	ASVTG	LQLPATVVF	D N R N P E N L	AAWLRSELAAQ
Lucensomycin_ACP-TE_S.achromogenes_NBRC-14001(LucE)/1-3	372 VEL	RNRL	ADTVG	LRLPGTVVL	DHKTPAEL	ARWLRGQLAEH
Lucensomycin_ACP-TE.S.cyanogenus(LcmE)/1-372	VEL	RNRL	AGTV <mark>G</mark>	LRLPSTVVL	DHKTPAEL	ARWLRGRLADH
Tetramycin_B_ACP-TE_S.hygrospinosus_CGMCC-4.1123(TetrE)/	1-360 VEL	RNRL	AAATG	LQLPATVVF	ENR <mark>T</mark> TEK <mark>L</mark>	ATWLDGELVAR
Pentamycin_ACP-TE_S.sp.S816/1-385		RRRL	SAATG	LRLPAGLAF	DHPTPARL	ARHLLTLLRGA
Selvamicin_ACP-TE_Pseudonocardia_sp.HH130630-07/1-376	VEL	RNHL	AGELD	LTLPASVVF	DNETPDRL	ASWLHEELAGH
NPP_A1_ACP-TE_Pseudonocardia_sp.AL041005-10(CPPK)/1-37	76 <mark>VG</mark> L	RNQL	GEVLG	L R L AG S I V F I	DSTTPVKL	ARRLHELVEQA
Auroramycin_ACP-TE_S.roseosporus_NRRL-15998/1-371	VEL	RNGL	NAATG	LRLSATVAFI	D H Q T P G G L	ARHMAAQIGAV
BE-14106_ACP-TE_S.sp.DSM-21069(becG)/1-371	MEL	RTAL	IAATG	AKLPTMAVF	O S K T P A N L	ARLLADEMESG
Bombyxamycin_A_ACP-TE_S.sp.SD53(bomP1)/1-377	MEL	RNAL	MKDTG	LRLPPMVVF	OSKSPTEL	ARLLRTDLLAA
cremimycin_ACP-TE_S.sp.MJ635-86F5(cmiP6)/1-358	TQL	RSRI	NAATG	LRLPPMVVF	OSKTPARL	VQHIRTQWHTG
Heronamide_C_ACP-TE_S.sp.CMB-0406(hmG)/1-371	MEL	RTAL	SAATG	VKLPTMAVF) SKNPANL	ARLLVAEMESG
Hitachimycin_ACP-TE_S.scabrisporus(hitP3)/1-381	тоц	RAAL	NAATG	LRLPAMVVF	DSKTPAEL	ARLVGAELAAS
ML-449_ACP-TE_S.sp /1-371	MEL	RTAL	SAATG	VKLPTMAVF	DSKNPSNL	ARLLVEEMESG
Sceliphrolactam_ACP-TE_S.sp.SD85(SceT)/1-373	TEL	RHAL	AGATG	LTLPAGVVF	EHGTPAAL	AAHLRTRLADG
Sipanmycin_ACP-TE_S.sp.CS149(Sip-P5)/1-371	VEL	RNGL	NAATG	LRLSATVAF	DHQ TPGGL	ARHMAAEIGAV
Lineamycin_A_ACP-TE_S.sp.Mg1(InyHI)/1-369	VEL	RNQL	NAATG	LRLPATLLFI	O H P T P L L V	AARLRAELAGA
Mediomycin_A_ACP-TE_S.blastmyceticus(med 9)/1-356	VEL	RNRL	NAASG	LRLSPTLLF	DHGTPALV	AGHMLTQLVDA
Neomediomycin_A_ACP-TE_S.sp.RK95-74(nmd9)/1-366	VEL	RNRL	NAATG	LRLPPTLLF		ARQLLTGLAGS
Pikromycin_ACP-TE_S.venezuelae(pikAlV,Non-polyene)/1-379	VDF	RNRV	NRLTG	LQLPPTVVF	2 HP TPVAL	AERISDELAER
Erythromycin_ACP-TE_Saccharopolyspora_erythraea(Non-polyen	e)/1-363 VG	RNQL	QQATG	LALPATLVE		ADHIGQQLD
٨		10	20	30		40 50
A						
		_	al		all	
			ui		MI	
					THE R. L.	
Pim TE 1	FLPAAAPET	U SLE	RMPLDAL	ESGRIPEAQR	ALSALGAL	CP SPENTAELED
Amph TE 1	VAGTTVHPD		GLFHNAV	RGGKLVEAMR	MUKAVANT	RP TFETPADLEE
Nys TE 1	DARPAVRSD	DILE	GLFYNAV	RGGKLVEAMR	MLKAVANT	RP MEDTPAELEE
CanFR-008 TE 1	GAAGGPTEN	D SME	RLFLDGL	AQGKVREAQR	MLATVAAL	RP SFEVTAELED

Pik TE 1 DEBS TE 1	S G A D T G A G A G S G T P A R E A S S	M F R AL F R Q A V AL R D G Y R Q A G	EDDRYGEFLD VSCRVRSYLD	VLAEASAF RP LLAGLSDFRE	Q FASPEACSE H FDGSDGFS -
		100	110	120	130
Pimaricin_ACP-TE-S.chattanooyensis_L-10(ScnS4)/1-363	FLPAAAPETD	SLERMFLDAL	. ESGRIPEAGI	RMLSALGALR	SFENTAELED
Amphotericin_B_ACP-TE-S.nodosus_ATCC-14899(AmphK)/1-373	VAGTTVHPDD	TLVGLFHNAV	RGGKLVEAM	RMLKAVANTRI	PTFETPADLEE
Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(NysK)/1-361	DARPAVRSD	TLEGLEYNAV	RG <mark>G</mark> KLVEAM	RMLKAVANTRI	PMFDTPAELEE
Candicidin_ACP-TE_S.sp_FR-008(fscFy1-376	GAAGGPTEND	SMER <mark>LF</mark> LDGL	AQGKVREAQI	RMLATVAALRI	PSFEVTAELED
Filipin_ACP-TE_S.avermitilis_MA-4680(pteA5)/1-371	SAAAEERPAD	MLVQLYRHAS	ENGTAAEAM	3 M L M E A A R F R I	PSFERPEELDR
Pimaricin_ACP-TE_S.natalensis_ATCC-27448(Pim S4)/1-364	FLPVAAPETD	SLERMFLDAL	. D S <mark>G</mark> K V P E A Q I	RML SALGAL RI	PSFENTAELED
Reedsmycins_ACP-TE_S.Sp_OUC6819(rdmJ)/1-366	VRPGDGTSLD	SLTSLYGRAT	LS <mark>G</mark> QIDVATI	NLLIAAARLRI	PTFETPEQLRR
Eurocidin_D_ACP-TE_S.eurocidicus_ATCC-27428/1-373	RGPAAGQEID	SLER <mark>LF</mark> LD <mark>A</mark> N	IEIGRIQEAQ	ML R S L S A L <mark>R</mark>	PSFENAAELED
Lucensomycin_ACP-TE_S.achromogenes_NBRC-14001(LucE)/1-372	PATTSGQSG	TVGKVYFDAV	RAGKVDEGWI	ELLKAIALTRI	PLFEAPAELEE
Lucensomycin_ACP-TE.S.cyanogenus(LcmE)/1-372	PTAAPGQSG	TVGTLYFDAV	RAGKVDEGWI	ELLKAVALTRI	PLFEAPAELEE
Tetramycin_B_ACP-TE_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-360	ARSGSADQD	WLVRHFLTSI	REDRISEARI	RLVSAMGGVG	PSYENTAELED
Pentamyoin_ACP-TE_S.sp.S816/1-385	GGAAQERPT	MLVQLYRHAS	R T G T A A E A M	3 M L M E A A R F <mark>R I</mark>	PSFEHPEDLAE
Selvamicin_ACP-TE_Pseudonocardia_sp.HH130630-07/1-376	AVAVDTDSEE	TLVGLFLAAV	<pre>/RRDKSVEAM</pre>	DAVAAL RI	PTFSRTSELER
NPP_A1_ACP-TE_Pseudonocardia_sp.AL041005-10(CPPK)/1-376	AGRPAQPGEE	TLVGLFHAAV	/RG <mark>G</mark> KLV <mark>E</mark> AMI	RMLKAAANTRI	PMFESPAELEE
Auroramycin_ACP-TE_S.roseosporus_NRRL-15998/1-371	EREPAPAAGE	SVPELFRAMV	/RAGQVQEGL(3 L L NWVVRTR:	SQFTSSADLER
BE-14106_ACP-TE_S.ap.DSM-21069(becG)/1-371	AAEPSEEDDE	TVTEMFRRAV	RAGDTTGAL	JLMSAVAAL RI	PRFVTPADLAR
Bombyxamycin_A_ACP-TE_S.sp.SD53(bomP1)/1-377	VAPSADRQPE	TLRDMFHAAV	/VS <mark>G</mark> RADKGF/	ALLQAAADVRI	PGFGSVDEIDR
cremimycin_ACP-TE_S.sp.MJ635-86F5(cmiP6)/1-358	GHLVGSGAAE	TLTALWREGI	LAGDVPKTF	TMLRAVADLRI	PEFASLAELES
Heronamide_C_ACP-TE_S.sp.CMB-0406(hmG)/1-371	SDEPSPGQDE	TVTELFRGAV	RAGDSTGAL/	ALASAVAALRI	PRFTTPAELGS
Hitachimycin_ACP-TE_S.scabrisporus(hitP3)/1-381	STSNDAGSAD	TLTALFRAGU	ETGDIPRTF/	AMLRAVADIRI	PQFAAPTDLER
ML-449_ACP-TE_S.ap /1-371	SDEPAPGQDE	TVTELFRGAV	/RAGDSTGAL/	ALASAVAALR	PKFTTPAELGR
Sceliphrolactam_ACP-TE_S.sp.SD85(SceT)/1-373	PAAQEDAESE	SVSG <mark>LLRR</mark> AA	AGGNMMKGM/	ALLNAVAEIL	PGFSSAAELGA
Sipanmycin_ACP-TE_S.sp.CS149(Sip-P5)/1-371	EREPVPVAGE	SVAELFRAMV	/RAGQVQEGL(3 L L NWVVRTR:	SQFTSSADLER
Linearmycin_A_ACP-TE_S.sp.Mg1(InyH)/1-369	AEQGGEEDAG	V F G S M L <mark>R E A</mark> G	, TQ <mark>G</mark> ASGQFMI	ELLMQASRF RI	PSFASAAELRK
Mediomycin_A_ACP-TE_S.blastmyceticus(med9)/1-356	EPAGGEQQTG	V F G A M M R E A G	ET <mark>G</mark> RQE <mark>E</mark> FTI	RMLMDVSRF RI	PSFNGTADLAK
Neomediomycin_A_ACP-TE_S.sp.RK95-74(nmd9)/1-366	AEAAGAEPSA	VFGAMMREAE	ELGQQGEFTI	RLLMDVSRFRI	P S F A S A A E L D K
Pikromycin_ACP-TE_S.venezuelae(pikAlV,Non-polyene)/1-379	SGADTGAGAG	MFRALFRQAV	EDD RYGEFLI	DVLAEASAF RI	PQFASPEACSE
Erythromycin_ACP-TE_Saccharopolyspora_erythraea(Non-polyene)/1-363	SGTPAREASS	ALRDGYRQAG	VSGRVRSYLI	DLLAGLSDFR	EHEDGSDGFS-

		60 · · · · · · · ·	70 · · · · · · · ·	80 	90 · · · · · ·	100 • • • • • • • •
		β2		β3	αΑ	
Can	Pim TE 51 Amph TE 51 Nys TE 51 FR-008 TE 51 Pik TE 51 DEBS TE 49	LPLPATLAEG LSEAVTLATG LSEPVTLADG LPWPVTLAEG RLDPVLLAGG LDLVDMADG	P G A P R P G S P R P G R P R P A P T R P T D R A E G R A V P T D R A E G R A V P G E V T	LICVSTPTAN LIFVSAPGAT LIFVSAPGAT LVCVSAPTAN LVGCTGTAAN VICCAGTAA	GGVHEYARLA GGVHOYARLA GGVHOYARLA GGVHOYALLS GGPHEFLRLS SGPHEFTRLA	ASFRGERHVS AHFRGKRHVS AHFRGSRHVS GHFRGRROVT TSF0EERDFL GALRGIAPVR
			150	160	170	180 16
Pimaricin_ACP-TE-S.chattanoogensis_L-10(Scn S4)1- Amphotericin_B_ACP-TE-S.nodosus_ATCC-14899(Am, Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(NysK) Candicidin_ACP-TE_S.np_FR-008(9scF)1-376 Filipin_ACP-TE_S.avermithis_MA-4680(bteA5)1-371 Pimaricin_ACP-TE_S.natalensis_ATCC-27448(Pim S4) Reedsmycins_ACP-TE_S.p_OUC6819(dm J)1-366 Eurocidin_D_ACP-TE_S.eurocidicus_ATCC-27428(1-3) Lucensomycin_ACP-TE_S.eurocidicus_ATCC-27428(1-3) Lucensomycin_ACP-TE_S.eurocidicus_ATCC-27428(1-3) Lucensomycin_ACP-TE_S.eurocidicus_CGMCC-4.1 Pentamycin_ACP-TE_S.p.S816(1-385 Selvamicin_ACP-TE_S.p.S816(1-385 Selvamicin_ACP-TE_S.p.S816(1-385 Selvamicin_ACP-TE_S.p.S816(1-385 Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Selvamicin_ACP-TE_S.p.S816(1-385) Beronamide_C_ACP-TE_S.p.CM8-0406(pm G)(1-371) Heronamide_C_ACP-TE_S.p.CM8-0406(pm G)(1-371) Heronamide_	363 ohKy/1-373 /1-361 /1-364 /1/LucEy/1-372 123(TetrEy/1-360 07/1-376 CPPKy/1-376 371	L P L P A T L A L S E A V T L A L S E P V T L A R P A P V R L S L P L P V T L A R P A P V R L A L P L P V T L A L P L P V T L A L P A S V T L A L P A S T T T L S P A S T T T L S P K T V R L A L P E P T T L S P K T V R L A	E G P G A P T G P G S P D G P G R P E G P G T P G G P A G T P D G P A G T P D G P A G T P D G P A S . P Q G D D L . P D G P T T A . P D G P T P D G P T P D G P T	- R L I C VS T P T - R L I F VS A P G - R L I F VS A P G - R L V C VS A P T - R L I C VS T P T - R L I C VS T P T - R L I C I S T P T - R L I C I S S P V - R L I C I S S P V - R L I C I S S P V - R L I C I S T P T - R L I C I S T P T - R L I C I S T P T - R L I C L S T P M - R L I C L S T P M - R L I C L S T P M - R L I C V S T P M - R L V S T V S	A NG G V H E YAR L A TG G V H Q YAR I A TG G V H Q YAR I A TG G V H Q YAR I A NG G V H Q YAR I A NG G V H E YAR I A NG G V H Q YAR I A NG G Y H Q YAR I A N N Y YAR I A N N Y YAR I A N N YAR I A N	A A S F R G E R H V S A A H F R G K R H V S S G H F R G R R D V T A A P F R D R D V T A A S F R G R H V S A A S F R G R H V S A A H F R G D R G V Q A A H F R G D R G V Q A A H F R G D R G V Q A A F R C R N V S A A P F R D V D M W A A F R G S R H V S A A P F R D V M V D M W A A F R G S R H V S A A P F R D V M V D M W A A F R G S R H V S A A P F R D V M V D M W A A F R G S R H V S A A P F R D V M V D M W A A F R G S R H V S S A A F R G S R H V S S A A F R S S S S S S S S S S S S S S S S S S
⊢ataenim yoin_ACP-TE_S.seaan sporus(nitP3y1-361 ML-449_ACP-TE_S.sp/1-371 Sceliphrolactam_ACP-TE_S.sp.SD85(SceTy/1-373 Sipanmycin_ACP-TE_S.sp.CS149(Sip-P5)/1-371 Linearmycin_A_ACP-TE_S.plastmycehicus(med 9)/1-350 Mediomycin_A_ACP-TE_S.blastmycehicus(med 9)/1-350	3	PPKTVQLA LEQPVRLA PPVSVRMA APSLVRLS APALVRLS	D G T S G . P D G A R R . P R G G E G . P D G D E A . P R G G T R . P G A G . A E P V P . P	- RLICUSTPM - RLICEPSPM - KLICEPSPM - HLVCLCTPA - GLVCESSIL - ALVCESSIL	AGGGVHQHARL AGGGVHQHARL ALGGAQQYARF AMGGAYQYARL SISGPHQYARF PISGPHQYARF	AAHFROVRPVS GSEFRDVRPVS AARFRGRREVV I SAFKGARTVT ASAFRGRRDVH AAGFRGHRDVW
Neomediomycin_A_ACP-7E_S.sp.RK95-74(nmd9)/1-36 Pikromycin_ACP-7E_S.venezuelae(pikAlV,Non-polyen Erythromycin_ACP-7E_Saccharopolyspora_erythraea(6 e¥1-379 Von-polyene¥1-3	APTLVRLS RLDPVLLA 63 - LDLVDMA	R <mark>G</mark> E T · · · · G · <mark>P</mark> G G P T D R A E G R A D G P · · · · · G E V	 ALVCFPSIL VLVGCTGTA TVICCAGTA 	SIGGPHQYARF ANGGPHEFLRL AIS <mark>G</mark> PHEFTRL	AAGFRGHRDVW STSFQEERDFL AGALRGIAPVR

	B)	αB		β5
Pim TE 96 Amph TE 96 Nys TE 96 Canv/FR-008 TE 96 Pix TE 101 DEBS TE 94	ALLPA GGFAAFGGEALLPA AALGGESALLPA AALPAGGESALLPA AALPAGGESALLPA AALPAGGESALLPA AALPAGGESALLPA AALPA	TPETAVRVVA EST TSEAAARIVA EST TSEAAARIVA EST HAEAAARIIA DCI DLDTALDAGA RAT SMAAVAAVGA DAT	L R ASDGE K ASSEGK L Q A AGDA I R T Q G D K	PFVUVGHSG PFVUVGHSTG PFVUVGHSTG PFVUVGHSG PFVUVGHSG PFVVQHSAG
DEBSTE 94 Pimaricin_ACP-TE-S.chattanoogensis_L-10(Scn S4)/1-363 Amphotenicin_B_ACP-TE-S.nodosus_ATCC-11495(Amph/Ky1-373 Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(Nys/Ky1-361 Candicidin_ACP-TE_S.ap_FR-008(50Fy1-376 Filipin_ACP-TE_S.ap_FR-008(50Fy1-376 Pimaricin_ACP-TE_S.ap_CMC8019(dm Jy1-366 Eurocidin_D_ACP-TE_S.ap_OUC6819(dm Jy1-366 Eurocidin_D_ACP-TE_S.aporgense_NBRC-14001(LucEy1-372 Lucensomycin_ACP-TE_S.aporgense_NBRC-14001(LucEy1-372 Tetramycin_B_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_B_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Tetramycin_ACP-TE_S.aporgense_LomEy1-372 Selvamicin_ACP-TE_S.aporgense_LomEy1-376 Auroramycin_ACP-TE_S.aporgense_LomEy1-371 BE-14106_ACP-TE_S.aporgense_LomEy1-371 Be-14106_ACP-TE_S.ap.CM8-0406(bm Gy1-371 Htachimycin_ACP-TE_S.aporgense(LomEy1-376 McL-449_ACP-TE_S.aport_371 Sceliphrolactam_ACP-TE_S.aporgense(LomEy1-373 Distribution_ACP-TE_S.aporgense(LomEy1-373 Distribution_ACP-TE_S.aporgense(LomEy1-376 McL-449_ACP-TE_S.aporgense(LomEy1-373 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distribution_ACP-TE_S.aporgense(LomEy1-376 Distributi	200 211 ALPLVOFAAGE RLPA AIPLMOFAPOE LLPA ALPLVOFAAGE LLPA ALPLMOFAPOE LLPA ALPLNOFAPOE LLPA ALPLNOFAPOE LLPA ALPLNOFAPOE LLPA ALPLNOFAPOE RLPA ALPLVOFATOE RLPA ALPLVOFATOE RLPA ALPLVOFATOE RLPA ALVHPOYEKGE PVPSI ALPLVOFAAGE RLPA ALVNPOYEGE RLPA ALPLVOFAAGE CLPA ALPLVOFAAGE SLPG ALPLVOFAAGE CLPA ALPLVOFAAGE SLPG ALPLVOFAAGE CLPA ALPLVOFAAGE CLPA ALPLVOFAAGE CLPA ALPLVOFAAGE CLPA ALPLVOFAAGE CLPA ALPLVOFAAGE CLPA ALPLVOFAAGE SLPA ALPLVOFAAGE CLPA ALPLVOFAAGE SLPA ALPLVOFAAGE CLPA AL	BMANY AVO DAY 220 TPETAVRVVAESTL TSEAAARIVAESVL HAEAAARIVAESVL HAEAAARIVAESVL HAEAAARIVAESVL HAEAAARIVAESVL TAETAVRVIAESAL OPDVVFRLHAQTVE TAETAVRVIAESAL SSRAITRAAASSVL TAECAARTIAESVL TAECAARTIAESVL TAECAARTIAESVL TSEAAARIVAESVL SVPALDVLAATVE SV	R 230 RA SDON MA SEO QA ADOK EC AGOK RA SDON RA ADON RA	240 PFVLVGHSS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PFVLVS PF
Sipanmyoin_ACP-1E_3.ap./C5149(SipA-79)7-377 Linearmyoin_A_ACP-TE_S.ap./Kg1(fnyHY)1-369 Mediomyoin_A_ACP-TE_S.blastmyoeticus(med 9)/1-356 Neomediomyoin_A_ACP-TE_S.ap.RK95-74(nmd 9)/1-366 Pikromyoin_ACP-TE_S.evenezuelae(pikAlV,Non-polyene)/1-379 Erythromyoin_ACP-TE_Saccharopolyspora_erythraea(Non-polyene)/1-363	AMAAPOFLOGEQLPA AMANPOFVAGEQLPA ALGNPOFVAGEQLPA AVPLPOYGTOTGTGTALLPA AVPDPOYGTGTGTGTALLPA	TAEAVIEAQAEAVL TPEAVIEAQAEAVL NPEAVIEAQAEAVL DLDTALDAQARAIL SMAAVAAVQADAVI	RH - ADGE RR - TDGS RQ - TDGA RA - AGDA RT - QGDK	PFVLLGHSSG PFVLLGHSSG PFVLLGHSSG PVVLLGHSGG PFVVLLGHSGG

		170 · · · · · · · · · · · · ·	180 190 · · · · · · · · · · ·	
	αC	β6		αL1 —
Pim TE 141 Amph TE 141 Nys TE 141 Can/FR-008 TE 141 Pik TE 151 DEBS TE 139	GAFAYLAAAL L GSLAYLAAGV L GSLAYLAAGV L GSLAYLAAGV L ALLAHELAFR L ALLAHELAFR L ALMAYALATE L	ENTWGIRPE AVVLLD EDTWGVKPE AVVLLD EDTWGVKPE AVVLLD ENTWGIRPE AVVLLD ERANGAPPA GTVLD L-DRGHPPR GVVLD	LSL RIEQNETIDY A TASI RYNPSEGNNL D TASI RYNPGEGNDL D LSI QTKSDEGVDY N PYPP GOQEPIEVWS F VYPP GOQEANNAWL E	AGLMRRH MV OQTTRFYLAD NRTTRFYLAD NGMMKFNFTA RQLGEGLFAG EELTATLFDR
Pimaricin_ACP-TE-S.chattanooyensis_L-10(Scn S4)/1-363 Amphotericin_B_ACP-TE-S.nodosus_ATCC-14899(AmphK)/1-373 Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(NysK)/1-361 Candicidin_ACP-TE_S.sp_FR-008(fscF)/1-376 Filipin_ACP-TE_S.avermitilis_MA-4680(pte A5)/1-371 Pimaricin_ACP-TE_S.natalensis_ATCC-27448(Pim S4)/1-364 Reedsmycins_ACP-TE_S.p_OUC6819(rdmJ)/1-366	250 G A F A Y L A A G V L G S L A Y L A A G V L G S L A Y L A A G V L G S L A Y A A A G V L G W I A H G V A A H L G W L A H S T A V H L	260 ENTWG I RPEAVVLLD EDTWG VKPEAVVLLD EDTWG VRPEAVVLLD EHTWG I RPEAVVLLD EA.MGERPAAVVMVD ENTWG I RPEAVVLLD EAL-GVKPAGLVLLD	270 280 TLSLRHEQNETIDYA- TASIRYNPSEGNNLD- TASIRYNPGEGNDLD- TLSIGHKSDEGVDYN- SFS-REVPFD-HQVLN TLSLRHEQNESIDYA- TYLPLAEQLERL-SA-	290 GLMRRHFMV QTTRFYLAD GMMKFNFTA GMMKFNFTA GLMRRHFMV GWLRRGWD
Eurocidin_D_ACP-TE_S.eurocidicus_ATCC-27428/1-373 Lucensomycin_ACP-TE_S.achromogenes_MBRC-14001(LucE)/1-372 Lucensomycin_ACP-TE_S.cyanogenus(LcmE)/1-372 Tetramycin_B_ACP-TE_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-360 Pentamycin_ACP-TE_S.sp. S816/1-385 Selvamicin_ACP-TE_Pseudonocardia_sp.HH130630-07/1-376 NPP_A1_ACP-TE_Pseudonocardia_sp.AL041005-10(CPPK)/1-376 Auropende_ACPTE_S.monocardia_sp.AL041005-10(CPPK)/1-376	G SLAYAAAGLL G ALALAVAGLL G ALALAVAGLL G AF SYAAAGVL G WI AHGVAAHL G SLAYEAAGVL G SLAYEAAGVL	ENTWGIKPTAVVLLD ESMWGVRADGVIMLD ESMWGVRADGVIMLD ESTWGIKPAGLVLLD EAM-GERPAAVVMVD EERWGVQPEAVIMLD EETWGVRPEAVVLLD	TLSIRHDRNEDIDYA- TLSLRHEHGDSVDYR- TLSLRHEHGDSVDYR- TLSIRHEENEDIDYG- SFSRALPFDRQV-LN- TMSLRYAEGEGADYE- TASIRWDAAEGNDLD-	GLMRQNFLV QLARRFMGE GLARRFMGE GLLKANFLA AMA - Q - AQS GVGRYYLAD RTTRFYLAD
Autoramycin_ACP-TE_S.toseosponus_NKRL:159597-1571 Be=14106_ACP-TE_S.sp.DSM-21069(becG)/1-371 Bombyxamycin_A_ACP-TE_S.sp.SD53(bomP1)/1-377 cremimycin_ACP-TE_S.sp.MJ638-86F5(cmiP6)/1-358 Heronamide_C_ACP-TE_S.sp.CMB-0406(tmG)/1-371 Htachimycin_ACP-TE_S.sp.cMB-0406(tmG)/1-381 ML-449_ACP-TE_S.sp./1-371	G I IGHIIARH G TLAYATAGH G ILAYATAGH G ILGYAVAHL G I IGHVLARH G I IGHVLARH G I IGHVLARH	EEU OKPPAGUVLLD EREYOVRPAGVILLD EHYGEQARPAGVVLLD EETVORPAGLVLID EVVGIRPAGLVLID EETVOVPPAGLVLID	TFRVEDTAM - NVGFD TFRVEDTAM - NVGFD SYAVADHGM - PAGFE TFRVEDTAM - NVGFG SYAVADNAM - PEGFE TFRVEDTAM - NVGFG	HLM - GELLT GLA - LGMFE HMT - HRLME HLM - GELLT HMT - YRMLE HLM - GELLT
Sceliphrolactam_ACP-TE_S.sp.SD85(SceT)/1-373 Sipanmycin_ACP-TE_S.sp.C5149(Sip-P5)/1-371 Linearmycin_A_ACP-TE_S.sp.Kg1(nyH)/1-369 Mediomycin_A_ACP-TE_S.blastmyceticus(med 9)/1-356 Neomediomycin_A_ACP-TE_S.sp.RK95-74(nmd 9)/1-366 Pildomycin_ACP-TE_S.sp.RK95-74(nmd 9)/1-379 Endthmycin_ACP-TE_Sacetampolyconsendthcast(1-363	OQFAHATAEVL GLLAHATAGLL GMLAHAVAGRL GMLAHEVAARL GLLAHEVAARL ALLAHELAFRL	EK - AG TPARAVVLLD EE - QG RP PVGVALD EG - AG VF PEAVVLVD EA - TG VF PQAVVLLD EG - TG VF PDAVVLLD ERAHGAPPAG IVLVD	TYLPGDGK · DELWR TYLVEQTAG · FQDFAA IYSHDDDAI · MGIQP IYSHDTDAA · TGIQP IYSHDREAA · MALQP PYPPGHQEPIEVWSR VYPPGHQDAMNAWI	QMF - HGMLD GLS - QGVDE GLS - ASVGE GLR - AGIDE QLGEGLFAG

			αL2		β7	
	Pim TE 11 Amph TE 12 Nys TE 12 CarvFR-008 TE 12 Pik TE 22 DEBS TE 12	91 DEVSPVRMT 91 IDSPSVTLN 91 IDSPSVTLN 91 VDDSPVRLT 91 ELPMS 88 ETVRMD	N S S S S S S S S S S S S S S S S S S S	GMLNQLEVRH MAMTDIDAPA MAMTDIQAPA VLLNALOVHP RFLAGPRPGR RLTGQWRPRE	TTVPVLIIRA TTAPTLLLRA PTAPTLLVRA TTVPVLEIKC SSAPVLLVRA TGLPTLLVSA	AKETFG IG TQANNG ARALDG TRALIEGVPA SEPLGD - WQ GEPMGP WP
		300	310	320	330	340
Pimaricin_ACP-TE-S.chattanoogensis_L-10(Scn S4) Amphotericin_B_ACP-TE-S.nodosus_ATCC-14899(A Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(Nysi Candicidin_ACP-TE_S.sp_FR-008fscFY1-376 Fillipin_ACP-TE_S.atalensis_ATCC-27448(Pim S Reedsmycins_ACP-TE_S.sp_OUC6819(dm))/1-366 Eurocidin_D_ACP-TE_S.eurocidicus_ATCC-27428(Lucensomycin_ACP-TE_S.eurocidicus_ATCC-27428(Lucensomycin_ACP-TE_S.eyanogenes_NBRC-14 Lucensomycin_ACP-TE_S.sp.S816/1-385 Selvamicin_ACP-TE_S.hygrospinosus_CGMCC-4 Pentamycin_ACP-TE_S.sp.S816/1-385 Selvamicin_ACP-TE_S.ps.S816/1-385 Selvamicin_ACP-TE_S.sp.S816/1-385 Selvamicin_ACP-TE_S.sp.S816/1-385 Selvamicin_ACP-TE_S.sp.S816/1-385 Selvamicin_ACP-TE_S.sp.S516/069(becGy1-371 Bombyxamycin_A_CP-TE_S.sp.S553(bom P1/1-371 Bombyxamycin_ACP-TE_S.sp.CMB-0406(hm G)1-371 Hatchimycin_ACP-TE_S.sp.S1085(SeeT)1-373 Sipanmycin_ACP-TE_S.sp.S108(Sip-65)1-371 Soeliphrolactam_ACP-TE_S.sp.S108(Sip-65)1-371 Linearmycin_ACP-TE_S.sp.S136(Sip-65)1-371 Sipanmycin_ACP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_ACP-TE_S.sp.S136(Sip-65)1-373 Sipanmycin_A_CP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_A_CP-TE_S.sp.S136(Sip-65)1-371 Seeliphrolactam_ACP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_A_CP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_A_CP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_A_CP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_A_CP-TE_S.sp.S136(Sip-65)1-371 Linearmycin_A_CP-TE_S.sp.S136(Sip-65)1-371	(1-363 ImphKy/1-373 Ky/1-361 1-373 001(LucEy/1-372 .1123(TetrEy/1-360 0-07/1-376 0(CPPKy/1-376 1-371 7 3 1-371	DEVSPVRMT DEVSPVRMT IDSPSVTLN VDDSPVRLT SQRLDFMRS DEVSPVRMT RAENYNYLD DEVSPVRMT TDSATVTVD SEATQVVD SEATQVVD SEATQVVD SEATQVF CDSATVTVD CDSATVTVD SEATQVF CDSATVTVD CDSATVTD CDSATVTVD CDSATVT	310 N SRLS AMARWM SARMSAMAHWF SARMSAMAHWF NSRLSAMGRWM G EQLTAMGRYM G EQLTAMGRWM SNRLSAMAHYL SNRLSAMAHYL SNRLSAMAHYL SNRLSAMAHYL SNRLSAMAHYL SARMSAMAHYL SARMSAMAHYF SARMSAMAH	320 MG ML NQ L - E - N MAM T D I - Q - A MAM T D I - Q - A MK L - F D DW - A A MG L NQ L - E - V MR L - F GQ L WK - F MG L L NR V - D - V NR MS AL - E - V NR MS AL - E - V NR MS AL - E - V MH L L NS I - D - V MR L F D DW - A - A YN R AAAL - R P V MT TE I - D - S YAL MP E I - E - L Q V L AG F - D - F H L L P D F - T - F Q V L AG F - D - F S Q V L AG F - D - F S Q L L TG C - L - F S Q L L TG C - L - F S Q L L TG C - L - F S Q L TG G W - K - F S L F G G W - K - F	330 I I R PATTAPTLLUR PATTAPTLLUR I PATAPTLLUR I PATTAPTLLUR I PATAPTLLUR I PATAPTLLUR I PATAPTLLUR I PATAPTLLUR I PATAPTLLUR I PATAPTLUR I PATAPTAPTLLUR <td>340 A KETFGIG A TQANNG A TQANNG CTRALIEGVPA S.EPMLAGAG A TKETFGIG A TELVDGEG A TKETFGIG A TELVDGEG C SVPLLGDPDT A TRUPGGLGPDT A SERFATGPED A SERFATGPED A SERFATGPED A SERFATGPEG A G ERFFOWTRA A G ERLFOWTRA</td>	340 A KETFGIG A TQANNG A TQANNG CTRALIEGVPA S.EPMLAGAG A TKETFGIG A TELVDGEG A TKETFGIG A TELVDGEG C SVPLLGDPDT A TRUPGGLGPDT A SERFATGPED A SERFATGPED A SERFATGPED A SERFATGPEG A G ERFFOWTRA A G ERLFOWTRA
Neomediomycin_A_ACP-TE_S.sp.RK95-74(nmd9)/1-	366	R S T G P <mark>V</mark> P V D	· DARLLAMGAYF	RLFGQW-E-P	REVKTPTLLVR	A E E Q F F D W S R -
Pikromycin_ACP-TE_S.venezuelae(pikAlV,Non-poly Erythromycin_ACP-TE_Saccharopolyspora_erythrae	ene)/1-379 a(Non-polyene)/1-3	ELEP • • • MS 363 ET • • • VRMD	- DARLLAMGRYA	A R F L A G P - R - P D R <mark>L</mark> T G Q W - R - P	GRSSAPVLLVR RETGL <mark>PTL</mark> LVS	ASEPLGD WQ AGEPMGP WP
	280	270	29	28	300	
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		68		αD		
Pim TE 239 Amph TE 236 Nys TE 236 Can/FR:008 TE 241 Pik TE 246 DEBS TE 233	TDTGIYGEDH FMLDTSA FRLDTSS PDPERLHLPV EERGDWRAHW DDSWKPTW	GSPVDVRSVD VPADVVRDIE VPADEVRDID VPDAEVRPLD DLPHTVAVP FFEHDTVAVP	ADH FSMVRDD ADH LSLAMEN ADH LSLAKEN SDH LSLAKEN GDH FTMMRDH GDH FTMVQEN	APETARIVKE SDLTAEAIEN SALTAGAIEG SGPAADLMDA APAVAEAVLS ADAIARHIDA	WLDSLGNA WLAELPAGEA WLAELPDPAA WLAELPDPAA WLTALETTAT WLDAIEGIEG WLGGGN-SSS	
	350	360	370	380	390	400
Pimaricin_ACP-TE-S.chattanooyensis_L-10(ScnS4)/1-363	TDTGIYGED	HGSPVDVRSVD	DADHESMVRDD	APETARIVKE	WLDSLGNAX · ·	
Amphotericin_B_ACP-TE-S.nodosus_ATCC-14899(AmphK)/1-373	FMLDTS/	AVPADV <mark>VR</mark> DIE	EADHLSLAMEH	SDLTAEAIEN	WLAELPAGEA >	£
Nystatin_A1_ACP-TE_S.nodosus_ATCC-11455(NysK)/1-361	F R L D T S 3	S V P A D E <mark>V R</mark> D I D	DADHLSLAKEH	SALTAQAIEG	WLAELPDPAA>	
Candicidin_ACP-TE_S.spFR-008#scFy1-376	PDPERLHLP	VVPDAEVRPL	SDHLSLIRED	SGPAADLMDA	WL TALETTATT	DAPETAPA
Filipin_ACP-TE_S.avemitilis_MA-4680(pteA5)/1-371	- DGDGRAAP	PEHVDTIVEVI	F G N H Y S M L E D H	AGTTAAAVDS	WLADAVTDASG	PVAPADAT
Pimaricin_ACP-TE_S.natalensis_ATCC-27448(PimS4)/1-364	TDTGIYGED	H	DADHESMVRDD	APETARIVRE	WLDSLGNAX · ·	
Reedsmycins_ACP-TE_S.Sp_OUC6819(rdmJ)/1-366	A - WQ - S SW -	- DFPHTAIDVF	PGNHFSIMDEH	AATTARVVEE	NIAAQDSX	
Eurocidin_D_ACP-TE_S.eurocidicus_ATCC-27428/1-373	E-EL-SSL-	- I P S A D I R <mark>V</mark> (DADHLSIVKAD	AGLAAEAVEN	WLDSVTGAX	
Lucensomycin_ACP-TE_S.achromogenes_NBRC-14001(LucE)/1-372	HGQQ - ELL -	· VPAE TVR TIC	DADHESLAQRD	SHVTATVMKE	WLATLX	
Lucensomycin_ACP-TE.S.cyanogenus(LcmE)/1-372	HGQQ - ELL -	- VPAE TVRTIC	DADHESLAGRD	SNVTATVMKE	WLATLX	
Tetramycin_B_ACP-TE_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-360	E - G L - S E M	- IPSAEIVKL	DADHLSIVRSD	ASAASQIADD	WLAATFGEX	
Pentamycin_ACP-TE_S.sp.S816/1-385	G - GRAAPP -	- EHVDTTVEN	I G N H Y S M L E D H	AGTTAAAVHS	WLADVATEATR	PAASEDTT
Selvamicin_ACP-TE_Pseudonocardia_sp.HH130630-07/1-376	QEAPI	PLDTDAVLTIC	DADHLTMAKEH	SGVTAEAMEE	WL TSLQAATR>	4
NPP_A1_ACP-TE_Pseudonocardia_sp.AL041005-10(CPPK)/1-376	• • • • • T S A • •	- VPADTVVDI	DADHLSLAMEH	SALTADAIET	WLAEL PTPTTE	DASX
Auroramycin_ACP-TE_S.roseosporus_NRRL-15998/1-371	D - WQ - T T W -	- SLATRVETVF	OD HF SLVEQG	AETTAAVVEN	WLGTLAX • • • •	
BE-14106_ACP-TE_S.sp.DSM-21069(becG)/1-371	E - MRARPW -	- DSEHTLRTVE	EGNHF SLGQDH	APATARVIEE	WLETLDX	
Bombyxamycin_A_ACP-TE_S.sp.SD53(bomP1)/1-377	D - GRAEPW -	- EAAHTLRTVF	RANHFTLVEDR	AEETAQVIDA	WLASQESAPA A	EX
cremimycin_ACP-TE_S.sp.MJ635-86F5(cmiP6)/1-358	EFPKARPW-	- DPAHDFVAS	SANHFTLIEED	AEETAGIIDQ	WLASHEX	
Heronamide_C_ACP-TE_S.sp.CMB-0406/hmGy1-371	E - MRAQPW -	- D D E H T L R T V E	EGNHFSLGQEH	APATARAIEE	WLETLDX	
Hitachimycin_ACP-TE_S.scabrisporus(hitP3)/1-381	F - L R A Q P W -	- DPAHTLRLSP	PGNHFGLVEED	AESTARIIED	WLAEVGAD SAP	IDRRLX
ML-449_ACP-TE_S.sp /1-371	E - MRAQPW -	- D T E H T L R T V E	EGN <mark>hfsl</mark> gqeh	APATARAIEE	WL ETL DX · · · ·	
Sceliphrolactam_ACP-TE_S.sp.SD85(SceTy/1-373	D - WR - A T W -	- DGAHVLREVF	OT HF TILEES	AASTAAAVDD	WL PSLGDCPLK	IGX
Sipanmycin_ACP-TE_S.sp.CS149(Sip-P5)/1-371	D - WQ - T T W -	- SLATRVETV	GDHFSLVEQG	AET TAAVVEN	WLGTLAX · · · ·	
Lineamycin A ACP-TE S.co.Mg10/nyHV1-369	D-WR-SYW-	- DLE <mark>HT</mark> AVD <mark>V</mark> A	AGN <mark>HF</mark> TMMEQH	ATTAGTVEE	WL DAHLX	
Mediomycin A ACP-TE S.blastmyceticus/med9y1-356	D - WR - SYW -	- DLEHTALDV	GNHF TMMEQH	AGT TAQAVAG	WL SAX	
Neomediamycin A ACP-TE S.so. RK95-74mmd9V1-366	D-WR-SYW-	ELPHTVRDV	GHHF TMMEDH	AATTARVVED	WLADX	
Pikonevoin ACP-TE S venezuelae/bik/AIV Non-nolveneV1-379	EERGDWRAH	NDLPHTVADV	GDHF TMMRDH	APAVAEAVLS	WL DAIEGIEG/	GKX
Catherine AOD TC Construction of the colores V4.00	DD. SWKPT	NPEENDTVAN	CONFTMUOR	ADALARHIDA	WI GOONSX	

Figure 27: Multiple sequence alignment of amino acid residues of TE type I gene associated with ACP of the last module of polyketide synthase (PKS). The result of the alignment of thioesterase gene was compared to the results of the previous study [29]

The results demonstrated that the ACP gene is highly conserved among both polyene and nonpolyene polyketide synthases, since it codes for important functional domains that are crucial for its activity. Moreover, the analysis of the multiple sequence alignment of the comprehensive dataset and its comparison to the aligned sequence in previous literature indicated that the cyclic (macrolactone and macrolactam) and linear polyenes share conserved domains with some variability in this region. Although different amino acids may be present within the conserved regions, these variations do not alter the electric charges and the overall geometry of the conserved domain.

Then the highly conserved region of the thioesterase for the polyenes from amino acid 45 to 387 was extracted and translated back into nucleotide sequences for further analysis at the nucleotide level and for the purpose of designing primers.

	10	20	30	40	50	60	70	80	90	100
Pimaricin_TE_nc_S.chattanoogensis_L-10(ScnS4)/1-705	OCCOAACTGOAGOAT	TOCCOCTC	CCOOCCACCCTC	OCCOA00	occcc	0 COCO CCO	- COOCTOATCTOT	OTCAGCACAC	CCACCO	CCAACOOCOOCOTACA
Amphotenicin_8_TE_nc_S.nodosus_ATCC-14899(AmphK)/1-690	OCODAC CTODAGOAG	TOTOCOAG	GCCOTCACCCTG	GCCACOO	0000000	BCTCCCCG	- COOCTOATCTTC	GTCAGCGCGC	000000	CCAC COOCOO TOTCCA
Nystatin_A1_TE_nc_S.nodosus_ATCC-11455(NysK)/1-690	OCCOACCTGOAGGAD	TCTCCGAG	CCOOTGACGCTC	GCCGACG	0000000	acceecc.	- COOCTOATCTTC	GTCAGCGCCC	CGGGGCG	CCACCGGCGGCGTCCA
Candicidin_TE_no_S.sp_FR-008#scFy1-711	OCCOAACTOGAGGAC	TOCCCTGG	COOTGACOCTO	OCCGAGO	000000	CACCGACC	- COCCTOOTOTOC	GTCAGCGCCC	CCACCO	CCAACGGCGOCGTCCA
filipin_TE_nc_S.avemitilia_MA-4680/pteA5/1-693	GAGGAACTGGACCGG	COCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCGGTGCGGTTG	TCGCAAG	GC G/	ACCATCCCCI	COCOCTGATOTOC	TTOTCGCCGT	ACGTCG	TACCOCCOCCOCA
Pimaricin_TE_nc_S.natalensis_ATCC-27448@imS4y1-706	OCCOAACTGGAGGAT	TGCCGCTC	CCGGCAACCCTC	GCCGAGG	6000060	GCACGCCG	· COOCTOATCTOT	GTGAGCACAC	CCACCO	CCAACGGTGGCGTACA
Reedomycina_TE_nc_S.Sp_OUC6819(rdmJV1=693	GAGCAGCTACGGCGG	GTCCCGAT	GTOGTOCCOCTO	GTCAAGO	GTGAGAGCG	ACGCGGCT	· CCOCTOGTCTOC	TTTGCCCGGC	GAACG	CATTCOCCOOGCCOCA
Eurocidin_D_TE_no_S.eurocidicus_ATCC-27428/1-696	OCCOAG CTGGAGGAC	C T G C C G C T G	COGACCACGCTC	GCCGAGG	GCTCC···GC	GCACACCG	· COOCTOATCTOC	ATCAGCACGC	CCACCO	CCAACGGCGGAGTCCA
Lucensomycin_TE_nc_S.achromogenes_NBRC-14001(LucE)/1-702	GCCGAACTGGAGGAG	TGCCCGAG	CCCGTCACCCTC	GCGGACG	GCCGGCCGG	9.G C C G	. CGGCTGATCTGC	ATCAGCTCAC	CCGTGT	CCGT CGGCGG TGCGCA
Lucensomycin_TE_nc.S.cyanogenus(LonE)/1-702	GCCGAGCTGGAGGAG	TGCCCCAG	CCCGTCACCCTC	GCGGACG	GCCGGCCG	AG CCA	· COOCTOATCTOC	ATCAGCTCAC	GGTGT	CCGTCGGCGGCGCGCA
Tetranycin_B_TE_no_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-699	GCCGAGCTGGAGGAC	CTGCC GCTG	CCGACCACGCTC	GCCGAGG	GCCCGGCGT	CA CCG	COGETGATETOC	ATCAGCACGC	CCACCO	CCAACGGCGGCGTCCA
Pentamycin_TE_nc_S.ap.SB16/1-693	GAGGAC CTGG CCGAA	TCCCCGCC	COGGTACGGCTG	TCCCAGG	GCGACGACCI	T C + + + C C G T /	ACACOG TOATO TOC	TTCTCGCCGT	ACGTCG	TACCOOCCOOCACA
selvanicin_TE_nc_Pseudonocardia_sp.HH130630-07/1-699	T COBAG CT CBAGCOG	CCGCCTCC	CCGGTGGTGCTC	GCCGACG	GGCCGACCA	CA CCG	AAGCTGATCTTC	GTCAGCGCGC	GGGGAG	CGACCGGCGGCGTGCA
NPP(Nys-like)_A1_TE_nc_Pseudonocardia_sp.AL041005-10(CPPK)/1-690	GCCGAGCTGGAGGAG	TGTCCGAG	CCGGTCACGCTC	GCCGAGG	GCCCGCCG	c c <mark>c c</mark> c	COGCTGATCTTC	GTCAGCGCCC	CCGGCG	CCACCGGCGGGGGTGCA
Auroramycin_TE_nc_S.roseosporus_NRRL-15998/1-696	GCGGAC CTGGAACGG	сс <mark>всс</mark> ветс	TCCOTGCGGATG	GCCGACG	GCGACGAGG	CG <mark>CC</mark> G	· CACCTOGTGTGC	CTGTGCACCC	CGGCCG	CGATGGGCGGCGCGTA
BE-14106_7E_nc_S.sp.DSM-21069(becG)/1-696	GCCGACCTCGCGAGG	ACC <mark>CC</mark> GAAG	ACGOTGCGGTTG	GCGGACO	0000000	GCCGC <mark>CC</mark> C	- COGCTGATCTOC	CTGGCCACAC	CCATOO	COGGCOGCOGCOTOCA
Bombyxamycin_A_TE_nc_S.sp.SD53(bomP1)/1-699	GACGAGATCGACCGG	стс <mark>сс</mark> сосо	GCGGTCCGCCTC	GCGGACG	CCCCCGAGGG	6 A <mark>C C</mark> G	- CATCTCATCTGC	CTCAGTACCC	CCATOG	CCAC COOCOO TOTCCA
creminycin_7E_nc_S.ap.MJ635-86F5(cmiP6)/1-699	OCCGAACTGGAGTCC	T T G C C G G A A	CCGACCACGCTC	TCCGAGO	O G C C G C G G G G	3 G <mark>C C</mark> G	- COOCT CATCTOC	GTCTCCACAC	CCATOO	TOGCCOGCOGGGGGCA
Heronamide_C_TE_nc_S.sp.CMB-0406/hmGy1-696	GCCGAACTCGGCAGC	COCC GAAG	ACGOTOCAACTO	OCCGACO	O TGCCCGCCC	8 C <mark>C C</mark> C	- COOCTGATCTOT	CTGTCCACAC	CCATGO	CCGG CGGCGG TG TG CA
Hitachimycin_TE_nc_S.scabrisporus@itP3y1-696	ACCOACCTCOAACOO	TTC <mark>CC</mark> GGAG	CCCCCCCCCCCCC	OCCGACO	GACGAGCOC	8 G C C G	- COOTTGATCTOC	GTCTCGACCC	GATOG	TCOCCOOTOCCOTOCA
ML-449_TE_nc_S.ap / 1-696	OCCOAACTCOCCCOC	CCCCGAAG	ACCOTCCAOCTC	GCCGACO	GCGCCCGCCC	8 C <mark>C C</mark> C	- COOCTOATCTOT	CTOTCCACCC	CCATOO	CCOG COG COG TO TO CAS
Sceliphrolactam_TE_nc_S.sp.SD85(SceT)/1-684	OCCOAACTOOCCOCO	TOGAGCAG	CCCOTACOCCTC	0000000	O C G G C G A G O G	8 C C C C	AAGCTGATCTGT	TTCCCCTCGC	GATOS	CCCTCGGCGGCGCCCA
Sipanmycin_TE_nc_S.sp.CS149(Sip-P5)/1-696	OCOGAC CTOGAACOO	COCCOGTO	TCCOTOCOGATO	OCCOACO	C C A C G A G G	co <mark>cc</mark> o	- CACCTOOTOTOC	CTOTOCACCO	COOCCO	COATOBOCOOCOCOTA
Lineamycin_A_TE_nc_S.sp.My1¢nyHV1-690	OCCOAOCTOCOCAAO	CACCGAGC	CTOOTOCOOCTC	TCGCGCG	CCOOOACGCC	00 <mark>00</mark> C	- GOACTOOTCTOC	TTCTCCTCCA	TCCTGT	COATCTCCOOTCCOCA
Mediomycin_A_TE_nc_S.blastmyceticus(med 9)/1-687	O COGAC CTGG C CAAG	CACCOCC	CTOOTGAGGCTC	T C G G G T G	CC000 CI	стссб	. OCOCTOOTCTOC	TTOTCOTOCA	TCCTGC	COATCTCOOOCCCOCA
Neomediomycin_A_TE_nc_S.sp.RK95-74/nmd9y1-687	OCCOACTODACAAO	COCCACO	CTOOTOCOOCTO	TCCCGCG	GGAGACCOC	0 A C C C	- GCACTOSTOTOC	TTOCCCTOGA	TCCTGT	COATCOGACCCCCCA
Pikromycin_TE_nc_S.venezuelae(pikAIV,Non-polyene)/1-699	O AGO CCTOCTCOOAD	COOCTCOAC	CCOOTOCTOCTC	0000000	O T C C G A C G G	CCGTGC	- GTTCTCGTCGOC	TOCACCOOCA	00000	COAACOOCOOCCCOCA
Erythromycin_TE_nc_Saccharopolyspora_erythraea(Non-polyene)/1-681	GACOGGTTCTCC	CTCGAT	CTCOTOGACATO	OCCGACO	OTCCC OC	AGAGGTC -	ACOGTOATCTOC	TOCOCOGAA	Ceecee	COAT CTC COO TCCOCA

	110	120	130	140	150	160	170	180	190	200	210
Dination TE as Cabattanananana / 40/2as 5494,706	A TARACA	a teo coo	ATCOTTORO	a no no no h	COACA TOACT	Descrete dest	AT PORCT TO	conneda	Andonetin	Concencia	CRARARCACO.
Anabalaria B TE as Conducts ATCC (1999)(Anabala)(1.00)	C A C A C C C A C	CATCOCCO	CCACTTOCO		CONTRACTO	S S S S S S S S S S S S S S S S S S S	ATOPOCTTCO	0000000000	ACTOCIÓN	COCCACCA	TALABOARCA
Amphotencin_B_TE_nc_5.nodosus_ATGC-14830(Amphi(y)-630	CAGTACOCOCO		COACTICCO	COOLAAGE	CONTRICTOR	OCOATACCOCT	ATODOCTICO	000000000		COCCACCAC	STUROUCOUCO
Nystatin_A1_TE_nc_S.nodosus_ATGC-11456(NysK)/1-690	CAGTACOCOCO	CATCOCCOC	OCACITCCO	COOCAGEEE	SCCATO TOTOC	acacracecert	ATOBOCTICO	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	AGGTCETCET	OBCCACCAC	COAGOCCOCO
Candicidin_TE_nc_S.apFR-008flacFV1-711	CAGTACGCCAC	TCTCTCCGG	CCACTTOCO	GEGECGCC	REGACOTCACE	SCOCTOCCCCT	CATCOGCTTC	ACACCOCC	AGGCCCTGC	COCCCACGO	COAGOCGGCC
filipin_TE_nc_S.avemitilis_MA-4680(pteA5)/1-693	CAGTACOCOCO	STTCOCCOC	ACCUTTCOG	COACCOOC	TEGACETOTEG	GCGCTGGTCCA	CCCCGGCTACO	AGAAGGGCG	AGCCGGTCC	GTCCGACCO	COCACO TCOTO
Pimaricin_TE_nc_S natalensis_ATCC-27448@imS4y1-705	GAATACOCCAD	SCTCOCCOC	OTCCTTCCO	AGGTGAGCO	OCACOTCAGE	OCOCTOCCOCTO	GTCOOCTTTC	CCACOOGOO	AGCOTCTOC	OGCCACTO	COCOCOCOCO
Reedsmycins_TE_nc_S.Sp_OUC6819pdmJy1-693	CAGTACTACCA	STTCSCTCG	GGCGTTCCC	COGACACC	COAGOTCOCO	CTCTACACGAT	BCCTOOCTTC	GCGATGGGG	AGACGCTGC	GOCCGACTI	TO ACO TACTC
Eurocidin_D_TE_nc_S.eurocidicus_ATCC-27428/1-696	GAGTACGTCAG	STTCGCCGC	GCACTTCCG	GGGCGAGC	CCACGTCTGC	O C G G T G C C A C T C	GTCGGCTTCG	CGGCGGGGGG	AGCOGCTOC	GOCCAACCO	CGAGACCGGG
Lucensomycin_TE_nc_S.achromogenes_NBRC-14001(LucEy1-702	CAGTACGCCCG	TATCOCCO	CCACTTCCG	CGGCGACCO	CGGTGTGCAG	GCGTTGCCACTO	GTCGGGTTCG	CGGCGGGGGGG	AGAGCCTGC	CGGCTCGGC	CACAGGCCATC
Lucensomycin_TE_no.S.cyanogenus@cmEV1-702	CAGTACOCCCO	TCTCOCCOC	CCACTTCCO	COGCOACCO	TOOCOTOCAG	OCATTOCCACTO	OCT COOS TTCC	COOCCOGAO	AGTOCCTOC	CAGCTCOTO	CCCOOCCATC
Tetranycin B TE nc S.hygrospinosus CGMCC-4.1123(TetrE)/1-699	GAGTACOCCAG	STTCOCCOC	CCGCTTCCG	COGCCGAC	GAACGTCAGT	OCCCTOCCOCTO	GTCOGCTTCO	CCCCGGGGGG	ACCCGCAGC	GOCCACTO	COAGTGCGCC
Pentamycin TE nc S.ap.S816/1-693	CAGTACGCGCG	STTCSCCGC	GCCCTTCCG	CGACCOGO'	TGGACATGTGG	GEGETGGTCAA	CCCCGGCTACG	AGCAGGACO	AACCGGTCC	COCCGACCO	GGACGCCGTG
Selvanioin TE no Pseudonocardia ap.HH130630-07/1-699	CAGTACGCGCG	SCTGGCGGG	GCACTTCCG	TOGCCGCCC	CCGGGGTGCTC	GCGCTGCCGCTC	GTCGGGTTCG	AGCCCGGTO	AGACGCTGC	GGCCACCGC	COAGOCGOCG
NPP(Nys-like) A1_TE_nc_Pseudonocardia_sp_AL041005-10(CPPKy1-690	CAGTACOCCCO	CATCOCCOC	OCACTTCCO	COOCAGCCO	OCACOTOTCC	OCOCTOCCOCTO	ATGOOCTTCO	CACCCOCCO	AGCTOCTOC	TOCCACCAC	COAGOCCOCO
Auroramycin_TE_nc_S.rozeosporus_NRRL-15998/1-696	CAGTACOCGAA	BCTCOTCTC	COCCTTCAA	0000000000	OACG OT CACC	O T C C T O C C O A T O	SCCOBBCTTCO	occccocc	AGCAGCTOC	COCCTCCO	CCCCGCCGCC
BE-14106_TE_nc_S.sp.DSM-21069(becG)/1-696	CAGCACGCCCG	SCTCGGTTC	CGAATTCCC	GGACGTGC	BOCACOTOTCO	GCGG TGGCACTO	CCCGGATTCC	ACCOGGACO	AGCCACTOC	CGACTCCG	COAGO TOCTO
Bombyxamycin_A_TE_nc_S.sp.SD53@omP1V1-699	CAGCACGOGCG	TCTGGTGTC	CCACTTOGG	AGGCCGGC	ACAAGATATCC	GEGETGECTGT	ACCCGGATTC	TCGCGGGCG	AGAGCCTGC	CACGTCCTO	CCACGCGGCG
Creminycin_TE_nc_S.sp.MJ635-86F5(smiP6)/1-699	CAGCACOCCCO	SCTCOCCOC	OCACTTCCO	COGCAAGCO	CAGCOTCACO	GOACTOGCATTO	CCCCCCTTCC	GGGACOOCO	AGCGACTOC	GOCCACOO	00000000000
Heronamide C TE nc S.sp.CMB-0406dhm GV1-696	CAACACOCCCO	CCTCOOCTC	COAGTTCCO	COACATCO	OCCCOTOTCC	OCOCTOOCOCTO	SCCCOGATTC	AGCODOGCO	ASCCOCTOC	COAATCCOI	COAGO TOCTO
Hitachimycin_TE_nc_S.scabrisporus/hitP3y1-696	CAACACGCCCG	CCTGGCCGC	GCACTTTCG	COGCOTCC	GCCGGTAAGC	GCGCTGCCGTTC	GTCGGCTTCG	CTCGGGGAG	ACCTOCTOC	COCCACOOO	000000000000000000000000000000000000000
ML-449_TE_ne_S.ap./1-696	CAGCACGCCCG	CCTCGGCTC	CGAATTCCG	GGACGTCCC	GCCCGTGTCC	GCGCTGGCACTO	CCCGGGATTO	AGCGGGGCG	AGCCGCTGC	CGAGTCCGI	CGAGG TGCTG
Sceliphrolactam_TE_nc_S.sp.SD86(SceTy1-684	CAGTACOCOCO	CTTCGCGGC	CCGTTTCCG	GGGCCGCCC	GGAGGTCGTC	O TOCCCOCCO TO	CCOGGCTTCC	GCAAGGGCG	ACOCCCTOC	CCTGTCGG	COACOCCOCC
Sipanmycin_TE_nc_S.sp.CS149(Sip-P5y1-696	CAGTACOCOAA	BCTCATCTC	COCCTTCAA	obococcc	GACGOTCACC	OTOCTOCCOATO	CCOBOCTTCO	OCCCCOOTO	AACAGCTOC	COCCTCCOT	reccescece
Lineamycin_A_TE-nc_S.ap My16nyHV1-690	CAGTACGCCCG	CTTCGCGTC	COCCTTCCO	COGCCGGA	GGACGTCCAC	GCGATGGCCGC	CCCOGTTTCC	TOCOGOCCO	AGCAACTOC	CTCGACGGO	CGAAGCGGTG
Mediomycin_A_TE_nc_S.blastmyceticus/med/9/1-687	CAGTACGCCCG	STTCGCCGC	CGGCTTCCG	TOGCCACCO	GGACGTATGG	GCGATGGCCAA	CCCCGGCTTCG	TGGCGGGGG	AGCAGCTCC	GGCGACCCC	GGAGGCGGTG
Neomediomycin_A_TE_no_S.sp.RK96-74/nmd9y1-687	CAGTACOCOCO	STTCOCCOC	COGATTOCO	COGCACCO	BOGACOTCTOG	GCGCTGGGCAA	CCCCGGTTTCC	TCOCCOOOO	AGCTGCTTC	GOCGAACCE	GOAGOCGOTG
Pikronycin TE nc S.venezuelae@ikAlV.Non-polyeneV1-699	GAGTTCCTOCO	OCTCAOCAC	CTCCTTCCA	O AGGAGC	BOOACTTCCTC	OCCOTACCTCT	CCCCGGCTACO	OCACOOO TA	COCTCCTCC	OBCCOATCI	COACACCOCO
Erythromycin_TE_nc_Saccharopolyspora_erythraea(Non-polyene)/1-681	GAGTTCACCCO	SCTESCESS	66C6C16C6	COGAATCO	CTCCGGTTCGG	GCCGTGCCCA	ACCCOGCTACO	AGGAGGGCG	AACCTCTGC	GTCGTCGAT	66 C66 C66 T6

	220	230	240	250	260	270	280	290	300	310	320
Pimaricin TE nc S.chattanoogensis L-10(ScnS4)/1-705	стссс <mark>сст</mark> сс	тс <mark>ссс</mark> баба	<mark>сасст</mark> тссс	GCG A	GCGACGGCAACC	ссттсетссте	TCGGGCACT	CGTCCGCCGG	CGCGTTCGCC	ТАССТСССС	CGGCCCTG
Amphotericin_B_TE_nc_S.nodosus_ATCC-14899(AmphK)/1-690	G C C C G <mark>G</mark> A T C G	тс <mark>ссс</mark> баба	<mark>ссстс</mark> стсатс	G C G A	GCGACGGCGAAC	с с ттс с тс Атс с	TGGGCCACT	CCACCGGCGG	ттссс <mark>тссс</mark> с	Т <mark>АССТС<mark>ССС</mark></mark>	CCGGGGGTG
Nystatin_A1_TE_nc_S.nodosus_ATCC-11455(NysK)/1-690	G C C C G T A T C G	тс <mark>бсс</mark> баба	G С <mark>G Т</mark> С <mark>С Т </mark> G А Т G	GCC···A	G C G A G G G C G A A C (с оттс отс Ато о	TCGGCCACT	CCACCGGCGG	стс <mark>сст</mark> б <mark>сс</mark> с	TACCTCGCCG	CCGGCGTC
Candicidin_TE_nc_S.spFR-008(fscF)/1-711	G C G C G <mark>G</mark> A T C A	тс <mark>ссс</mark> аст	G C A C C <mark>C T</mark> C C A G	GCCG	CCGACGGCAAGC	с <mark>стт</mark> сетссте <mark></mark> с	TCGGCCACT	сстссвесве	стсс <mark>стс</mark> сс	T <mark>A C G C G G C G G</mark>	CCGGAGTC
filipin_TE_nc_S.avermitilis_MA-4680(pteA5)/1-693	T T C C G <mark>G</mark> C T G C	A C <mark>G C</mark> G C <mark>A G</mark> A	C C <mark>G T</mark> A <mark>C</mark> G G G A G	төтө	C C G G C G G A A A G C (с в в с <mark>с в т</mark> с <mark>с т в</mark> с	TCGGCTACT	CCTCGGGCGG	CTGGATCGCC	C <mark>A C G G C G T G G</mark>	CGGCACAC
Pimaricin_TE_nc_S.natalensis_ATCC-27448(PimS4)/1-705	G T G C G <mark>G G T</mark> C A	ТС <mark>СС</mark> БАБА	<mark>сс</mark> сс <mark>стс</mark> сс	G C G A	G C G A C G G C A A C C (с <mark>от</mark> тсот <mark>осто</mark> с	TCGGGCACT	CGTCCGCCGG	CGCCTTCGCC	TACCTGGCTG	CGGCCCTG
Reedsmycins_TE_nc_S.Sp_OUC6819(rdmJ)/1-693	G G C A G <mark>G</mark> C T C C	A G <mark>G C</mark> G <mark>G A G</mark> G	с <mark>сет</mark> ст <mark>тс</mark> ссс	C G <mark>G</mark> T A C C	C G A A C <mark>G G</mark> G G A G <mark>C</mark> (з <mark>остсстссто</mark> с	C G <mark>G G</mark> C A T G T	CGTCCGGTGG	A T G G C T G <mark>G C</mark> A	C <mark>A</mark> T T C <mark>G</mark> A C G <mark>G</mark>	CCGTACAT
Eurocidin_D_TE_nc_S.eurocidicus_ATCC-27428/1-696	G T C A G <mark>G </mark> G T C A	T C <mark>G C </mark> G <mark>G A G</mark> A	с <mark>сс</mark> сс <mark>стс</mark> ссс	G C G A	G C G A C G G C G A G C (с <mark>с ттс с тс</mark> с т с <mark>с</mark>	TCGGCCACT	CCTCCGGCGG	G T C G C <mark>T C G C</mark> C	TACGCGGCCG	CCGGCCTG
Lucensomycin_TE_nc_S.achromogenes_NBRC-14001(LucE)/1-702	A C A C G <mark>G G</mark> C C G	T C <mark>G C</mark> G <mark>G A</mark> A A	G Т <mark>G Т </mark> G <mark>С Т G</mark> С А С	GCC···G	GCGACGGGAACC	с с ттс с т <mark>с</mark> с т с с	TCGGTCACT	CGTCGGGCGG	TGCGCTGGCC	ст <mark>бссбс</mark> тб <mark>б</mark>	CCGGGCTG
Lucensomycin_TE_nc.S.cyanogenus(LcmE)/1-702	A C A C G <mark>G G</mark> C C G	C C <mark>G C </mark> G <mark>G A G</mark> A	<mark>стстс</mark> сас	GCC···A	G C G A C G G G G A C C (с <mark>оттсо</mark> тосто	TCGGCCACT	CGTCGGGCGG	TGCGC <mark>T</mark> G <mark>GC</mark> C	с <mark>т </mark>	CCGGGCTG
Tetramycin_B_TE_nc_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-699	G C C C G <mark>G</mark> A C G A	T C G C <mark>G G A </mark> G A	<mark>сссссстс</mark> сс	GCC · · · A	G C G A C G G C G A A C (C C T T C A T C C T G G	TCGGACACT	CCTCCGCCGG	CGCGTTCTCC	TACGCGGCCG	CGGGCGTG
Pentamycin_TE_nc_S.sp.S816/1-693	с т <mark>с с б б</mark> с т б с	A C <mark>G C</mark> C C G <mark>G</mark> A	C C <mark>G T</mark> A <mark>C </mark> G C G A G	төсө	C G G G C G G A A A G C (с <mark>батс бтс</mark> с т б	TCGGCTACT	CCTC <mark>G</mark> GGCGG	C T G G A T C G C C	C <mark>a c g g c g t c g</mark>	CCGCACAC
Selvamicin_TE_nc_Pseudonocardia_sp.HH130630-07/1-699	A T C G A <mark>G</mark> T C G G	T C <mark>G C </mark> G A G A	G C <mark>G T </mark> G <mark>C T </mark> C C G G	GCC···G	C G G A C G G A G C A C (с о т т с о т <mark>о с</mark> т о о	TCGGGCACT	CCACCGGCGG	T T C G C T G G C C	TACGAGGCGG	CCGGGTTG
NPP(Nys-like)_A1_TE_nc_Pseudonocardia_sp.AL041005-10(CPPK)/1-690	G C C C G <mark>G</mark> A T C G	ТС <mark>ССС</mark> АААА	<mark>сс с т</mark> с т с с т с	G C C A	G T G A C G G C G A G C (C G T T C G T <mark>G A T G</mark> G	TCGGGCACT	CCACCGGCGG	GTCGCTGGCC	TACCTGGCCG	CAGGGGTG
Auroramycin_TE_nc_S.roseosporus_NRRL-15998/1-696	C T C G A C G T C C	тссссссс	C C G T C A A G G A C	A T C G	T C G G T G A C G C A C (с <mark> б т т с б с с с т с</mark> с	TCGGGCACT	CCGGCGGCGGCGG	GCTCCTCGCG	CACGCCACCG	CCGGGCTC
BE-14106_TE_nc_S.sp.DSM-21069(becG)/1-696	A C A C A <mark>G G T</mark> G C	T G <mark>G </mark> G C <mark>G A</mark> C G	сс <mark>ст</mark> сстсссс	GCG···G	C G G A C G G T G A G C (C G T T C G T A C T G C	TCGGCTACT	CCTCCGGCGG	CATCATCGGC	CACATCATC	CCCGTCAC
Bombyxamycin_A_TE_nc_S.sp.SD53(bomP1)/1-699	G Т <mark>С</mark> С А <mark>G</mark> G С С С	T C <mark>G C</mark> C C G <mark>G</mark> A	G C <mark>G T G C T G</mark> G A C	A C G G	C C G G A <mark>G G </mark> G G A G C (C G T T C G T A C T G C	TGGGCTACT	CCTCCGGAGG	CACCCTCGCC	TACGCCACCG	CCGGCCAT
Cremimycin_TE_nc_S.sp.MJ635-86F5(cmiP6)/1-699	G T <mark>C</mark> G A <mark>G</mark> C G G	T G <mark>G C</mark> G <mark>G A G</mark> G	A G <mark>G T G C T</mark> C C G C	<u> </u>	C G C G G <mark>G</mark> A A G A G C (C G T T C G T G C T C G	TCGGCCTCT	CGTCCGGCGG	CATCCTCGCG	T <mark>a c </mark> c c c c c t c c	CCCACCTC
Heronamide_C_TE_nc_S.sp.CMB-0406(hm G)/1-696	Τ C A C A <mark>G G T</mark> C C	T C <mark>G </mark> G C <mark>G A G</mark> G	C C <mark>G T</mark> T <mark>C T</mark> C G C G	<u> </u>	C G G A C G G C G A C C (C G T <mark>A C G T A</mark> C T G C	TCGGCTACT	CCTCCGGCGG	CATCATCGGC	C A T G T C C T C G	CGCGTCAC
Hitachimycin_TE_nc_S.scabrisporus(hitP3)/1-696	G C A C A <mark>G </mark> G T G G	T C <mark>G C</mark> G A G A	A C <mark>G T</mark> C <mark>C T</mark> C C G G	<u> </u>	C C C C C C C C C C C C C C C C C C C	с	TCGGGTATT	сстссббсбб	TGTCCTGGCC	T A T G C G G C G G	CGCACCAC
ML-449_TE_nc_S.sp./1-696	T C A C A <mark>G G T</mark> C C	T C <mark>G </mark> G C <mark>G A G</mark> G	C C <mark>G T A C T</mark> C G C G	<u> </u>	C G G A C G G C G A T C (C G T A C G T C C T G C	TGGGCTACT	CCTCCGGCGG	CATCATCGGC	C A T G T C C T C G	CGCGCCAC
Sceliphrolactam_TE_nc_S.sp.SD85(SceT)/1-684	G T C G A <mark>G</mark> G T G T	T C <mark>G C C G A</mark> A G	А	<u>G C</u> C G	C C G C G G G G C G A G C (с <mark>сттсет</mark> ссте <mark></mark>	TCGGCTACT	CCTC <mark>G</mark> GGCGG	GCAGTTCGCC	CACGCGACCG	CGGAGGTG
Sipanmycin_TE_nc_S.sp.CS149(Sip-P5)/1-696	C T C G A C <mark>G T</mark> C C	T C <mark>G C </mark> G G <mark>G C </mark> G A	C G <mark>G T</mark> C <u>A A </u> G G A C	A T C G	T C G G C G A C G G A C (с <mark>бттсбс</mark> стбс	TCGGGCACT	CCGGGGGGCGG	GCTCCTCGCG	CACGCCACCG	CCGGGCTC
Lineamycin_A_TE-nc_S.sp.Mg1(InyHI)/1-690	A T C G A <mark>G G</mark> C G C	A G <mark>G C </mark> C <mark>G A G</mark> G	с <mark>батсстб</mark> сбб	C A C G	C G <mark>G A C G G </mark> G G A G C (с оттсотсстос	TCGGACACT	CCTCCGGCGG	CATGCTCGCC	C <mark>a c g c g g t g g</mark>	CCGGCCGG
Mediomycin_A_TE_nc_S.blastmyceticus(med9)/1-687	A T C G A <mark>G G</mark> C G C	A G <mark>G C </mark> C <mark>G A G</mark> G	с <mark>с т</mark> с т с с с с с с с с с с с с с с с с	C G <mark>G</mark> A	C G G A C G G T T C A C (с <mark>сттсстс</mark> стс	TGGGCCACT	CCTC <mark>G</mark> GGCGG	CATGCTCGCG	C A C G A G G T C G	CGGCGCGG
Neomediomycin_A_TE_nc_S.sp.RK95-74(nmd9)/1-687	A T C G A <mark>G</mark> G C G C	A G <mark>G C </mark> C <mark>G A G</mark> G	C G <mark>G T G C T G</mark> C G A	CAG A	C C G A C G G G G C G C (с <mark>от</mark> тсот <mark>о</mark> стос	TGGGCCACT	CCTC <mark>G</mark> GGCGG	GCTGCTCGCC	C <mark>A T G A G G T G G</mark>	CCGCGCGG
Pikromycin_TE_nc_S.venezuelae(pikAIV,Non-polyene)/1-699	С Т <mark>С</mark> G A C <mark>G</mark> C C C	A G <mark>G C</mark> C C G <mark>G</mark> G	C G A T C C T C C G G	<u> </u>	C C G G G G A C G C C C C	с <mark>ботсо</mark> тсстос	TCGGGCACT	CCGGCGGCGC	CCTGCTCGCG	C <mark>A C G A </mark> G C T G <mark>G</mark>	CCTTCCGC
Erythromycin_TE_nc_Saccharopolyspora_erythraea(Non-polyene)/1-681	G C G G C <mark>G G T</mark> G C	A G <mark>G C </mark> C <mark>G A</mark> T G	C G <mark>G T</mark> C A <mark>T</mark> C A G G	ACA···C	A G G G G G A C A A G C (CGTTCGTGGTGG	CCGGTCACT		A C T G A T G G C C	TACGCGCTGG	CGACCGAA

	330	340	350	360	370	380	390	400	410	420	430
Pimaricin_TE_nc_S.chattanooyensis_L-10(ScnS4)/1-705	CTGGAG <mark>AACAC</mark> C	TGGGGCATCA	AGG <mark>CCCG</mark> AG <mark>G</mark> C	CGTGGTGCT	G C T G G A C A C C	C T C A G C C T C C G G	C <mark>ac</mark> gagca	G A A C G A G A C G	ATCGACTACGCG	GGGCTGA	A T <mark>G</mark> C G A
Amphotericin_B_TE_nc_S.nodosus_ATCC-14899(AmphK)/1-690	C T G G A G A C A C C	тө <mark>өөө</mark> сөтө <i>А</i>	A A G C C G <mark>G</mark> A G <mark>G</mark> C	GGTCGTCCT	GCTCGACACG	G C G T C <mark>C</mark> A <mark>T C</mark> C G G	TACAACCC	стс <mark>ба</mark> ббб	AACAACCTCGAC	C A G A C <mark>G</mark> A	ACCCGC
Nystatin_A1_TE_nc_S.nodosus_ATCC-11455(NysK)/1-690	C T G G A G A C A C C	тб <mark>бб</mark> асбтс (C G G C C C G A A G C	б б Т б <mark>б Т</mark> С С Т	C C T C <mark>G A C A</mark> C C	G C G T C <mark>C A T C C G</mark> C	TACAACCC	C G G C G A G G G <mark>C</mark>	AACGACCTGGAC	C G C A C C A	A C <mark>G</mark> A G G
Candicidin_TE_nc_S.spFR-008(fscF)/1-711	C T G G A C A C A C C C	TGGGGCATCO	C G G C C C G A G G C	сстсстсст	C C T C <mark>G A C A</mark> C C	C T C A G C A T C C A G	C <mark>a</mark> caagag	C G A C G A G G G <mark>C</mark>	GTCGACTACAAC	GGCATGA	A T <mark>G</mark> A A G
filipin_TE_nc_S.avermitilis_MA-4680(pteA5)/1-693	CTGGAAGCC	A T G G G G G A G (c g g <mark>c c g g</mark> c g g c	CGTGGTGAT	G G T C G A C A G C	TTCAGC CGC	G A G G T C C C	CTT <mark>CGA</mark> C	CACCAGGTGCTC	AAC · · · GC <mark>G</mark> A	A T <mark>G</mark> G C C
Pimaricin_TE_nc_S.natalensis_ATCC-27448(PimS4)/1-705	C T G G A G A A C A C C	TGGGGCATC	C G G C C C G A G G C	CGTCGTGCT	G C T G G A C A C C	C T C A G C C T C C G G	C <mark>acga</mark> gca	G A A C G A G A G C	ATCGACTACGCC	G G C C T G A	A T <mark>G</mark> C G A
Reedsmycins_TE_nc_S.Sp_OUC6819(rdmJ)/1-693	C T G G A G G C G C T G	<mark>бб</mark> ббТб/	A A G C C C G C G G G	CTTGGTGCT	GCTCGACACG	TACCTGCCGCTG	G C <mark>C G A</mark> G C A	G T T G <mark>G A</mark> G C G G	с т G А G <mark>С G</mark> С G	<mark>бб</mark> бтб <mark>б</mark> С	ат <mark>с</mark>
Eurocidin_D_TE_nc_S.eurocidicus_ATCC-27428/1-696	C T G G A G A A C A C C	тб <mark>ббб</mark> атси	A A <mark>G C C</mark> C A C G <mark>G</mark> C	сстостост	G C T G G A C A C C	C T C A G C A T C C G G	CACGACCG	G A A C G A G G A <mark>C</mark>	ATCGACTACGCC	G G T C T G A	A T <mark>G</mark> C G G
Lucensomycin_TE_nc_S.achromogenes_NBRC-14001(LucE)/1-702	<mark>ς τος ας</mark> ας ς α τς	то <mark>сс</mark> тстс	C G C G <mark>C C G</mark> A C <mark>G</mark> G	CGTGATCAT	G C T G G A C A C G	C T C A G C C T G C G G	C <mark>acga</mark> gca	C <mark>G G G G A</mark> C A G <mark>C</mark>	G T G G A C T A C C G G	C A A C T G G	3 C C A G G
Lucensomycin_TE_nc.S.cyanogenus(LcmE)/1-702	<mark>стобаб</mark> абсатб	т <mark>с с с с</mark> с с т с (C G C G <mark>C C G</mark> A C <mark>G</mark> G	CGTGATCAT	GCTCGACACG	C T C A G C C T G C G G	C <mark>acga</mark> gca	C <mark>G</mark> GG <mark>GA</mark> CAGT	<mark>g t g g a c t a c</mark> c g g	C A G C T G G	3 C C A G G
Tetramycin_B_TE_nc_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-699	C T G G A G A G C A C C	Т G <mark>G G G</mark> C A T C A	A A G C C C G C G G G	ACTGGTGCT	G C T G G A C A C C	C T C A G C A T C C G G	C <mark>a</mark> cgagga	G A A C G A G G A <mark>C</mark>	ATCGACTACGGC	<mark>G G C C T G</mark> C	CT <mark>G</mark> AAG
Pentamycin_TE_nc_S.sp.S816/1-693	C TGGAG <mark>G</mark> CCATG	<mark>G G </mark> G G A G (c g g <mark>c c c g</mark> c g g c	CGTGGTCAT	G G T C G A C A G C	TTCAGCCGCGCG	ст <mark>с</mark> ссстт	C <mark>G</mark> A <mark>C</mark> C G C C A G	G T G · · · C T C A A C	<mark>G</mark> C G A <mark>T G</mark> G	эсс
Selvamicin_TE_nc_Pseudonocardia_sp.HH130630-07/1-699	A TGGAG GAGCGG	тсссссстс	C A G C C G G A G G C	GGTGATCAT	GCTCGACACG	A T G A G C C T G C G C	Т <mark>АС</mark> БССБА	G G G G G A G G G <mark>C</mark>	GCCGACTACGAG	<mark>66</mark> 66 <mark>7</mark> 66	3 G C C G C
NPP(Nys-like)_A1_TE_nc_Pseudonocardia_sp.AL041005-10(CPPK)/1-690	C T G G A G A G A C C	т б <mark>б б б</mark> б б т б (C G G C C C <mark>G</mark> A G <mark>G</mark> C	G G T G <mark>G T</mark> G C T I	G C T C G A C A C C	G C C T C G A <mark>T C</mark> C G G	TGGG <mark>A</mark> CGC	G G C C G A G G G <mark>C</mark>	AACGACCTGGAC	C G C A C G A	ACCCGG
Auroramycin_TE_nc_S.roseosporus_NRRL-15998/1-696	<mark>стобаб</mark> баасаб	<mark>GG</mark> ACGG	сс <mark>ссс</mark> сст <mark>с</mark> с	CGTCGCGCT	<mark>g c t g g a c a </mark> c g	ΤΑ C C T C G T C G A A	C <mark>A</mark> GACCGC	с <mark>в</mark> вс тт <mark>с</mark>	CAG <mark>GACTTCG</mark> CG	G C C <mark>G A G G T</mark> C G	э <mark>с</mark>
BE-14106_TE_nc_S.sp.DSM-21069(becG)/1-696	C T G A A G G A G A C G	C T C A A G G T C (c c <mark>c c c c c</mark> c c <mark>c</mark> c	ACTCGTCCT	GATCGACACC	TTCAGGGTCGAG	G <mark>AC</mark> ACGGC	G A T G • • • A A C	G T C G G G T T C G A C	CACCTCA	АТ <mark>G</mark>
Bombyxamycin_A_TE_nc_S.sp.SD53(bomP1)/1-699	C T G G A A C G G G A G	TAC <mark>GG</mark> GGTG	c g c <mark>c c g g</mark> c g <mark>g</mark> g	CGTCATCCT	G T T G G A C A C G	T T C A A G G T G C A C	G <mark>ACGGGGG</mark> G	ТА <mark>С G А</mark> С G G <mark>С</mark>	GTGCCCCTCGAC	<mark>66 C C T</mark> C G	Э <mark>С</mark> А
Cremimycin_TE_nc_S.sp.MJ635-86F5(cmiP6)/1-699	C T G G A G C A C G G C	G A <mark>G</mark> C A G G C C (c g g <mark>c c </mark> c g g g g g g g g g g g g g g g g g	AGTCGTGCT	<mark>g c t c g a c t</mark> c c	TACGCGGTCGCC	3 <mark>a</mark> t c <mark>a</mark> c g g	G A T G • • • C C C	G C C G G G T T C G A G	CACATGA	A C <mark>G</mark>
Heronamide_C_TE_nc_S.sp.CMB-0406(hm G)/1-696	C T G G A G A G A C G	G T C <mark>G G</mark> C A G G (c c <mark>c c c c c c c c</mark> c	GCTCGTCCT	GATCGACACC	TTCCGCGTCGAG	G <mark>a</mark> caccgc	G A T G - · · A A C	GTCGGCTTCGGC	CATCTCA	АТ <mark>G</mark>
Hitachimycin_TE_nc_S.scabrisporus(hitP3)/1-696	CTGGAG CACGTG	C T <mark>G G G</mark> C A T T (C G G C C C G C A G G	CGTCGTCCT	G T T G G A C T C G	TATGCCGTCGCG	3 <mark>A C A A</mark> C G C	GATG C C G	GAGGGCTTCGAG	CACATGA	A C G
ML-449_TE_nc_S.sp /1-696	C T G G A G A G A C G	стс <mark>сс</mark> сстс	c c <mark>c c c c c c c</mark> c c <mark>c</mark> c	GCTCGTCCT	GATCGACACC	TTCCGCGTCGAG	G <mark>a</mark> caccgc	CATG · · · AA <mark>C</mark>	GTCGGCTTCGGC	C A T C T C A	A T G
Sceliphrolactam_TE_nc_S.sp.SD85(SceT)/1-684	CTGGAG <mark>AA</mark> G	G C <mark>G G G</mark> C A C C (c c <mark>e e c</mark> e c e <mark>e e</mark> c	CGTCGTCCT	G C T G G A C A C C	TATCTGCCCGGC	9 <mark>a c g </mark> g c a a	g G A <mark>C</mark>	G A G C T C T G G C G C	C A G A T G T	гтс
Sipanmycin_TE_nc_S.sp.CS149(Sip-P5)/1-696	CTGGAGGAA	CA <mark>GGG</mark> ACGG	C C <mark>G C C C G</mark> T G <mark>G</mark> G	CGTCGCGCT	<mark>g c t g g a c a c g</mark>	TACCTCGTCGAA	C <mark>A</mark> GACCGC	с <mark>сс</mark> стт <mark>с</mark>	C A G <mark>G A C T T C G</mark> C G	G C C <mark>G A </mark> G G <mark>T</mark> C G	3 C G
Lineamycin_A_TE-nc_S.sp.Mg1(InyHI)/1-690	CTGGAG <mark>G</mark> GC	G C C <mark>G G G G T</mark> C 1	ттс <mark>сс</mark> б <mark>бабб</mark> с	б б Т б <mark>б Т С С Т</mark>	C G T C G A C A T C	TACTCGCACGAC	G <mark>ac</mark> gacgc	сат <mark>с</mark> ат б	<mark>g g c</mark> a t <mark>c</mark> c a g c c <mark>c</mark>	G G G C T G T	Г С <mark>С</mark>
Mediomycin_A_TE_nc_S.blastmyceticus(med9)/1-687	C T G G A G G C C	A C <mark>G G G </mark> C G T C 1	T T C <mark>C C</mark> G C A G G C	GGTC <mark>GTC</mark> CT	C C T C G A C A T C	TACTOCCACGAC	ACGG <mark>A</mark> CGC	6 6 C C A C <mark>C</mark>	<mark>g g c</mark> a t <mark>c</mark> c a g c c <mark>c</mark>	G G C C T G A	4 <u>GC</u>
Neomediomycin_A_TE_nc_S.sp.RK95-74(nmd9)/1-687	CTGGAGGGC	A C C <mark>G G </mark> A <mark>G T C</mark> 1	T T C <mark>C C </mark> G <mark>G</mark> A C <mark>G</mark> C	GGTGGTGCT	GCTCGACATC	TACTCGCACGAC	C G G G <mark>A</mark> G G C	GGCC · · · ATG	G C C C T C C A G C C C	GGTC <mark>TG</mark> C	3 G <mark>G</mark>
Pikromycin_TE_nc_S.venezuelae(pikAIV,Non-polyene)/1-699	CTGGAG CGGGCG	CAC <mark>GG</mark> CGCG	c c <mark>c c c </mark> g <mark>g</mark> c c <mark>g</mark> g	GATCGTCCT	G G T C G A C C C C	TATCCGCCGGGC	CATCAGGA	GCCCATCGAG	<mark>g t</mark> g tgg ag <mark>c</mark> agg	· · · CAGCTGG	∋GCGAG
Erythromycin_TE_nc_Saccharopolyspora_erythraea(Non-polyene)/1-681	CTGCTCGAT	CGCGGGCAC	C	TGTCGTCCT	GATCGACGTC	TACCCGCCCGGT	CACCAGGA	C <mark>G</mark> CGATGAA <mark>C</mark>	G C C T G G C T G G A G	GAGCTGA	ACCGCC

	440	450	460	470	480	490	500	510	520	530 54
Pimaricin_TE_nc_S chattanoogensis_L-10(Scn S4V1-705	COCCACTICATO	GTCGACGAGGT	TCGCCGGTACG	GATGACG	AACTCCAGG	CTOTOGOO	GATGOCOCOCTO	GATGGGCATGC	GAACCAGCT	C GAA GTG
Amphotenicin_B_TE_nc_S nodosus_ATCC-14899(AmphKy1-690	TTCTACCTCGCC	GACATCGACTC	CCGTCCGTGAC	GCTCAAC	AGCGCCCGG	ATGTCCGC	GATOGCOCACTO	GTTCATGGCGA	GACGGACAT	C GAC GCG
Nystatin_A1_TE_nc_S.nodosus_ATCC-11455(NysK)/1-690	TTCTACCTOOCC	GACATCOACTCO	CCCTCGOTGAC	OCTCAAC	AGCOCCCOG	ATOTCCOC	CATOOCCCACTO	GTTCATGGCGA	GACCGACAT	C C A O O C O
Candicidin_TE_nc_S.apFR-008(facFy1-711	TTCAACTTCACC	OCCOTCOACOAC	TCCCCCOTOCO	CCTGACC	AACTCCCCC	CTOTCCOC	CATOGOCCOCTO	GATGGTGCTCC	CAACOCCCT	C GAC GTG
filipin_TE_nc_S.avemitiliz_MA-4680(pte A5)/1-693	· · · CAG · · · GCG	CACTCOCAGCO	CTGGACTTCAT	GAGGTCCGGG	GGCGAACAG	CTCACCGO	GATGGGCCGCTA	TATGCGCCTG .	. TTCGACGA	CTGGGCCGCG
Pimaricin_TE_no_S.natalensis_ATCC-27448(PimS4)/1-708	COCCACTICATO	GTCGACGAGGT	TCGCCGGTACG	GATGACG	AACTCCAGG	CTGTCGGC	GATOGCOCOCTO	GATGGGCATGC	GAACCAGCT	C GAA GTG
Reedsmycins_7E_nc_S.Sp_OUC6819pdmJy1-693	COTOAGOOTTOO	GACCOGOCOGA	AACTACAACTA	CCTOGAT	- GOCCGGGCA	CTGACCOC	CACOGGTTOGTA	CATOCOCCTOT	TOGOCAGTT	GTOGAAG CCC
Eurocidin_D_TE_nc_S.eurocidicus_ATCC-27428/1-696	CAGAACTICCTO	OTCGACGAGGT	TCCCCCOTOCO	GATGACG	AACTCCCOG	CTOTCCOC	CATOGOACGOTO	OAT 000 C C TO C	GAACCOGGT	C 0 AC 0 TO
Lucensomycin_TE_nc_S.achromogenes_NBRC-14001(LucE)/1-702	AGGTTCATGGGC	GAGACOGACTCO	GCCACCGTGAC	GGTGGAC	AGCAACCGG	CTOTOOOC	CATOGCOCACTA	CCTCAACCGCA	GTCCGCCCT	G G A G G T T
Lucensomycin_TE_nc.S.cyanogenus(LowE)/1-702	AGGTTCATGGGC	GAGACGGACTC	GCCACCGTGAC	GGTGGAC	AGCAACCGG	CTGTCGGC	CATGOCGCACTA	CCTCAACCGCA	GTCCGCCCT	G GAA GTT
Tetramycin_B_TE_nc_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-699	GCGAACTTCCTG	GCATCOGAGOCO	ACCCAGOTCCG	GATCCTG	AACGCCAAC	CTGTCCGC	GATGGGGGGGGTG	GATGCACCTGC	GAACAGCAT	C GAC GTG
Pentamycin_TE_nc_S.sp.S816/1-693	CA0 0 CO CA0	TCOGAOCOCCT	GACTICATOAG	0 T C C 0 0 C	OO COAO CAA	CTCACCOC	GATOGOCCOCTA	CATOCOCCTOT	COACOACTO	· · · · · · · · · · · · · · · · · · ·
Selvamicin_7E_nc_Pseudonocardia_sp.HH130630-07/1-699	TACTACCTCOCC	GACATCGACTCA	ACCOGCOGTCGC	GCTCACC	AGCACCCGG	CTCACCOC	GATGOTOCACTO	GTACAACCGCGC	GOCCOCOCT	G · · · CGCCCGGTG
NPP(Nya-like)_A1_TE_nc_Pseudonocardia_sp.AL041005-10(CPPK)/1-690	TTCTACCTGGCC	GACATCGACTC	CCGTCGGTCAC	CCTCAAC	AGCGCGCGG	ATGTCGGC	GATGGCGCACTG	GTTCATGACGA	GACCGAGAT	C GAC TCG
Auroramycin_TE_nc_S.roseosporus_NRRL-15998/1-696	GTCGGAGCCCTG	ACCCGGGGGGAGAT	TCCTACOGCCA	CICGGCAAI	GCGACCTCG	TGAGCOC	CATOGCOCAGTA	COTOGCCCTGA	GCCGGAGAT	C GAG CTC
BE-14106_7E_nc_S.sp.DSM-21069(becG)/1-696	OG COAAC TOCTO	ACOGTOGAGACO	ACCCTCOGCAA	CTACGAC	OCOOCOCOA	CTOTCCOC	GATOCCOCACTA	CTTCCAGGTAC	GOCGOOCTT	C GAC CCC
Bombyxamycin_A_TE_nc_S.sp.SD53(bomP1)/1-699	CTCGGCATGTTC	GAGAAGGAGGCG	GTCTTCOGCCG	GTTCGAC	AGCAGCAGG	CTCTCCOC	GATEGOCCOCTO	GO TOGAGCTOG	TCCGCAACT	C CCG CTC
Cremin yoin_TE_nc_S.ap.MJ635-86F5(bmiP6)/1-699	CATCOGCTCATO	GAATTGGAGACO	CGATTCOGCCC	GTACCGC	AGCGCGGAA	CTCACCOC	CATGAGCCGGTA	TTTCCACTTCC	GCCGGAATT	C ACG CGG
Heronamide_C_TE_no_S.ap.CMB-0406(hmG)/1-696	OGCGAAC TOCTC	ACGGTGGAGACO	ACGGTCGGCAG	CTTOGAC	ACGGCCCGG	CTGTCCGC	GATGCCGTACTA	CTTCCAGGTGC	GGCGGGCTT	C GAC CCC
Htachimycin_TE_nc_S.scabrisponus/hitP3y1-696	TACCOGATOCTO	GAGATOGAAGCO	ACCTTCOGTCC	OTACGAC	AGCOCOCAA	CTCTGCGC	GATGOCCCOCTA	CTTCCATCTOC	OCCODATT	C ACC COO
ML-449_7E_nc_S.sp /1-696	GO COAAC TOCTC	ACGOTCGAGACO	ACGGTCOGCAG	CTTCGAC	ACGGCCCGG	CTGTCCGC	GATOCCOTACTA	CTTCCAGGTGC	GGCGGGCTT	C GAC CCC
Sceliphrolactam_TE_nc_S.ap.SDB5(SceT)/1-684	CACGGCATGCTG	GACCOGGAGTE	C T C C T T C C G C C G	GTTCAGC	GCCGCCCGG	CTCGCCGC	GATGAGCCGCTA	CAGCGACCTCA	CACCOGCTG	C C T G C C C
S(panmyoin_TE_no_S.ap.CS149(Sip-P5)/1-696	GTCGGAGCCCTG	ACCCGGGAGAT	TCCTACOGCCA	GCTCGGCAA	GCGACCTCG	CTGAGCGO	CATOGCOCAGTA	COTGOCCCTGA	GCCGGAGAT	C GAG CTC
Lineamycin_A_TE-nc_S.sp.Mg1@nyHV1-690	CAGGGAGTOGAC	O AG COO CAGOO	CAGCTACOTACC	OGTCGAC - · ·	GACAGCCGG	CTOCTOOC	GATOOCOCOTA	CTTCAGGCTGT	COOCOOCTO	0 · AA0 · CCO
Mediomycin_A_TE_nc_S.blastmyceticuz(med 9)/1-687	OCGAGEG TOGOC	OAGCOGAGCGA	AGCCATOTOCC	OCTOGAC	OACOCOCOO	CTOCTTOC	CATGOOCOCCTA	CTTCCOCCTCT	COOCOAOTO	· · · · · · · · · · · · · · · · · · ·
Neomediomycin_A_TE_nc_S.ap.RK95-74(nmd9)/1-687	GCCGGGGATCGAC	GAGCGGAGCAC	GGCCCGGTGCC	GGTGGAC · · ·	GACGCCCGG	CTGCTGGC	CATGOGCGCGTA	CTTCCGGCTGT	COGACAGTO	
Pikromycin_TE_no_S.venezuelae(pikAIV,Non-polyene)/1-699	GGCCTGTTCGCG	GGCGAGCTGGAG	CCG	ATGTCC	GATGCGCGG	CTOCTOOC	CATGOGCCOGTA	COCOCOCTCC	COCCOOCCC	.G CGG CCG
Erythromycin_TE_nc_Saccharopolyspora_erythraea(Non-polyene)/1-681	ACOCTOTICOAC	COCGAGACO		GATGGAC	GACACCAGO	CTCACCOC	CCTOGGCOCCTA	COACCOCCTCAC	COOTCASTO	0 · · · COA · · · CCC

	550	D .	560		570	580		590	600		610	620
Pimaricin_TE_nc_S.chattanoogensis_L-10(ScnS4)/1-705	CGGCACACGAC	ссттс	CCGTGCT	IGAT CA	тссстс	CCGCCAAG	GAGACCI	TCGGC	A	тсссана	GACAC	с д <mark>д</mark> а а т с т а
Amphotericin_B_TE_nc_S.nodosus_ATCC-14899(AmphK)/1-690	CCCGCCACGAC		CCACCCI	гсстсс	төсөсө	CACACAG	GCCAACA	ACGGC				TTCATGCT
Nystatin_A1_TE_nc_S.nodosus_ATCC-11455(NysK)/1-690	CCCGCACCGAC		CCACCCI	гсстсс	төсөсө	c c c c c c c c c	вссстсе	ACGGC				ттссббст
Candicidin_TE_nc_S.spFR-008(fscF)/1-711	CACCCCACCAC	ссстсс	CCGTCCT	T G G A G A	т с а а б т	GCACCCG	зосостся	TCGAG	GCGTCC	с с <mark>с</mark> с с с с	CGACCC	с G A <mark>G</mark> С G C C T
filipin_TE_nc_S.avermitilis_MA-4680(pteA5)/1-693	CCGCAGATCG	c c <mark>c c c c</mark>	CGACCCI	гөстсө	TACGG	GAGC	GAGCCCA	TGCTG	o c c o o c o	сссст.	GACGG	TGACGGGCG
Pimaricin_TE_nc_S.natalensis_ATCC-27448(PimS4)/1-705	CGGCACACGAC	сссттс	CCGTACT	TGATCA	тссстс	CACCAAG	GAGACCI	тсссс	A	T C <mark>G</mark> G G A (CGACAC	с <mark>с</mark> аатста
Reedsmycins_TE_nc_S.Sp_OUC6819(rdmJ)/1-693	TCGGAGATCG	CACCC	CGACGCI	TGCTAG	төсөө	CACCGAG	этт <mark>в с т</mark> ве	ATGGC	<mark>.</mark>	A G <mark>G</mark> G T <mark>G</mark> (с G Т <mark>G (</mark>	Э <mark>с</mark> ад тс
Eurocidin_D_TE_nc_S.eurocidicus_ATCC-27428/1-696	CAGCACACGAC	GGCCC	CCGTGCT	CAACC	TCCAGG	с <mark>сстссс</mark>	CAGCACO	CCGGGG	<mark>стт с</mark> ,	А G T C <mark>C </mark>	4 G G A (Э <mark>С</mark> Т <u>Б</u> АБ
Lucensomycin_TE_nc_S.achromogenes_NBRC-14001(LucE)/1-702	CCGCCGACCAC	CGCGC	CGACGT	гөстөө	төсөтт	GCAGCGT	SCCCCTGC	тссст	GACCCCG	ACGCGC	A C G G G C A C	3 <mark>C</mark> A G G A
Lucensomycin_TE_nc.S.cyanogenus(LcmE)/1-702	CCGCCGACCAC	c c <mark>c c c c</mark>	CGACGC	гөстөө	тосост	GCAGCGT	всссство	TCGGA	GATCCC <mark>G</mark>	ACACAC	A C G G G C A C	G C A G G A
Tetramycin_B_TE_nc_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-699	CCCCAGATGA	ACACCC	CCGTGCT	CAGCA	TCCAGG	GACCCG	S C T G T A C C	CGGGC	ATCGCGG	C C G G C G A	A G G G	Э <mark>С</mark> Т <u>Б</u> АБ
Pentamycin_TE_nc_S.sp.S816/1-693	CCCGAGATCAC	ccc <mark>c</mark> cc	CGACCCI	гсстбб	TACGGG	GAGCGAG	CCCGTAC	CG <mark>G</mark> CC0	GCCGCC <mark>G</mark>	CG <mark>G</mark> AC <mark>G</mark> G	3 C G G (s <mark>c</mark> gggggggg
Selvamicin_TE_nc_Pseudonocardia_sp.HH130630-07/1-699	GGCGAGACGAC		CCACCCI	гөстөө	тссссс	G T C G A T C	ссс <mark>в</mark> ство	с <mark>сс</mark> ст	GGGAAG <mark>G</mark>	G <mark>C</mark> CCG		C A
NPP(Nys-like)_A1_TE_nc_Pseudonocardia_sp.AL041005-10(CPPK)/1-690	CCGCCGACGAC	GGCGC	CGACGCI	TG C T G G	TGCGCG	CCGCGCGCGC	CGACG <mark>TC</mark> G	AGGC	ТТСАТ <mark>С</mark> С	т		A C
Auroramycin_TE_nc_S.roseosporus_NRRL-15998/1-696	AAGGAGCTCG	GACGC	CGACGCI	T C C T G G	TGAAGG	G T C C G A A	ACGGTTCG	CCACCO	с с т с с с <mark>с</mark> ,	AGGACGA	4 C T G (∋ <mark>с</mark> аg Ас
BE-14106_TE_nc_S.sp.DSM-21069(becG)/1-696	G T A C G G C T G G A	ACACAC	CGACCCI	TG T T C G	TCCAGG	GTCCGAG	эсс <mark>с</mark> ттсе	TTCAG	CCCCCCC	A G <mark>G</mark> G G <mark>G /</mark>	4 G A T (∋ <mark>с</mark> ссссссс
Bombyxamycin_A_TE_nc_S.sp.SD53(bomP1)/1-699	AACCCGGTCGA	AGTCCC	CGGTGCT	гсттсс	TGCAGT	GCACCCAG	этссттсе	TCCCG	GACGGGG	AC <mark>G</mark> ACGA	4 C G G (C <mark>CGG</mark> GCCGA
Cremimycin_TE_nc_S.sp.MJ635-86F5(cmiP6)/1-699	GCGAGCCTCA	A G G C T C	CCGTGCT	TGTTCG	TCGGAG	CCGAGAA	Э А С Ө Т Т С А	TACCG	GAAACCG	G T T C C <mark>G ∕</mark>	GTTTCC	CAA <mark>G</mark> GCCCG
Heronamide_C_TE_nc_S.sp.CMB-0406(hmG)/1-696	GTACGGCTGG	GGCGC	CGACGCI	гсттсс	TCCAGG	GTCCGAG	SCCGTTCG	TGCAG	CCCCCGG	A G <mark>G </mark> G C <mark>G A</mark>	4 G A T (G C G G G C G C A
Hitachimycin_TE_nc_S.scabrisporus(hitP3)/1-696	GAAAGCGTCGG	C G C A C	CCGTGCI	гөттсө	тссстс	CCGGGAAA	ΑΤΟΟΤΤΟΑ	AGCCG	GAAACCG	G G <mark>G</mark> A T T 1	гтст(C <mark>G</mark> TGCCCA
ML-449_TE_nc_S.sp /1-696	GTACGGCTCG	CGCGC	CCACCCI	гсттсс	TCCAGG	C G T C C G A G	эсс <mark>еттс</mark> е	TCCAG	CCCCCGG	AGGGCGA	4 G · · · A T (G C G G G C C C A
Sceliphrolactam_TE_nc_S.sp.SD85(SceT)/1-684	GGTGC <mark>G</mark> CTGT	CGCGC	CCGTCCI	гсттсс	TCCGGC	CGGCGGAG	этссттсе	CGACCO	GGAGCG <mark>G</mark>	G C G A	<u>ч</u> сто(3 <mark>C G</mark> C G C
Sipanmycin_TE_nc_S.sp.CS149(Sip-P5)/1-696	AAGGAACTCG	GACGC	CGACGCI	T C C T G G	TGAAGG	G T C C G A A	ACGGTTCG	CACCO	GGCCCG <mark>G</mark>	AAGGCGA	<u>ч</u> сто (G C A G A C
Lineamycin_A_TE-nc_S.sp.Mg1(InyHI)/1-690	GAAGCCGTG <mark>A</mark> A	AGGCGC	CCACGCI	TGCTGG	TCCGGG	GCAGGAG	SCAGTTC1	TCGAC	TGGA <mark>CC</mark> C	GCGCCGA	<u> т</u> с с	Э <mark>сс</mark> ттс
Mediomycin_A_TE_nc_S.blastmyceticus(med9)/1-687	CGCGAGATC <mark>A</mark> A	AGA <mark>C</mark> CC	CGACGCI	гөстөө	TACGGG	CGGGGGAG	CGGCTG1	TCGAC	TGGA <mark>CC</mark> C	ст <mark>ссс</mark> и	<u>ч</u> сто (Э <mark>с</mark> өс тс
Neomediomycin_A_TE_nc_S.sp.RK95-74(nmd9)/1-687	CGCGAGGTCA	AGACGC	CGACCCI	TGCTGG	TCCGGG	CCGAGGAG	SCAGTTC1	TCGAC	төөт <mark>сс</mark> с(G C <mark>G</mark> A	<u> т</u> с с	Э <mark>с</mark> бс тс
Pikromycin_TE_nc_S.venezuelae(pikAIV,Non-polyene)/1-699	GGCCGCAGCAG	GCGCGC	CCGTGCI	ттстбб	тссстс	CCTCCGAA	ACCGCTGG	GCGAC	т (G G C A <mark>G </mark>	AGGAGCGG	G G C G A C T G
Erythromycin_TE_nc_Saccharopolyspora_erythraea(Non-polyene)/1-681	CGGGAAACCGG	GCTGC	CGACGC	TGCTGG	TCAGCG	CCGGCGAG	G C C G A T G G	GTCCG	т(GGCC <mark>CG</mark> /	ACGAC	AGCTG

	630	640	650	000	670	080	690	700	710	720	730
Pimaricin_TE_nc_S.chattanoogensis_L-10(ScnS4y1-705	ACGGCGAGGAC	CACOGCAGCCCGG	TCOACOTO	COTAGCOTOGA	COCGOACCAC	TTOTOCATOO	CCOCOACOAC	OCCCCOGAGAC	COCOCOGAT	COTGAAGG!	AATOOCTC
Amphotericin_B_TE_no_S.nodosus_ATCC-14899(AmphKy1-690	TGGACACCTCC	GCGGTGCCGGCGG	ACGTGGTG	CGTGACATCGA	GCCGACCAT	CTGTCCCTGG	CATGGAGCAC	TCGGATCTGAC	CGCCGAGGC	CATAGAGAI	ACTOOCTC
Nystatin_A1_TE_nc_S.nodosus_ATCC-11455(NysKV1-690	TCGACACCTCG	TCCOTCCCCCCC	ACOAGOTO	COOGACATCOA	COCCOACCAC	CTCTCCCTCO	CCAAGGAGCAC	TCOOCACTOAC	COCOCAGOC	CATCOAGO	SATOOCTC.
Candicidin_TE_ne_S.apFR-008#seFV1-711	TOCACCTTCCG	GTCGTCCCCGACG	CGGAGGTO	COCCCCTOGA	CTCCGACCAC	CTOTOCCTCA	T C C G T G A G G A C	TCCGGCCCCGC	CGCCGACCT	CATGGACGO	CGTGGCTG
filipin_TE_nc_S.avemitilis_MA-4680(bteA5)/1-693	GCGCGGGCACCG	CCCGAGCACGTGG	ACACGATO	GTCGAAGTGAC	COGAAACCAC	TACTOGATOT	TOGADGACCAC	GCCGGCACCAC	COCOGCOGC	GGTGGATTC	CCTGGCTG
Pimaricin_TE_nc_S.natalensis_ATCC-27448(PimS4)/1-705	ACOGCGAGGAC	CACOGCAGCCCOG	TCGACGTO	COCAGCOTOGA	COCGGACCAC	TTCTCCATGO	CCGCGACGAC	GCCCCGGAGAC	COCOCOGAT	COTOCOOO/	AATOOCTC
Reedowycine_TE_ne_S.Sp_OUC6819#dmJV1-693	CCTCGTGG	· · · GACTTCCCGC	ATACCOCO	ATCGACGTACC	SGTAATCAC	TTCTCGATCA	TOGACGAGCAC	GCCGCCACGAC	GGCGCGCGT	GGT CGAGG/	AGTGGATC
Eurocidin_D_TE_nc_S.eurocidicus_ATCC-27428/1-696	OCTCCCTO	ATTCCOTCOO	COACATO	COCAGOOTCOA	COCOGACCAC	CTCTCGATCG	CAAGO COGAC	GCCGGACTOG	000000000	GOTODAGA	ACTOOCTC
Lucensomycin_TE_nc_S.ach/omogenes_NBRC-14001(LucE)/1-702	AGTTGCTC	· · · OTCCCCGCGG	AGACGGTO	COCACCATCOA	COCCOACCAC	TTCTCGCTGG	COCAOCOGOAC	TCCCACGTCAC	GGCGACCGT	CATGAAGG/	AGTGGCTG
Lucensonycin_TE_nc.S.cyanogenus@cmEy1-702	AGCTOCTC	OTCCCCCCCC	AGACGGTO	COCACCATCOA	COCCOACCAC	TTCTCCTTOO	COCAOCOGOAC	TCCAACGTCAC	GOCGACCOT	CATOAA667	AGTOSCTO
Tetramycin_B_TE_nc_S.hygrospinosus_CGMCC-4.1123(TetrE)/1-699	GCGAGATG	· · · ATCCCGTCGG	CCGAGATO	GT CAAGC TOGA	COCOGACCAC	CTOTOCATCO	TCCGO TCCGAC	GCCTCCGCGGC	CTCCCAGAT	COCGOACO/	ACTOOCTC
Pentamycin_TE_nc_S.ap.S816/1-693	CACCOCCC	BAGCACOTOG	ACACGACO	GTCGAGGTCAC	COGAAACCAC	TACTOGATOC	EGGAGGACCAC	GCCGGTACCAC	COCCOCCOC	GGTGCACTO	CCTOOCTO
Selvanicin_TE_nc_Pseudonocardia_sp.HH130830-07/1-699	AGGAGGCGCCT	CCOCTOGACACCO	ACOCOGTO	CTCACGATCGA	COCCOACCAC	CTGACGATGO	CCAAGGAGCAC	TCCGGTGTGAC	CGCCGAGGC	GATOBAGG/	AGTOSCIC
NPP(Nys-like)_A1_TE_nc_Pseudonocardia_sp.AL041005-10(CPPK)/1-690	CCTCCGCC	· · · · · · · · · · · · · · · · · · ·	ACACCGTG	GTCGACATCGA	COCOGACCAC	CTGTCGCTGG	CCATEGASCAC	TCCGCGCTCAC	COCCGACOC	GATOGAGAG	CCTGGCTC
Auroramycin_TE_nc_S.roseosporus_NRRL-15998/1-696	COACATOO	TCOCTODCOA	CCCCCCCCCC	GAGACOBTOCC	COGCGATCAC	TTCAGCCTCG	COAO CAGOOT	6 COGAGACGAC	OBCOOCCOT	GOTOBAGA	ACTOOCTC
BE-14106_TE_nc_S.ap.DSM-21069(becGy1-696	GCCCGTGG	· · · GACTCCGAGC	ACACCCTG	COCACCOTCOA	AGGCAACCAT	TTCTCGCTCG	GCAGGACCAC	GCCCCGGCGAC	COCCGAGT	CATCOAGO/	AATGGCTG
Bombyxamycin_A_TE_nc_5.sp.SD53(bomP1)/1-699	AGCCCTOG	0 A00 COD C T C	ACACCCTO	COGACCOTCCO	COCCAACCAC	TTCACCCTCO	TOGADGACCO	GCCGAAGAGAC	COCACAGOT	CATCOACO	CATOOCTO
Cremimycin_7E_nc_5.sp MJ635-86F5(bmiP6)/1-699	GTCCCTGG	GATCCGGCGC	ACGACTIC	GTCOCATCCTC	COCAAACCAT	TTCACGCTGA	ECGAGGAAGAC	GCCGAGGAAAC	GOCOGGAT	CATCOACC	AGTOGCTC
Heronamide_C_TE_no_S.ap.CMB-0406(hm G)/1-696	AGCCGTGG	GACGACGAGC	ACACCCTO	COCACCOTCOA	AGGCAACCAT	TTCTCCCTCG	ACAGGAGCAC	GCCCCGGCGAC	CGCCCGAGC	CATCOAGO	AGTOOCTO
Hitachinycin_TE_nc_S.scabrisporus/hitP3y1-696	AACCCTOG	· GATCCCGCAC	ACACCTTO	COOCTCTCOCCO	BOGAAATCAC	TTCOGAT TOO	COAGGAGGAC	SCOBAGTCGAC	COCOCOCAT	CATCOAGO/	ACTOOTTO
ML-449_7E_no_S.ap /1-696	AGCCGTGG	· · · GACACCGAGC	ACACCCTG	COCACCOTCOA	AGGCAACCAT	TTCTCCCTCG	ACAGGAGCAC	GCCCCGGCGAC	COCCGAGO	CATOGAGG/	AGTOOCTO
Sceliphrolactam_TE_nc_S.sp.SD85(SceTy1-684	CCACCTOO	0ACOOCOCOC	ACOTOCIC	COCOAGOTOCCO	BOGCACCCAC	TTCACGATCC	TOGAOGAOTCO	SCCOCCTCCAC	coccocco	COTODACO/	ACTOOCTO
Sipanmycin_7E_nc_S.ap.CS149(Sip-P5)/1-696	COACCTOG	TCGCTGGCGA	CCCCCCCCCC	GAGACGGTCCC	COGTOATCAC	TTCAGCCTCG	CGAGCAGGG T	GCGGAGACGAC	GGCGGCCGT	GGTCGAGA	ACTOOCTC
Linearmycin_A_TE-nc_S.sp Mg1@nyHy/1-690	CCTACTOG	GACCTOGAGC	ACACGOCO	GTOGACOTCOC	GOCAACCAC	TTCACGATGA	TOGAGCAGCAC	GOGACGACGAC	COCOGOCAC	COTCOAGO/	AGTOOCTO
Mediomycin_A_TE_nc_S.blastmyceticus/med/9/1-687	CCTACTOG	GATCTCGAGC	ACACCOCO	CTCGATOTOCC	COGCAACCAC	TTCACGATGA	TGGAGCAGCAC	GCCGGGACGAC	6 8 C 6 C A 6 6 C	667666666	OCTOOCTO
Neomediomycin_A_TE_no_S.ap.RK95-74#md9y1-687	CGTACTOG	· · · GAGCTGCCC	ACACGGTG	CGCGATGTGCCC	BOGCCACCAC	TTCACGATGA	TGGAGGACCAC	GCCGCCACGAC	GOCGCGTGT	GOTGGAGG/	ACTOOCTO
Pikonnycin_TE_nc_S.venezuelae@ikAlV,Non-polyeney/1-699	GOCOTOCCAC	TOODACCTTCCOC	ACACCOTO	OCOGACOTOCC:	OGCOACCAC	TTCACGATGA	TOCODOACCAC	OCOCCOOCCOT	COCCOADOC	COTOCTOTO	CCTOOCTC
Erythromycin_7E_nc_Saccharopolyzpora_erythraea(Non-polyene)/1-681	GGAAGCCGACG	TGGCCCTTCGAGC	ACGACACO	GTCGCCGTCCC	CGGCGACCAC	TTCACGATGG	TGCAGGAACAC	GCCGACGCGAT	CGCGGGGCA	CATOGACOC	CCTOSCTG

Figure 28: Multiple sequence alignment of the conserved region of TE type I nucleotide sequences. The regions of interest that could be used for designing the primers are indicated with black boxes.

The analysis of the coding region of the thioesterase gene showed that there is a sequence variability at the level of nucleotides so that the degenerative positions needed to be used.

4.5.4. Designing of primers

Factors such as G+C content, length, melting temperature (Tm) and degeneracy should be considered during designing of primers as they provide optimal specificity and efficiency in PCR amplification.

The results of the designed primers are listed in table 26, analyzed and compared. Then the best option for the primer pairs was selected based on the factors mentioned above.

Table 26: List of the ten primers that were designed by Geneious software version R8.1.9. The best primer pair is highlighted in green.

Primer	Sequences	length	Interval	%GC	Tm	Hairpin	Dimer	Product
pairs						Tm	Tm	size
1	TE-Forward 5`GCCGAGCTGG AGGAGC3`	16	1-16	75.0	59.5	49.6	6.4	501
	TE-Reverse 5`GCCCATCGCGG ACAG3`	15	501-487	72.0	62.9	None	None	
2	TE-Forward 5`GCCGAGCTGG AGGAGC3`	16	1-16	75.0	59.5	49.6	6.4	499
	TE-Reverse 5`CAGCAGGTGG AAGTAGCGC3`	19	499-481	63.2	61.1	None	None	
3	TE-Forward 5`GCCGAGCTGG AGGAGC3`	16	1-16	75.0	59.5	49.6	6.4	499
	TE-Reverse 5`CAGCAGGTGG AAGTAGCG3`	18	499-482	61.1	57.8	None	None	
4	TE-Forward 5`GGCCCGCGGCT GATC3`	15	51-65	80.0	60.3	49.6	19.3	500
	TE-Reverse 5`GGCCCGGACG AGCAG3`	15	550-536	80.0	59.4	None	2.8	
5	TE-Forward 5`GGCCCGCGGCT GATC3`	15	51-65	80.0	60.3	49.6	19.3	499
	TE-Reverse 5`GCCCGGACGA GCAGC3`	15	549-535	80.0	60.2	None	None	
6	TE-Forward	16	53-68	75.0	60.7	43.7	22.9	500

	5`CCCGCGGCTGA TCTGC3`	1.5	550 500		50.7	N	NT	
	TE-Reverse	15	552-538	80.0	59.7	None	None	
	GAGC3`							
7	TE-Forward	18	61-78	61.1	59.7	50.4	2.7	502
	5`TGATCTGCGTC							
	AGCACCC3`							
	TE-Reverse	16	562-547	75.0	60.5	47.9	5.1	
	5`GAACGGCTCG							
	GTGGCC3`							
8	TE-Forward	18	61-78	61.1	59.7	50.4	2.7	501
	5`TGATCTGCGTC							
	AGCACCC3`							
	TE-Reverse	15	560-546	80.0	61.6	48.8	8.6	
	5°ACGGCTCGGTG							
	GCCC3`							
9	TE-Forward	18	61-78	61.1	59.7	50.4	2.7	500
	5°TGATCTGCGTC							
	AGCACCC3				T O O	4 - 0		
	TE-Reverse	15	561-547	73.3	59.2	47.9	5.1	
	5°AACGGCTCGGT							
10	GGCC3	10	<i>(1.50)</i>	<i>c</i> 1.1		50.4	0.5	=0.1
10	TE-Forward	18	61-78	61.1	59.7	50.4	2.7	501
	5 TGATCIGCGIC							
	AGCACCC3	16		75.0	(2) 2	40.0	0.0	
	TE-Keverse	16	561-546	/5.0	62.2	48.8	8.6	
	5 AACGGCTCGGT							
	GGCCC3							

The analysis of the results indicated that primer pair 1 is the optimal choice. This is because not only does it fulfill the required criteria, but upon examining the binding regions for this primer pair, it was observed that these regions were highly conserved. Consequently, designing a less degenerate pair was possible.

Then the sequences of the best primer pair were utilized to design degenerate primers with the objective of targeting a minimum of 50% of the polyenes present in the dataset.

Sequence	Length	Tm	%GC	Hairpin	Self-Dimer	Degeneracy
				Tm	Tm	
TE-Forward	16	52.5	66.7-	45.2	6.4	4
5`GCCGARCTGGAGSAGC3`		-	73.3			
		56.6				
TE-Reverse	15	56.3	73.3	36.6	None	2
5`SCCCATCGCGGACAG3`		-				
		57.1				

Table 27: Degenerate PCR primer pair properties.

4.5.5. In silico PCR

The specificity of the primer pair against the *Streptomyces* genomes was assessed by conducting *in silico* PCR amplification.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
															No					
100 bp															band	s				
DNA ladder																				
2000	_																			
1500																				
1000	=																			
1000	_					CDA	L	AT	P/G1	TP F	Ribon	ucleo	side-			Ribon	icleo:	side-		
800	_		Hyp	othet	ical	pepti	ide -	bi	ıding	d	liphos	phate	2			diphos	phate		rR	NA
600	_	ΤE	prot	ein		synti	hase l	pro	otein	I	eauci	ase		_	1	reduct	ase		m	ethylt
000			_																_	
400	—			_			_						_							_
									=				_	_				_		
			_		—	—														
200	—		—	_																
									_				_	_						
					_	_														
100				_																
No Band	e	(1)	(6)	(4)	(4)	(4)	(1)	(2)	(3)	(2)	(1)	(1)	(5)	(5)		(2)	(2)	(1)	(3)	(4)
. to. Danu	3	(4)	(2)	(2)	(1)	(1)	(*)	(4)	(2)	(4)	(4)	(4)	(2)	(2)		(1)	(4)	(4)	(2)	(1)
		450	561 519	275 398 183	249 249 246	249	477	439	318	168	117	202		6490 6490 369		273	214	283	459 78	543 468
			498	93	132	132			_				348	348					_	6.4

Figure 29: *In-silico* PCR gel illustrating the amplified genes.

The results of the analysis showed that the primer pair, which was designed to target the thioesterase gene, exhibited some degree of non-specific binding. This non-specific binding resulted in the amplification of the intended target as well as other genes with the same band size (around 500 bp).

5. Discussion

The results of the bacterial isolation indicated that chitin and ATCC media were the most efficient media for promoting the growth of *Streptomyces* strains from studied samples. Most of the *Streptomyces*-like strains were isolated from chitin medium. This confirmed that chitin medium enhances the growth of *Streptomyces*, which have a slower growth rate compared to many other common bacteria [8]. One possible explanation is that *Actinobacteria* (e.g. *Streptomyces sp.*) have the ability to utilize chitin as a carbon and nitrogen source by secreting chitinases that degrade chitin into smaller molecules, which can then be taken up and utilized by the bacteria for growth and metabolism. This adaptation allows *Streptomyces* to grow in environments rich in chitin [47].

Moreover, the results of the morphological characterization of the isolates showed that isolates from samples S6, S10 and all of sample S18 except for isolates S18/8 and S18/9 exhibited similar morphological characteristics. Sample S2 appears to resemble *Streptomyces*, although it exhibits distinct morphology compared to samples S6, S10 and S18. Isolate S16/2 exhibited pale orange colonies that resemble actinomyces strain. However, isolates S18/8 and S18/9 exhibited unusual morphological features that were not typical of actinomycetes. Further analysis as BOX-PCR and 16S rRNA identification were done for confirmation of their identity after the DNA extraction of the isolates. A high-quality DNA sample will have a ratio of absorbance values, known as the A260/ A280 ratio, ranging from 1.7 to 2.0 [45] and the optimal A260/A230 ratio is approximately 2 [46]. The results of measuring the purity of the DNA extracts showed that the A₂₆₀/A₂₈₀ ratio of the DNA samples ranged from 1.87 to 1.97, indicating that they are of good quality. However, the DNA samples of isolates S10/25 and S18/5 had relatively low A260/230 indicating that the samples may contain some organic compounds. The results of the BOX-PCR showed that isolates S6/6, S10/3, S10/15, S10/20, S10/25, S18/1, S18/5, S18/15, and S18/17 shared the same genomic fingerprint on the 1% agarose gel. This suggests that although they are collected from different trees, it is very likely that they share the same BGCs, indicating that they belong to the same strain. Furthermore, the results of 16S rRNA identification via blastn analysis revealed that only two isolates (S16/2, S18/8) do not belong to Streptomyces species and the majority of the bacterial samples (S2, S6, S10, and S18 except for isolates S18/8 and S18/9) share the closest relatives within Streptomyces genus, in particular Streptomyces flavogriseus or Streptomyces flavovirens, but with variable percentage of identity. This suggests that these samples S6, S10 and S18 except for isolates S18/8 and S18/9 may belong to the same Streptomyces strain. On the other hand, sample S2 may belong to different species or strain. However, the identification of bacterial species based solely on 16S rRNA gene sequence

analysis has its limitations as the resolution to distinguish between strains at the species level is too low. Moreover, even if strains show more than 99% similarity, there is no guarantee that they belong to the same species [48]. To obtain a more comprehensive understanding of the taxonomic classification and relationships among these isolates, further analyses using additional analysis would be necessary. These could include whole-genome sequencing or multi-locus sequence analysis (MLSA), which provide a more detailed characterization of bacterial isolates at both the species and strain levels [48].

Furthermore, the bioassay results indicated that all strains, except for BCCO 10_1102, BCCO 10_1105, S2/1, S2/2, S2/3, and S2/5 isolates, exhibited a hemolytic zone. The presence of a hemolytic zone indicates that the strains have the potential to induce hemolysis, which involves the rupture of erythrocytes and serves as a virulence factor [19]. Hemolysins not only affect erythrocytes but can also damage other eukaryotic cells. They are typically lytic proteins, such as enzymes, channel-forming porins or, less frequently, polyene antibiotics [19]. In addition, the results demonstrated that the strains (BCCO 10_1102, BCCO 10_1105, S2/1, S2/2, S2/3, and S2/5) that have no hemolytic activity, possess no inhibitory activity against *Candida albicans*. On the other hand, the strains that showed a β -hemolytic activity also exhibited inhibitory effects against *Candida albicans*. This suggests a possible correlation between hemolysis and antifungal activity. Moreover, some samples (BCCO 10_1093, BCCO 10_1095, BCCO 10_1099, S6, S10, S18) inhibited the β -hemolysis of *Staphylococcus aureus*. The β -hemolysis exhibited by Staphylococcus aureus is attributed to the production of an enzyme called beta-hemolysin or sphingomyelinase [49]. The exact mechanism by which *Streptomyces* inhibits the beta hemolysis of Staphylococcus aureus is still unclear. However, it is known that Streptomyces species are well-known producers of various secondary metabolites, including antibiotics and other bioactive compounds so one possible mechanism could be the production of antimicrobial substances by Streptomyces as actinomycin D that directly target and inhibit the production of virulence factors of Staphylococcus aureus [50]. Another suggested mechanism could involve the production of enzymes by *Streptomyces* that degrade the β -hemolysin produced by *Staphylococcus aureus*.

Based on the results of the bioassays at different incubation periods, the isolates exhibit a significant inhibitory effect against *Candida albicans* as well as inhibition of the beta hemolysis of *Staphylococcus aureus* after 4 days of incubation. Furthermore, the highest inhibitory effect was observed on day 6, indicating that the cultivation of *Streptomyces* for 4 - 6 days prior to the inoculation of indicator strain is the optimal time for the production of tested antimicrobial activity. The growth of the *Streptomyces* can be related to the inhibitory effects observed against

Candida albicans and the β -hemolysis of *Staphylococcus aureus*. Bacterial growth is divided into four phases: lag, exponential, stationary, and death [51]. The results suggested that the studied *Streptomyces* strains reached the late exponential phase, which is characteristic by production of secondary metabolites with antimicrobial properties after cultivating these strains for 4-6 days on Muller-Hinton agar with sheep blood (5%) (KHM agars).

Moreover, the results of the bioassays indicate that the presence of both *Streptomyces* and *Staphylococcus aureus* on the same plate leads to a significantly larger inhibitory zone against *Candida albicans*, compared to when only *Streptomyces* is inoculated. This suggests that there is a synergistic effect of these two bacteria leading to enhanced production of antimicrobials targeting *Candida albicans*. One possible explanation for this enhanced antifungal activity is that *Streptomyces* may produce secondary metabolites, that have antifungal properties. In addition to that *Staphylococcus aureus* may produce volatile metabolites (e.g. N-(2,5-Dicyano-3,4-dihydro-2H-pyrrol-2-yl)-acetamide and Benzyl methyl ketone) that possess antifungal properties [52]. Therefore, when both bacteria are present together, their combined action of releasing antimicrobial compounds could potentially enhance the overall antifungal activity against *Candida albicans*. Another explanation is that *Staphylococcus aureus* is known for its ability to synthesize compounds that act as elicitors. These elicitor compounds play a crucial role in stimulating the biosynthesis of various antimicrobial secondary metabolites, such as antibiotics and antifungal agents in *Streptomyces* [53].

The results of the bioassay against *Bacillus subtilis* indicated the absence of halo zone, showing that none of the tested isolates demonstrated any inhibitory effects against *Bacillus subtilis*. This suggests that these isolates do not produce any compound with antimicrobial properties against this bacterium.

The results of the antifungal activity test showed that both BCCO 10_1099 (B1) and BCCO 10_ 1104 (B2) strains exhibited an inhibitory effect against the four fungal strains that were obtained from different areas (Czech Republic, Poland, and Hungary). This suggests that the antifungal compounds produced by these strains have a broad spectrum of activity and are not restricted to targeting only the fungi found in their local environment (Papua New Guinea). Moreover, BCCO 10_1106 (B3) demonstrated a weak inhibitory effect against the fungal strain *Akanthomyces muscarium*. However, it did not exhibit any inhibitory activity against the other three fungal strains (*Graphium asporum, Ophiostoma piceae*, and *Ophiostoma minus*). This suggests that the antifungal compound is relatively specific to certain fungal species. Additionally, the four isolates S6/6 (B4), S10/3 (B5), S10/25 (B6), and S18/15 (B7) that were collected from the Czech Republic, demonstrated a weak inhibitory effect against all fungal strains. Further tests are needed to determine if changing the incubation period of *Streptomyces* could potentially enhance its antifungal activity or if it only prevents fungal growth near its vicinity.

Moreover, two hypotheses were formulated to identify a specific gene marker crucial for the biosynthesis of polyenes in *Streptomyces* and evolutionarily related actinomycetes. The first hypothesis was that Cytochrome P450 could serve as a reliable gene marker for polyene production. The multiple sequence alignment results revealed that the CYP sequences of the six-polyene BGCs exhibited shared regions where the amino acid residues are highly conserved. This suggests that these conserved regions may correspond to important catalytic sites or binding domains involved in the biosynthesis of polyene antifungal compounds [30]. Furthermore, apart from the Candicidin and Rimocidin BGCs, the other 4 polyene BGCs possess two cytochrome P450 genes. The phylogenetic tree analysis revealed the presence of two distinct groups within the constructed tree. The possible explanation is that each group is involved in regiospecific oxidation [54]. The first group in figure 26, including PimG, AmphN, NysN, PteD, RimG and fscP are catalyzing the oxidation of the exocyclic methyl branch to the carboxyl group, whereas the second group including PimD, AmphL, PteC and NysL, are involved in oxidative modifications of the polyol segment. Example for the regiospecific oxidation by PimG and Pim D is illustrated in Figure 30 [55].



Figure 30: The biosynthesis of Pimaricin. The genes involved in regiospecific oxidation are marked with black boxes.

However, the results of searching for more polyene BGCs indicated that certain polyenes such as Reedsmycin [56], Linearmycin-A, Mediomycin-A, and Neomediomycin-B are devoid of cytochrome P450 genes. The biosynthetic gene clusters of those polyenes are listed in the appendix. This means that cytochrome P450 cannot serve as a reliable gene marker for polyene production as it is not a gene marker for all polyene biosynthetic gene clusters. Therefore, the hypothesis was rejected, and we have focused on alternative gene. The second hypothesis was that thioesterase type I can be used as an effective gene marker for identifying polyene-producing actinomycetes, particularly streptomycetes. Then the BGCs of 20 polyenes and 2 non-polyenes were included to the dataset

The results of multiple sequence alignment of thioesterase type I within the biosynthetic gene clusters (BGCs) of 26 polyenes and 2 non-polyenes were compared to the aligned sequences of TE type I of the cyclic (macrolactone) polyenes in previous literature [29]. The analysis revealed that the results in our study are similar to the results in the literature. Despite including additional sequences of cyclic (macrolactone and macrolactam) and linear polyenes, they still exhibited conserved domains with some degree of variability in these regions. However, these variations in the amino acids that present within the conserved regions do not significantly impact the electric charges or overall geometry of the conserved domain for example, at position 47 (Figure 27), both aspartate (D) and glutamate (E) amino acids have negative charge. These domains share low sequence similarity with non-polyenes at the amino acid level, suggesting that these domains are specific for cyclization and offloading of the polyhydroxylated intermediates with continuous conjugated double bonds [29]. As a result, the amino acid sequences of these domains are translated back to nucleotide sequences and used for designing the primers. The analysis of the domains at the nucleotide level indicated that those different amino acids, that have the same properties, are represented by different codons, and even identical amino acids have multiple possible codons. This poses a challenge when designing primers for thioesterase type I.

Based on the circumstances, 10 primer pairs could be designed. The results showed that primer pair 1 is the best option as both forward and reverse primers have the optimum GC %. This indicates the stability and specificity of primer binding to the target DNA sequence [42]. Furthermore, the melting temperatures (Tm) of the primers are close to each other. Having closely matched Tm values for the forward and reverse primers is important so that they are likely to anneal to the target DNA sequence at a similar temperature during PCR amplification [42].

Moreover, formation of dimers and hairpins are possible only for the forward primer, unlike primer pairs 7-10. This difference in behavior lowers the probability of the primers annealing effectively. The most important advantage of this primer pair over the others is that the analysis of the binding regions for this primer pair revealed a high degree of conservation for polyenes thus designing less degenerate primer pair.

Then the sequences of the best primer pair were used to design the degenerate primers. Degenerate primers provide flexibility in primer design, enabling them to bind to target regions with slight variations. However, degeneracy of the primers was kept low to ensure specific binding. In silico PCR was used to evaluate the specificity of the degenerate primer pairs by testing these primers against 19 Streptomyces strains. The results show that not only the thioesterase type I gene was amplified, but also other genes. The lack of specificity observed with this primer pair suggests that the sequences of the primer pair may have similarity with other genes within the genome. Moreover, the relatively short length of the primer pair (15 bp) may increase the probability of non-specific binding to other regions of the genome as shorter primers have a higher likelihood of encountering sequences with partial complementarity elsewhere in the genome, leading to unintended amplification during PCR. Therefore, the hypothesis was rejected. To enhance primer specificity and minimize non-specific amplification, it is recommended to design longer primers (18-30 bases) [42] that are more unique to the target gene sequence. Due to time constraints, the primer pair was only tested using in silico PCR. However, it is preferable to conduct experimental testing in order to facilitate further analysis, such as sequencing the amplicons.

6. Conclusion

Nowadays, the emergence of multi drug-resistant fungi has become a silent crisis which affects the prevention and treatment of fungal infections. Consequently, discovering novel antifungals has become a priority for the researchers. Among these compounds, polyenes are the 2nd most frequently used antifungals. However, they have drawbacks including drug resistance, low bioavailability, and significant cytotoxicity that limited their uses in the market.

Therefore, this thesis contributed to investigating unexplored niches, specifically bark beetles in order to discover new potential producers of polyenes or other antifungal agents that exhibit low cytotoxicity. Other aspects were to determine whether the antifungal effects exhibited by streptomycetes were specific to certain fungi or occurred randomly and to search for a specific gene marker, which is essential for synthesis of polyenes.

This thesis focused on isolating, characterizing, and identifying streptomycetes obtained from adult bark beetles, larvae, and detritus. The bioactivity of these strains was evaluated. The promising strains were also tested against fungi from different areas. Moreover, phylogenetic analyses of CYP 450 and TE type I enzymes were conducted to identify potential gene markers for polyene biosynthesis. Then a degenerate primer pair was designed for TE type I gene and tested against *Streptomyces* strains using *in-silico* PCR.

The results of the morphological characteristics showed that some isolates were Streptomyceslike strains, while others exhibited atypical morphology that did not resemble actinomycetes. The majority of *Streptomyces* strains exhibited inhibitory activity against *Candida albicans* and βhemolysis of Staphylococcus aureus. Cultivating Streptomyces strains for 4-6 days before inoculation resulted in a significant inhibitory effect, indicating that Streptomyces strains reached the late exponential phase at that time and produced the tested antimicrobial secondary metabolites. Moreover, the co-cultivation of Streptomyces and Staphylococcus aureus on the same plate resulted in a significantly larger inhibitory zone against Candida albicans compared to when only Streptomyces was present. This suggests a synergistic effect between the two bacteria, potentially due to the production of antifungal secondary metabolites by *Streptomyces* and Staphylococcus aureus. Additionally, Staphylococcus aureus may act as an elicitor, stimulating the biosynthesis of antimicrobial secondary metabolites in Streptomyces. The four most promising isolates only had a weak inhibitory effect on fungal strains from different areas. Furthermore, the analysis of cytochrome P450 sequences in the polyene biosynthetic gene clusters revealed two different groups. Each group leads to regiospecific oxidation. However, some polyene biosynthetic gene clusters did not have cytochrome P450 enzymes, making it an unreliable gene marker. Furthermore, the thioesterase type I gene was found in all polyene BGCs but was not effective as a gene marker due to the lack of specificity of the designed primer pair. Therefore, testing the primer pair in the lab and sequencing the products can provide additional information about the amplified regions.

7. References

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8. Appendix

Photo documentation for the results of antifungal test (Photos are taken from the bottom of plates)



Control



Bacterial strains (B1, B2 and B3)



Bacterial strains (B4 and B5)



Bacterial strains (B6 and B7)



Control



Bacterial strains (B1, B2 and B3)



Bacterial strains (B4 and B5)



Bacterial strains (B6 and B7)





Bacterial strains (B1, B2 and B3)

Control



Bacterial strains (B6 and B7)



Bacterial strains (B4 and B5)



Control



Bacterial strains (B1, B2 and B3)



Bacterial strains (B4 and B5)



Bacterial strains (B6 and B7)

Protein	Size (aa)	Proposed function	Homologs		
			Protein/organism	Accession no. (identity/similarity %)	
Orf(-3)	213	TetR/AcrR family transcriptional regulator	IF52_RS0105620/Streptomyces ruber	WP_030357119.1 (66/74)	
Orf(-2)	418	FAD-binding monooxygenase	BCAV_RS14010/Beutenbergia cavernae	WP_015883264.1 (59/69)	
Orf(-1)	267	Methyltransferase	H299_RS35255/Streptomyces sp. CNH287	WP_051262671.1 (68/80)	
RdmA	286	XRE family transcriptional regulator	ASD26_RS06115/Streptomyces sp. Root1319	WP_056788122.1 (57/65)	
RdmB	256	Methyltransferase	AB852_RS08905/Streptomyces uncialis	WP_073785832.1 (63/73)	
RdmC	218	LuxR family two-component system response regulator	KasW/Streptomyces kasugaensis	BAF79686.1 (84/91)	
RdmD	111	Two-component system sensor kinase	KasX/S. kasugaensis	BAF79687.1 (66/81, the 475th-585th aa)	
RdmE	410	Two-component system sensor kinase	KasX/5. kasugaensis	BAF79687.1 (68/75, the 31st-413rd aa)	
RdmF	233	PAS-LuxR regulator	AOM46_RS26735/Streptomyces sp. NBRC 109706	WP_062215992.1 (83/89)	
RdmG	4996	Type I polyketide synthase	AOM46_RS26740/Streptomyces sp. NBRC 109706	WP_078857234.1 (70/78)	
RdmH	6440	Type I polyketide synthase	AOM46_RS26745/Streptomyces sp. NBRC 109706	WP_078857210.1 (71/80)	
Rdml	9533	Type I polyketide synthase	SHXM_01039/Streptomyces hygroscopicus XM201	AQW47576.1 (52/63)	
RdmJ	7252	Type I polyketide synthase	AOM46_RS26755/Streptomyces sp. NBRC 109706	WP_062216000.1 (74/82)	
RdmK	498	MFS transporter	AOK24_RS06280/Streptomyces niveiscabiei	WP_055719056.1 (76/83)	
RdmL	398	Ferritin	AOK24_RS06275/S. niveiscabiei	WP_055719055.1 (84/91)	
RdmM	172	Paal family thioesterase	AOK24_RS06270/5. niveiscabiei	WP_055719054.1 (75/85)	
RdmN	82	Acyl carrier protein	AOK24_RS06265/S. niveiscabiei	WP_055719053.1 (54/75)	
RdmO	576	Acyl-CoA synthetase	AOK24_RS06260/5. niveiscabiei	WP_055719052.1 (66/76)	
Orf1	176	Hypothetical protein	AMJ94_18775/Deltaproteobacteria bacte- rium SM23_61 AOK24_RS06255/S. niveiscabiei AOK24_RS06250/S. niveiscabiei	KPK85730.1 (32/53) WP055719051.1 (78/84, the 1st–79th aa) WP055719050.1 (82/89, the 81st–174th aa)	
Orf2	207	DNA-binding response regulator	AOK24_RS06245/S. niveiscabiei	WP_063799404.1 (61/74)	
Orf3	361	Hypothetical protein	AOK24_RS06240/5. niveiscabiei	WP_079056694.1 (56/67)	

Table 28: Reedsmycin biosynthetic gene cluster from S. youssoufensis OUC6819 [53].

Table 29: linearmycin-A biosynthetic gene cluster from Streptomyces sp. Mg1 [57]

Identifiers	Position	Product
M444_04750 AKL64821.1 lnyT	1061319 - 1062308 (-)	agmatinase
M444_04755 AKL64822.1 lnyS	1062415 - 1063971 (-)	methylmalonyl-CoA carboxyltransferase
M444_04760 AMB20391.1 lnyR	1064457 - 1067375 (+)	transcriptional regulator
M444_04765 AKL64823.1 lnyQ	1067356 - 1068252 (+)	metallophosphoesterase
M444_04770 AKL64824.1 lnyP	1068249 - 1068992 (+)	4'-phosphopantetheinyl transferase
M444_04775 AKL64825.1 lnyO	1069184 - 1070845 (+)	amine oxidase
M444_04780 AKL64826.1 lnyN	1070939 - 1072351 (-)	acylCoA ligase
M444_04785 AKL69762.1 lnyM	1072435 - 1073028 (-)	thioesterase
M444_04790 AMB20392.1 lnyL	1073166 - 1073738 (-)	glucose-1-phosphate thymidylyltransferase
M444_04795 AKL64827.1 lnyK	1074063 - 1075127 (+)	daunorubicin ABC transporter ATPase
M444_04800 AKL69763.1 lnyJ	1075226 - 1075996 (+)	ABC transporter
M444_04805 AKL64828.1	1076160 - 1077197 (+)	ACP S-malonyltransferase
Identifiers	Position	Product
M444_04750 AKL64821.1 lnyT	1061319 - 1062308 (-)	agmatinase
M444_04755 AKL64822.1 lnyS	1062415 - 1063971 (-)	methylmalonyl-CoA carboxyltransferase
AMB20391.1 lnyR	1064457 - 1067375 (+)	transcriptional regulator
M444_04785 AKL64823.1 lnyQ	1067356 - 1068252 (+)	metallophosphoesterase
M444_04775 AKL64824.1 lnyP	1068249 - 1068992 (+)	4'-phosphopantetheinyl transferase
A444_04775 AKL64825.1 lny0 M444_04780	1069184 - 1070845 (+)	amine oxidase
AKL64826.1 InyN M444_04785	1070939 - 1072351 (-)	acylCoA ligase
AKL69762.1 lnyM M444_04790	1072435 - 1073028 (-)	thioesterase
AMB20392.1 lnyL M444 04795	1073166 - 1073738 (-)	glucose-1-phosphate thymidylyltransferase
AKL64827.1 lnyK M444_04800	1074063 - 1075127 (+)	daunorubicin ABC transporter ATPase
AKL69763.1 lnyJ	1075226 - 1075996 (+)	ABC transporter
AKL64828.1	1076160 - 1077197 (+)	ACP S-malonyltransferase

M444_04810 AMB20393.1 lnyHI	1077302 - 1094107 (-)	polyketide synthase
M444_04815 AKL64829.1 lnyHH	1094175 - 1116644 (-)	polyketide synthase
M444_04820 AKL64830.1 lnyHG	1116707 - <mark>1</mark> 126318 (-)	polyketide synthase
M444_04825 AKL64831.1 lnyHF	1126413 - 1142603 (-)	polyketide synthase
M444_04830 AMB20394.1 lnyHE	1142689 - 1158345 (-)	polyketide synthase
M444_04835 AKL64832.1 lnyHD	1158599 - 1163497 (-)	polyketide synthase
M444_04840 AKL64833.1 lnyHC	1163620 - 1183653 (-)	polyketide synthase
M444_04845 AKL69764.1 lnyHB	1183678 - 1193985 (-)	polyketide synthase
M444_04850 AKL64834.1 lnyHA	1194150 - 1228586 (-)	polyketide synthase
M444_04855 AKL64835.1 lnyG	1228902 - 1230452 (-)	membrane protein
M444_04860 AKL69765.1 lnyF	1230449 - 1230949 (-)	membrane protein
M444_04865 AKL64836.1 lnyE	1231012 - 1231545 (-)	LnyE
M444_04870 AKL64837.1 lnyD	1231711 - 1233006 (+)	histidine kinase
M444_04875 AKL69766.1 lnyC	1233071 - 1233631 (+)	LuxR family transcriptional regulator
M444_04880 AKL69767.1 lnyB	1233701 - 1236565 (-)	transcriptional regulator

Identifiers	Position	Product
BAW35626.1 medA	149 - 739 (-)	
BAW35627.1 medB	899 - 2029 (-)	sulfotransferase
BAW35628.1 medC	2543 - 5371 (+)	putative LuxR family transcriptional regulator
BAW35629.1 medD	5503 - 7134 (+)	putative L-arginine mono-oxygenase
BAW35630.1 medE	7211 - 8617 (-)	putative 4-guanidinylbutanoate:CoA ligase
BAW35631.1 medF	8681 - 9322 (-)	putative type II TE
BAW35632.1 medG	9842 - 10786 (+)	putative 4-guanidinylbutanoyl-CoA:ACP acyltransferase
BAW35633.1 med9	10945 - 22446 (-)	modular polyketide synthase
BAW35634.1 med8	22528 - 39621 (-)	modular polyketide synthase
BAW35635.1 med7	39677 - 49276 (-)	modular polyketide synthase
BAW35636.1 med6	49365 - 70760 (-)	modular polyketide synthase
BAW35637.1 med5	70824 - 86309 (-)	modular polyketide synthase
BAW35638.1 med4	86533 - 91488 (-)	modular polyketide synthase
BAW35639.1 med3	91616 - 116827 (-)	modular polyketide synthase
BAW35640.1 med2	116853 - 1 26989 (-)	modular polyketide synthase
BAW35641.1 med1	126996 - 151358 (-)	modular polyketide synthase
BAW35642.1 medH	151633 - 153189 (-)	putative membrane protein
BAW35643.1 medI	153186 - 153668 (-)	putative membrane protein
BAW35644.1 medJ	153755 - 154288 (-)	
BAW35645.1 medK	154456 - 155709 (+)	putative histidine kinase
BAW35646.1 medL	155702 - 156331 (+)	putative two-component system response regulator
BAW35647.1 medM	156389 - 159283 (-)	putative LuxR-family transcriptional regulator
BAW35648.1 medN	159372 - 160022 (-)	putative type II TE
BAW35649.1 medO	160405 - 161190 (+)	putative 4-guanidinobutyramide hydrolase
BAW35650.1	161231 - 161671 (-)	putative transcriptional regulator
mede		

Table 30: Mediomycin-A biosynthetic gene cluster from *Streptomyces blastmyceticus* [58].

Identifiers	Position	Product
BAW35598.1 nmdA	1987 - 3483 (+)	putative methylmalonic acid semialdehyde dehydrogenase
BAW35599.1 nmdB	3628 - 4071 (+)	hypothetical protein
BAW35600.1 nmdC	4156 - 6249 (+)	putative integral membrane protein
BAW35601.1 nmdD	6493 - 7251 (+)	putative type II TE
BAW35602.1 nmdE	7340 - 10183 (+)	putative transcriptional regulator
BAW35603.1 nmdF	10205 - 10837 (-)	putative DNA-binding response regulator
BAW35604.1 nmdG	10830 - 12110 (-)	putative two-component system sensor kinase
BAW35605.1 nmdH	12245 - 12820 (+)	hypothetical protein
BAW35606.1 nmdI	12889 - 13389 (+)	putative membrane protein
BAW35607.1 nmdJ	13494 - 14930 (+)	putative membrane protein
BAW35608.1 nmd1	15242 - 54211 (+)	modular polyketide synthase
BAW35609.1 nmd2	54262 - 65442 (+)	modular polyketide synthase
BAW35610.1 nmd3	65469 - 85640 (+)	modular polyketide synthase
BAW35611.1 nmd4	85708 - 90672 (+)	modular polyketide synthase
BAW35612.1 nmd5	90919 - 106437 (+)	modular polyketide synthase
BAW35613.1 nmd6	106489 - 122601 (+)	modular polyketide synthase
BAW35614.1 nmd7	122658 - 132275 (+)	modular polyketide synthase
BAW35615.1 nmd8	132332 - 149440 (+)	modular polyketide synthase
BAW35616.1 nmd9	149501 - 171664 (+)	modular polyketide synthase
BAW35617.1 nmdK	171777 - 172724 (-)	putative acyl transferase
BAW35618.1 nmdL	172765 - 174381 (-)	putative ABC-2 type transporter
BAW35619.1 nmdM	174378 - 175385 (-)	putative ABC transporter
BAW35620.1 nmdN	175705 - 176367 (+)	putative type II TE
BAW35621.1 nmdO	176424 - 177836 (+)	putative acyl CoA ligase
BAW35622.1 nmdP	177948 - 179606 (-)	putative amino oxidase
BAW35623.1 nmdQ	179646 - 182426 (-)	putative LuxR family transcriptional regulator
BAW35624.1 nmdR	183092 - 184135 (+)	putative sulfotransferase
BAW35625.1 nmdS	184328 - 185326 (+)	putative amidinohydrolase

Table 31: Neomediomycin-B biosynthetic gene cluster from Streptomyces sp. RK95-74 [59].