Chapter 1

General Introduction

1.1 General characteristics of *Streptomyces*

1.1.1 Actinomycetes phylogeny

Actinomycetes are gram positive bacteria with high GC content ranging from 54% (*Corynebacterium*) to 70% (*Streptomyces*) belonging to class Actinobacteria and order Actinomycetales. Actinomycetes mainly inhabit soil or aquatic ecosystem (1, 2). The Actinobacteria phylum includes *Bifidobacterium*, *Mycobacterium*, *Corynebacterium* and *Streptomyces* with morphology ranging from cocci to filamentous form (3). The unique molecular characteristics of Actinobacteria are presence of 100 nucleotides between helices 54 and 55 of 23S rRNA gene (4).

The two important traits of Actinomycetes which place them in scientific focus are the study on development and differentiation and secondary metabolite production (5).

1.1.2 Streptomyces

Streptomyces are abundantly present in soil and contribute significantly to degradation of dead plants and animals. They can effectively degrade insoluble polymers like protein, starch, chitin cellulose and xylan by forming immovable substrate mycelium and secreting various extracellular hydrolytic enzymes (6). The group of *Streptomyces* is strictly aerobic which forms differentiating hyphal growth and spore chains. Type I cell wall is common among *Streptomyces* having LL-Diaminopimalic acid in peptidoglycan composition and phosphatidiletanolamine phospholipid in cell membrane (CTT1) (7, 8). The very important and noticeable characteristics of *Streptomyces* are their ability to produce secondary metabolites with diverse biological activities. *Streptomyces* is considered as microbial factory for production of wide array of metabolites with many important applications for humankind like immunosuppresants, anti cancer compounds and antibiotics (9, 10).

1.1.3 Genetic Characteristics of *Streptomyces*

Streptomyces comprises of linear chromosome with size ranging from 8 to 10 Mb which is among the largest bacterial genomes. *S. coelicolor* being the best studied *Streptomyces* and model organism for antibiotic production. The first fully sequenced *Streptomyces* is *S. coelicolor* harboring 9.05 Mb of chromosome with 72% GC content, 8300 putative genes and 8153 putative proteins (10). As *Streptomyces* genome has high GC content the codons ending with T or A are rarely found (11).

The extremes of the chromosome presents number of inverted and repeated sequences also called TIR (Terminal Inverted Repeats) of size ranging from 24 to 600 kb also there is presence of direct repeats, insertion and transposable elements (12). This feature is cause of high genetic instability displayed by *Streptomyces* species. Hence *Streptomyces* are known to undergo major spontaneous mutations that include chromosome amplification or deletions or appearance of instable circular forms which are more instable than linear ones.

The genome sequence of *S. coelicolor* revealed that Actinomycetes carry much more genetic potential for natural products than those detected in laboratory conditions. Many of the gene clusters are 'Cryptic' in nature that means they are nor transcribed under laboratory conditions rather a trigger can activate such clusters (13). By means of genome mining the potential new molecules can be figured out and isolated. In last decades natural product research on Actinomycetes has been revolutionized by Next Generation Sequencing. In NCBI database good number of sequenced and annotated genomes can be found. The analysis of genomic organization of *Streptomyces* revealed that genes associated with primary metabolism are located in center of chromosome (10, 14). The extremities include genes related to adaptive responses to surrounding media i.e. genes for secondary metabolite biosynthesis. In core genes there is high degree of synteny but at the extremities there are few or no orthologues or low synteny (10, 14, 15). The secondary metabolite biosynthetic cluster can be found at extremities or out of core. The most common genes found are encoding proteins for the structure of the biomolecule, protein involved in intrinsic resistance of the compound, regulators which control transcription of gene cluster.

Along with chromosome in *Streptomyces* extra-chromosomal elements in form of linear or circular plasmids can be found out which include necessary elements to be transferred by conjugation to other microorganism (16). Some plasmid contains biosynthetic gene cluster, some plasmids also bear mobile genetic elements and conjugative transposon (17, 18). Presence of transposon is common feature of *Streptomyces*. Moreover, certain bacteriophage called Actinophage with double stranded DNA can infect and integrate in the chromosome of *Streptomyces* (16).

1.1.4 Life Cycle of *Streptomyces*

The life cycle of *Streptomyces* is complex resembling to fungi, as it combines stages of hyphal growth and differentiation with formation of spores which are non-motile unigenomic with thick cell walls resistant to dry and acidic environment. The life cycle begins when under favorable condition spore germinates germ tube, develops and grows apically into hyphae which undergo branching in order to explore nutrients. Finally vegetative mycelium results from intricate network of branched multi-chromosomic hyphae. When there is nutrient exhaustion in the surrounding medium along with other environmental signals vegetative mycelia undergoes morphological differentiation to generate white colored mycelia called as aerial mycelium. This process is accompanied with massive cell lysis called autolysis which provides necessary nutrients for morphogenesis (19). Along with morphological differentiation there is onset of antibiotic biosynthesis. When aerial mycelia stop growing aerial hyphae start forming multiple septa which gives rise to unigenomic spores which can in turn start the life cycle once again (Fig. 1.1) (20).

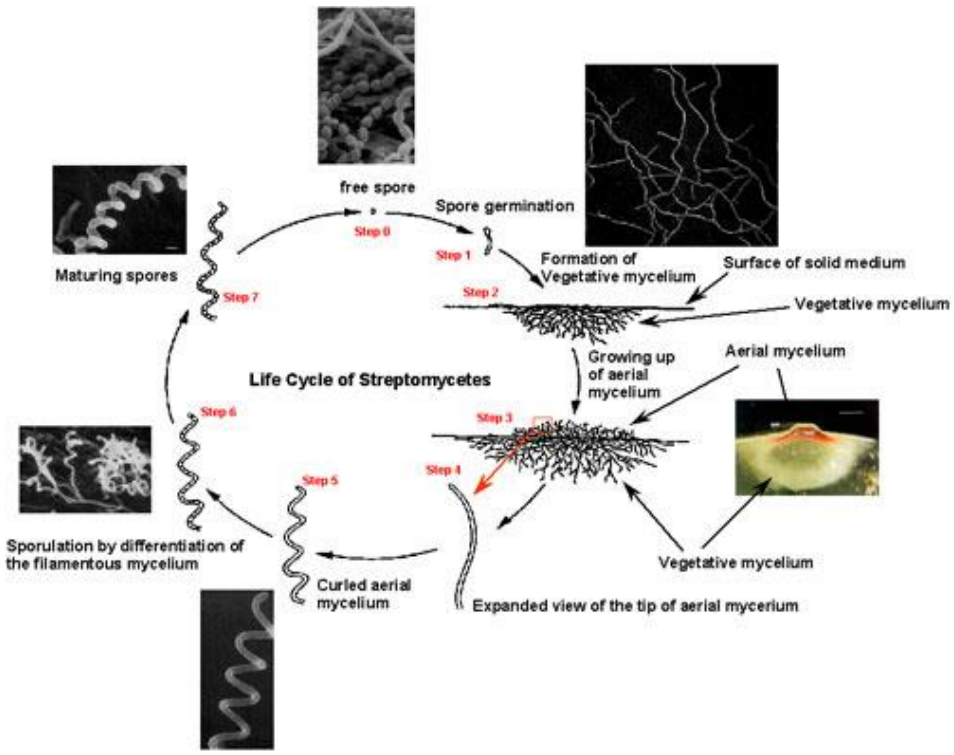

Figure 1.1 Life cycle of *Streptomyces*

(Adapted from http://home.hiroshima-u.ac.jp/mbiotech/hosenkin_lab/Fig(Stre)/Fig(Stre)3E.jpg)

1.1.5 Different phases of growth of Gram positive bacteria - *Streptomyces*

Streptomyces is considered to be multicellular prokaryotic model as it undergoes sporulation and programmed cell death (PCD). According to recent advances it is clear that the development of *Streptomyces* is different in solid and in liquid media. On solid media like R2YE and soyabean manitol, *Streptomyces* undergoes sporulation phase. According to traditional view, as described above on solid media, once the spore germinates it gives rise to viable vegetative mycelia called as substrate mycelia which grows on and under the surface of agar until it get differentiated into reproductive aerial mycelia which grows in the air, producing spores at the end of cycle (21). The basic understanding of the developmental programme on solid media is progressed in recent years, in terms of stages preceding aerial mycelium formation and sporulation. According to the new advancement, some compartmentalized mycelium (MI) dies early on by highly ordered programmed cell death. Consequently, remaining viable cells forms multinucleated second mycelium with sporadic septa (MII) (22, 23) (Fig. 1.2). This MII grows in the culture medium called as substrate mycelium until it initiates expression of hydrophopbic cover and grows into the air (aerial mycelium) and ends by forming spores. This MII stage produces antibiotics and sporulates on solid media (Fig. 1.2). It is reported that there is second round of programmed cell death prior to sporulation which affects substrate and aerial mycelium. These stages are now considered general to all *Streptomyces* genus. It is noteworthy that proteins involved in primary metabolism (protein synthesis, Kreb cycle, and energy production) are more in MI and proteins involved in secondary metabolism (actinorhodin and type II polyketide biosynthesis, β-lactamases, epimerases) are up-regulated in MII according to proteomics study.

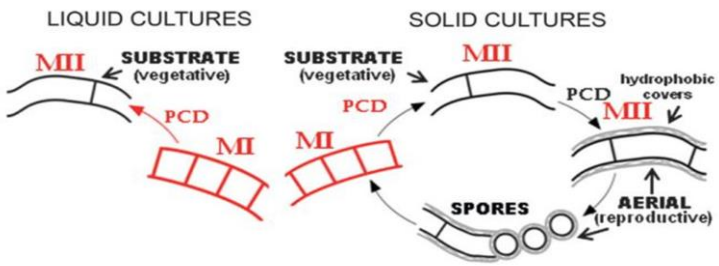

Figure 1.2 Developmental cycle of *Streptomyces* in solid and liquid cultures - MI-first compartmentalized mycelium, MII-second compartmentalized mycelium, PCD-programmed cell death [Image imported from reference(24)]

1.1.6 Differentiation in liquid culture

Majority of *Streptomyces* species do not sporulate in liquid culture moreover morphological changes do not occur in these conditions (25-29). There are few exception to this, there are reports where some species like *S. venezuela*e (30), *S. griseus* (31), *S. chrysomallus* (32), *S. antibioticus* ETHZ7451 (33), *S. albidoflavus* SMF301 (34), or *S. brasiliensis* (35) were found to sporulate in liquid medium. In liquid medium, after transient growth arrest substrate mycelium starts producing secondary metabolites. In model organism, *S. coelicolor* in response to nutritional shiftdown sporulation is activated (36, 37). It is observed that similar to solid culture the developmental phases are MI (young compartmentalized mycelium) and MII (multinucleated mycelium). Because of PCD in MI there is transient growth arrest followed by emergence of MII (Fig. 1.2). Thus there are only two phases MI and MII without sporulation. Manteca *et. al.*, (38) showed for the first time that MII is antibiotic producing mycelium. The longevity of MI is more in liquid media as compared to solid media. The proteomic (39) and transcriptomic (40) analysis revealed that the differentiation in solid and liquid medium is almost similar, MI is associated with primary metabolism and MII with secondary metabolism but non existence of sporulation and hydrophobic layer producing proteins.

1.1.7 The programmed cell death in *Streptomyces*

The studies done by Miguelez *et. al*., (41) and Manteca *et. al.,* (42) demonstrate that programmed cell death occurs in *Streptomyces* where a death phenomenon is associated with development. Manteca *et. al.,* (42), confirmed that there is existence of regulated active cellular suicide in *Streptomyces,* evidenced by altered membrane permeability, nucleic acid degradation and activation of degradative enzymes like nucleases. The proteomic studies by Manteca *et al*., (42) demonstrated that during PCD in *S. coelicolor,* there is abundance of cellular macromolecular degradation of proteins, stress induced proteins and regulatory proteins (39).

1.1.8 New perspective on germination, hyphal growth and sporulation

1.1.8.1 Germination

It has been found that spore germination involves “resuscitation promoting factor” (Rpf) which is specialized peptidoglycan hydrolases and is part of

peptidoglycan remodeling system (43). In *S. colelicolor,* Rpfs are well studied and it has been found out that one of the five Rpf is regulated at multilevel by nucleotide second messengers: transcription initiation, controlled by cAMP-binding protein previously associated with germination, riboswitch mediated transcription attenuation responsive to cyclic di-AMP and ppGpp dependent changing rates of proteolysis (44).

1.1.8.2 Hyphal Growth

The hyphal growth of *Streptomyces* occurs by tip extension through activities of protein complex called "polarisome". The key component of this protein complex is, DivIVA. The recent research on hyphal growth indicates that there is deposition of glycan through action of cellulose synthase like protein (ClsA) and GlxA (a copper oxidase which oxidizes glycan as it is secreted) (45, 46). *clsA* and *glxA* controls synthesis of extracellular polysaccharides soon after germination (47, 48). A new locus *matAB* is responsible for formation of aggregation of germlings which forms mycelial clumps in submerged culture (49).

1.1.8.3 Division of cytoplasm of vegetative hyphae into compartments

The recent advances of microscopic technique suggest that cross-membranes generates mycelial compartments which can be cytosol permeable or impermeable and provide plausible answer to question why hyphae of *ftsZ* null mutants do not die but can be fragmented and propagated. These cross membrane also created DNA free zones (24, 50, 51).

1.1.8.4 Hydrophobic cover formation, septation and sporulation

Streptomyces differentiation was studied by analyzing mutant strains defective in morphological development. *bld* mutant blocked in development forms substrate mycelia on rich media but can't produce antibiotic. *bld* gene products play important role in the regulatory cascade which triggers aerial mycelium formation. A special case is set of genes which encodes a leucyl-$tRNA_{UAA}$. Only 2-3% of *Streptomyces* genes contain TTA codon (52). Majority of genes are involved in morphogenesis and secondary metabolism. Mutants blocked at later stages of development are called white or *whi* which can form aerial hyphae but can't form spore chains and hence that don't exhibit grey color of mature spores (53, 54). Recent findings suggest existence of 'sky pathway' which controls expression of rodlins and chaplin genes that encode proteins for surface hydrophobicity to aerial hyphae and

spores (19). Another well characterized differentiation process is of hyphal septation during sporulation. Multiple sporulation septa are produced in coordination with sporogenic aerial hyphae. Along the aerial hyphae, FtsZ is localized at regular sites along aerial hyphal wall during first step of sporulation specific cell division. Next, the proteins required for bacterial divisomes are directed to form Z ring (55). ParA and ParB controls segregation of DNA into prespore compartments and FtsK play role in completion process at the septum (56). After the septum formation, wall of spore compartments thickens and pigmentation occurs. By the poorly understood process of autolytic cleavage, mature spores are separated.

1.2 Primary metabolism in *Streptomyces*

Primary metabolism involves catabolic and anabolic reactions which results in increase in biomass i.e. the reactions harness energy and reducing power to synthesize building blocks of protein, nucleic acids, lipids, polysaccharides structural and storage materials. Similar to other bacteria *Streptomyces* also have network of central carbon metabolic pathways for efficient utilization of carbon to generate energy. The main pathways are glycolysis (or Embden-Meyerhof-Parnas pathway), pentose phosphate pathway (PPP), Entner–Doudoroff pathway and tri-carboxylic acid cycle (TCA) .

1.3 Secondary metabolism in *Streptomyces*

'Secondary metabolite' term came in general use by Bu'Lock in 1961- a microbiologist, for the microbial products which do not fit in intermediary metabolism. They were termed as 'secondary' on the grounds that unlike the intermediates and cofactors which play essential role in cellular function, these substances have no apparent role in growth and reproductive metabolism. Secondary metabolites have diverse chemical structure and are usually distinctive products of group of organisms or single strain. The best known secondary metabolite is antibiotic. In nature the role of this compound is survival benefit because of killing role of these compounds. The precursors for the synthesis of these compounds are obtained from primary metabolism like acetyl-CoA and malonyl-CoA leading to formation of polyketides, terpens, steroids and metabolites derived from fatty acids (57). Intermediates from shikimic acid pathway, TCA and amino acids can also serve as precursor for secondary metabolites. Secondary metabolite production is also tightly regulated involving induction, feedback regulation and catabolite repression.

In laboratory conditions, secondary metabolites are produced in growth phase dependent manner. The biosynthesis of secondary metabolite occurs in growth phase or developmentally controlled manner. *Streptomyces* undergo intermittently starvation and adaptation (58). During starvation metabolic stress is elicited, differentiating *S. coelicolor* adapts primary metabolism and starts developmental programme leading to morphogenesis and antibiotic biosynthesis. In nature certain environmental and biochemical signals triggers antibiotic biosynthesis.

Streptomyces produces different class of antibiotics such as aminoglycosides, polyketides, non-ribosomal peptides, β-lactams and other antibiotics. This genus is best known as one of the polyketide producers. Polyketide contains alternating carbonyl and methyelene groups or derived from precursors containing these groups. Examples of polyketides produced by *Streptomyces* are rapamycin, oleandomycin, actinorhodin, daunorubicin and caprazamycin. Polyketides exhibit a broad range of bioactivities such as antibacterial (e.g., tetracycline), antifungal (e.g., amphotericin B), anticancer (e.g., doxorubicin), antiviral (e.g., balticolid), immune-suppressing (e.g., rapamycin), anti-cholesterol (e.g., lovastatin) and anti-inflammatory activity (e.g., flavonoids) (59-65). Biosynthesis of these polyketides is complex as the process involves multifunctional enzymes, polyketide synthases.

1.3.1 Polyketide Synthases

Successive condensation of short chain acyl-CoA results in polyketide biosynthesis through action of multi-function enzyme polyketide synthases (PKS) as fatty acid synthases (FAS) (66). PKS is divided into 3 main groups: type I, II and III.

Type I PKS also known as modular PKS, are large multifunctional enzymes which include different domains with different catalytic activities grouped in modules. The main domains of PKS responsible for elongation cycle is called minimal module: acyl transferase (AT) which selects extender unit in successive condensation steps, β –ketoacyl-ACP-synthase (KS) catalyses decarboxylation condensation of new extender molecule in the growing polyketide chain and acyl carrier protein (ACP) which acts as carrier of the growing polyketide chain and presents it to adjacent enzymatic domain. Reduction state of keto groups are altered by domains like ketoreductase (KR) which reduces keto to hydroxyl group, dehydratase (DH) responsible for dehydration of enoyl groups, enoylreductase (ER)

which catalyses reduction of enoyl groups to alkane group. Along with elongation process, the polyketide chain is presented successively to different modules in the way that the module operates only once over the polyketide chain. The last module is released from polyketide chain by ACP-thioesterase activity (67).

Type II PKS or iterative PKS are multienzyme complex, where different proteins interact with each other in the elongation of polyketide chain. Each PKS has 3 enzymes for elongation of polyketide chain: KS_a which presents KS and AT activities; KS_b also called as chain elongation factor (CEF) which controls number of cycles of elongations and ACP. Enzyme with KR activity causes reduction of keto groups. Hence, molecules synthesized by this PKS are in more reduced state than by type I PKS. Cyclase and aromatase is mainly for aromatic ring formation (68-70).

Type III PKS are mainly found in plants even though many bacteria like *Streptomyces* own it. It is very different from above PKS. These enzymes are interacting KS homodimers which uses precursor molecules acyl-CoA in ACP-independent way (71, 72).

1.3.2 Types of polyketides

Based on the chemical structure polyketides are of 3 types: aromatic, macrocyclic and polyether. Aromatic polyketides are synthesized by type II PKS. Starter and extender unit is mainly manonyl-CoA but acetyl-CoA, propionyl-CoA and isobutryl-CoA are also been reported. Examples of aromatic polyketides are actinorhodin and tetracyclin.

Macrocyclic polyketides are sythesized by type I PKS and consists of large macrocyclic rings. They are further divided into polyene (contains two to eight conjugated bonds, eg. pimaricin and nystatin) and non-polyene (macrocyclic rings with 12 to 16 carbons and absence of conjugated double bonds, erythromycin).

Polyether polyketide are synthesized by type I PKS and are linear molecules with polyene backbone that undergoes oxidative cyclization by epoxidase and epoxide hydrolase which generates ether bonds. Examples are Monensins A and B.

1.4 Regulation of antibiotic production

The biosynthesis of each antibiotic produced by *Streptomyces* is specified by large gene cluster which includes regulatory genes. The regulatory systems in these organisms are highly conserved which governs organisms' physiology, developmental states, population density and environment; these factors altogether determine the onset as well as the level of antibiotic production.

1.4.1 Transcriptional regulation of antibiotic production

The cluster situated repressors or activators which involve pleiotropic global regulators directly activate or repress the antibiotic biosynthesis gene cluster. A large number of regulatory proteins are involved in control of antibiotic production. Moreover cross regulation also occur leading to complex regulatory network to interpret the environmental signal and accordingly appropriate transcriptional responses are translated for antibiotic production along with developmental program. The well studies examples of cluster situated transcriptional regulators are actII-ORF4 for actinorhodin biosynthesis, RedD controlling undecylprodigiosin biosynthesis and CdaR controlling calcium dependent antibiotic biosynthesis in *S. coelicolor* (73) and StrR regulates streptomycin biosynthesis in *S. griseus*. In many cases the regulators are responsive to small molecule signals. Regulators responsive to autoregulatory molecules such as ϒ-butyolactone are well studied for example, for regulation of antibiotics such as jadomycin, actionorhodin and simocyclinone biosynthesis (74-78). The complete regulatory network of activation of biosynthetic pathway was studied initially for streptomycin in *S. griseus*. AdpA (A factor dependent protein), is a pleiotropic regulator which activates transcription of StrR. The transcription of AdpA depends on ϒ-butyrolactone also known as A-factor (79-81). AdpA is also involved in morphological differentiation (82, 83). AtrA is a pleotropic regulator of *strR*, for streptomycin biosynthesis and it has an orthologue in *S. coelicolor* which activates transcription of *act*II-ORF4 (84). Apart from AtrR many direct and indirect regulators regulating antibiotic biosynthesis have been identified. For instance, DasR, which controls uptake and metabolism of N-acetylglucosamine and degradation of chitin (85-87), AbsA2, which negatively regulates antibiotic production in *S. coelicolor* (88, 89), AbrC3, a response regulator of two component system having two histidine kinases and a positive response regulator of antibiotic production in *S. coelicolor* (90, 91) and Crp, a cyclic AMP receptor protein which mediate carbon catabolite

repression in *E. coli* and it also controls various cellular processes in different bacteria are found to be important regulators of antibiotic production, spore germination and colony development in *S. coeliolor* (92-94). There is direct regulation of *act*II-ORF4 by RelA, which is required for induction of stringent response during starvation (see below for detail). *Streptomyces* harbors large number of two component system (TCS) which regulate the secondary metabolism. For example phoRP TCS system play important role in controlling antibiotic production. AfsQ1/2 controls biosynthesis of ACT, RED, CDA in response to nitrogen limitation, DraRK TCS (nitrogen responsive) and OsdRK TCS (oxygen responsive) both are required for Act production.

1.4.2 Effect of amino acid limitation and ribosome mediated effect on antibiotic biosynthesis (effect of *relA* on antibiotic production)

In *E.coli* the role of highly phosphorylated guanosine, ppGpp and pppGpp in growth rate control of gene expression and stationary phase gene expression is well recognized. In *Streptomyces* also several studies have noted correlation of ppGpp synthesis and antibiotic production (95). In *E. coli,* (p)ppGpp is synthesized when an uncharged tRNA binds to the acceptor site of translating ribosome from ATP and GTP by ribosome bound RelA. In Actinomycetes after amino acid starvation accumulation of (p)ppGpp was first demonstrated in *S. hygroscopicus* (96). Correlation between (p)ppGpp synthesis and antibiotic production and morphological differentiation was observed in many *Streptomyces* spp. (97, 98). As shown by Bibb *et. al., relA* deletion mutant of *S. coelicolor* failed to make antibiotics ACT and RED in medium dependent manner. In another studies by Martinez *et. al., relA* deletion mutant of different *S. coelicolor* strain led to loss of ACT production and no apparent effect on RED synthesis. The earlier study concluded that *relA* is required for antibiotic production under nitrogen limiting condition (99). On the contrary, to the above observed phenotypes in *S. clavuligerus,* the relA null mutants showed antibiotic - clavulanic acid and cephamycin C overproduction (100).

1.4.3 Phosphate regulation of antibiotic production

Phosphate limitation in growing culture activates phosphate scavenging and induces growth transition preceding stationary phase and secondary metabolism. The two component system phoR-phoP is signal transduction system for phosphate control

in *S. coelicolor* and affects ACT and RED production influencing transcription of *act* and *red* genes (101). Similar effect on antibiotic production is also seen in *S. rimosus* (oxytetracyclin), *S. lividans* (ACT and RED), *S. natalensis* (pimaricin) and *S. griseus* (candicidin) (102-105). *phoP* regulon not only activate pathways for phosphate scavenging and cell wall polymer biosynthesis but also mediated transient shut down of central metabolic pathway of some secondary metabolic pathways and morphological differentiation. Further PhoP gets phosphorylated by phoR which is membrane bound sensor kinase which senses phosphate limitation further PhoP binds to specific sequence in target promoters for its expression. Some studies are contrary which shows that PhoP binds and represses promoter of *afsS*, encoding global activator of antibiotic production showing negative effect i.e. phosphate limitation activates ACT and RED production in phosphate limiting condition. In *S. lividans* on the contrary, the *phoP* deletion turns off ACT and RED production. This can be explained by many other genes affected by *phoP* including developmental genes (106).

1.4.4 Regulation of secondary metabolism by nitrogen

Nitrogen is essential for anabolism of amino acids, nucleic acids and other macromolecules. Ammonia, nitirite, nitrate, urea, amino sugars and amino acids are utilized as nitrogen source by *Streptomyces*. *Streptomyces* possesses regulatory system which enables them to adapt to nitrogen availability. It has been reported that the sources of nitrogen influence secondary metabolism. Nitrogen source which favors growth reduces production of secondary metabolite. GlnR is a global regulator which controls nitrogen metabolism, *glnR* null mutant of *S. coelicolor* exhibited blocked production of Act and Red (107-109). GlnR has also been shown to directly regulate synthesis of avermectin and oligomycin biosynthesis in *S. avermitilis* (110).

1.4.5 Control of antibiotic production by carbon source

The availability of glucose as carbon source promotes vegetative growth but suppresses morphological and chemical differentiation. Glucose represses Act production in *S. coelicolor* (111, 112), chloremphenicol in *S. venezuelae* (113), streptomycin production in *S. griseus* (114) and erythromycin production in *Saccharopolyspora erythraea* (115, 116). Carbon catabolite repression controls carbon utilization in *Streptomyces* as well for utilization of high energy carbon source

such as glucose, fructose and TCA cycle intermediates over lactose, glycerol and mannitol which are energetically less favourable. In *S. peucetius,* there is regulatory system which responds to glucose transport and metabolism and activated carbon catabolite repression (117). Glycolytic intermediates in growth media results in glucose repression of daunorubicin and doxorubicin biosynthesis in *S. peucetius* (118). Many antibiotics shows phase dependent control. For instance the *bld* mutants fail to produce antibiotic, they also exhibit defective CCR. Thus it becomes clear that there is link between regulation of carbon utilization and morphological differentiation. BldB is DNA binding protein mainly found in Actinobacteria, the paralogoues of the BldB in *S. coelicolor* found to play role both in antibiotic biosynthesis and development (119-121).

1.4.6 Regulation of "cryptic polyketide" gene cluster

The CPK (cryptic polyketide gene cluster) of *S. coelicolor* regulation involves gamma- butyrolactone (GBL) signaling molecule (SCB1) or its congeners (122, 123). The GBL autoregulators are membrane diffusible and accumulate in culture after certain concentration it binds to cytoplasmic protein and cause it to dissociate from end and there after derepresses promoters of target genes. Some *Streptomyces* autoregulators coordinate physiological changes across colony (124).

Part of the complex cluster-situated regulatory system for CPK biosynthesis is involved in cross talk with other antibiotic biosynthesis pathways and CPK biosynthesis is induced by entirely 3 different secondary metabolites (SCBs, Act and RED) through ArpA-like repressors. Phosphate response regulator (*phoP*) directly represses *cpkO* (biosynthetic gene of CPK cluster) and AfsQ1 activates promoter between biosynthetic genes *cpkO* and *cpkD (123).*

1.4.7 Regulation of antibiotic production by N-acetylglucosamine

As discussed above the autolysis of substrate mycelium provides nutrients for morphological differentiation and secondary metabolite biosynthesis (106, 125). During autolysis, the N-acetylglucosamine (GlcNAc) released in the medium is imported back and phosphorylated by phosphotranferase transport system. After import the GlcNAc~P is deacetylated resulting in accumulation of GlcN~P, which serves as signaling molecule and bind to DasR and relieves repression of DasR target genes. DasR target genes include genes for PTS for GlcNAc uptake and phosphate

uptake system. Other downstream targets are CSR genes for ACT and RED. This is evidenced by addition of GlcNAc to solid medium causing stimulation of antibiotic production and development (126-128). The *dasR* mutants exhibited difference in expression of many genes including *act*, *red* and *cpk* clusters.

1.4.8 *Streptomyces* Antibiotic Regulatory Proteins (SARPs)

SARPs are CSRs encountered in *S. colelicolor* and other *Streptomyces* spp. and mainly associated with antibiotic biosynthesis gene clusters for example the actII-ORF4 activates ACT biosynthesis (129), CcaR that activates the transcription of cephamycin and clavulanic acid biosynthetic gene clusters in *S. clavuligerus* (130). SARPs have N-terminal winged helix-turn-helix DNA binding motifs at 11 nucleotide spacing and an adjacent transcriptional activation domain which switches on expression of target antibiotic production genes (131). Many SARPs are less than 300 residues long referred as small "SARPs" (*actII*-ORF4 and RedD), those around 600 amino acids long are medium SARPs and other containing 1000 residues are large SARPs. The functional domains of SARPs include N-terminal SARP domain, central AAA domain (ATPase associated with diverse cellular activities) and conserved C-terminal domain of unknown function. *Streptomyces* Antibiotic Regulatory Proteins (SARP) and Cluster Situated Regulator (CSR) encoded by cdaR activate *cda* biosynthetic genes. Two component regulatory genes *absA1*/*absA2* regulates CDA biosynthesis where AbsA1 is membrane-located sensor histidine protein kinase and AbsA2 is the target which is a response regulator whose phosphorylation increases its binding efficiency to repression of the promoter of *cdaR* (132).

1.5 *Streptomyces flavisclerotic*us: A producer of antibiotic chromomycin

*Streptomyces flavisclerotic*us, a synonym for *Streptomyces minutisclerotic*us of the present study was first identified by Goodfellow *et. al.,* in 1986 (133). *S. flavisclerotic*us was procured from Microbial Type Culture Collection Center, Institute of Microbial Technology, Chandigarh, India as *Streptomyces* sp. 275. The molecule produced by this organism has been characterized by HPLC, ESI-MS and by NMR to be yellow colored antibiotic chromomycin, an aureolic class of antitumor compound, aromatic polyketide in nature, synthesized by polyketide synthase cluster of genes (PKSII) (Fig. 1.3). It is used *in-vitro* as G/C specific fluorescent DNA

binding dye, as an antibiotic and *in-vitro* as anti cancer drug that inhibits RNA and DNA synthesis.

The whole-genome of *S. flavisclerotícus* has been sequenced using Illumina platform NextSeq Illumina paired-end technology. The sequence has been deposited in Gene Bank at NCBI under the Accession no. MAZZ00000000. *Streptomyces flavisclerotícus* exhibits closest homology to *Streptomyces minutisclerotícus*. RAST annotation (Rapid Annotation using Subsystem Technology) shows that *S. flavisclerotícus* contains 3,071 characterized, 2296 hypothetical /putative and 1034 proteins with pathway annotation. AntiSMASH online tool has been used to identify other secondary metabolite gene(s) present in the genome. AntiSMASH predictor identified full complement of genes for chromomycin A3 biosynthesis and also gene clusters for spore pigment biosynthesis, desferrioxamine B biosynthetic and ectoine biosynthesis.

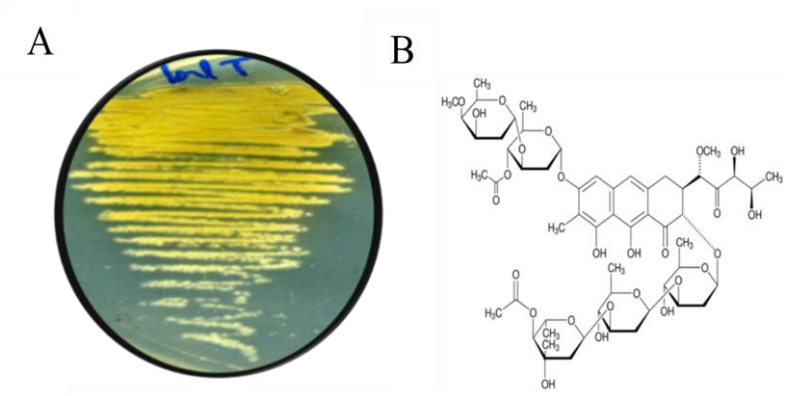

Figure 1.3 (A) *S. flavisclerotícus* producing yellow colored chromomycin on R2YE media incubated for 5 days; (B) Structure of chromomycin A3 (Figure source-Sigma)

1.5.1 Chromomycin chemical structure and its mode of action

Chromomycin belongs to an aureolic type of compound which contains tricyclic chromophore (aglycon) with two aliphatic side chains attached at C-3 and C-7. The aglycon is synthesized when one acetyl-CoA get condensed with nine malonyl-CoA units. The aglycons are further glycosylated by oligosaccharides of different chains sugar composition. Chromomycin and mithramycin both belong to same aureolic class of compound differs only in glycosylation patterns. Chromomycin A3 contains a trisaccharide of D-olivose (sugar C), D-olivose (sugar D) and 4-O-acetyl-L-chromose B (sugar E) and a disaccharide of 4-O-acetyl-D-oliose (sugar A) and 4-O-methyl-D-oliose (sugar B) at positions 2 and 6 of aglycon respectively. The biological

activity of chromomcycin is mainly contributed by carbohydrate moieties. The DNA binding property is contributed by mainly acetoxy groups of sugar A and E by providing H bond with 2-amino groups of G bases and specificity of DNA binding. Chromomycin A3 binds reversibly to GC-specific DNA ligand in minor groove which inhibits transcription, DNA gyrase and topoisomerase II activity. Moreover it is potent inhibitor of RNA polymerase. The sugar moiety present in chromomycin structure responsible for its inhibiting RNA polymerase activity and to a lesser extent it is found to inhibit DNA polymerase [Deoxynucleoside triphosphate: DNA nucleotidyltransferase] reaction.

1.5.2 Chromomycin biosynthesis

Proposed pathway for chromomycin biosynthesis is depicted in figure 1.4.

1.5.2.1 PKS genes:

The backbone of chromomycin aglycon of 20 carbon polyketide is synthesized by 3 genes namely *sflP* (ketosynthaseI, KS_{α}), *sflK* (ketosynthase II, KS_{β}) *and sflS* (Acyl Carrier Protein) which code for minimal PKS. The enzymes responsible for cyclization and aromatization processes are SflQ, SflX and SflY where *sfl*Q codes for cyclase/aromatase for cyclization and aromatization of first ring, *sfl*X and *sfl*Y are cyclases for fourth and second ring closure during chromomycin biosynthesis. Post-polyketide genes are *sfl*OI, *sfl*OII and *sfl*OIV involved in hydroxylation of aromatic polyketides (134).

1.5.2.2 Genes involved in glycosylation

Five deoxysugars are attached to the aglycon of chromomycin which are two D-olivoses, one 4-*O*-acetyl-L-chromose, one 4-*O*-acetyl-D-oliose, and one 4-*O*-methyl-D-oliose. Genes involved in first steps of deoxysugar biosynthesis are *sfl*D and *sfl*E. *sfl*D is NDP-D-glucose synthase and *sfl*E codes for NDP-D-glucose 4,6-dehydratase. Biosynthesis of first and second steps of chromomycin biosynthesis is catalyzed by products of *sfl*D and *sfl*E. Other downstream genes to *sfl*D and *sfl*E are *sfl*V (glucose dehydratase), *sfl*W (ketoreductase) and *sfl*UI (ketoreductase). The other genes are *sflUII* and *sflUIII* whose products are ketoreductase involved in biosynthesis of D-deoxysugars. The 2-deoxygenation step occurring during biosynthesis of all chromomycin of deoxysugars (sugars A-E) is carried out by *sflV* (dehydratase) and *sflW* (reductase). Downstream *sflUIII* is *sflC* that codes for

methyltransferase. Another gene function of *sflF* is an epimerase and contains the conserved domain for NDP-sugar-isomerase. The last step after sugar synthesis is its decoration by methyl (sugar B) or acetyl groups (sugar A and E) at C-4 hydroxy groups. *sflMIII* codes for **O**-methyltransferase. After the aglycons get attached to sugar they get methylated by pathway specific enzymes. The modifying enzymes include (i) *sflAI* that codes for acyltransferase acts on aglycon sugars (ii) *cmmIII* is methyltransferase for methylation of d-oliose of disaccharide (iii) *sflA* being acetyltransferase, transfers one or both acetyl groups to sugars A and E (iv) Finally the glycosyltransferase, *sflGIV* and *sflGIII* transfer sugars (first and second D-olivose, deoxysugars) to aglycon and (v) *sflGI* transfers third deoxysugar to aglycon unit (134).

1.5.2.3 Regulation of chromomycin biosynthesis

*sfl*RI and *sfl*RII are important genes involved in regulation of chromomycin biosynthesis. *sfl*RI is transcriptional activator and *sfl*RII is transcriptional regulator of chromomycin biosynthesis. These genes are cotranscribed with genes of ABC transport system (134).

1.5.2.4 Genes for self-resistance

Gene products of *sflA*, *sflB* and *sflX* are involved in self resistance to chromomycin. *sfl*A and *sfl*B are similar to ATP binding proteins and membrane proteins being part of type I ABC transport system. *sflX* gene product resembles one of the three subunits of ABC excision nuclease system for DNA repair. The product of ABC transport system (SflAB) and SflX are involved in self resistance (134).

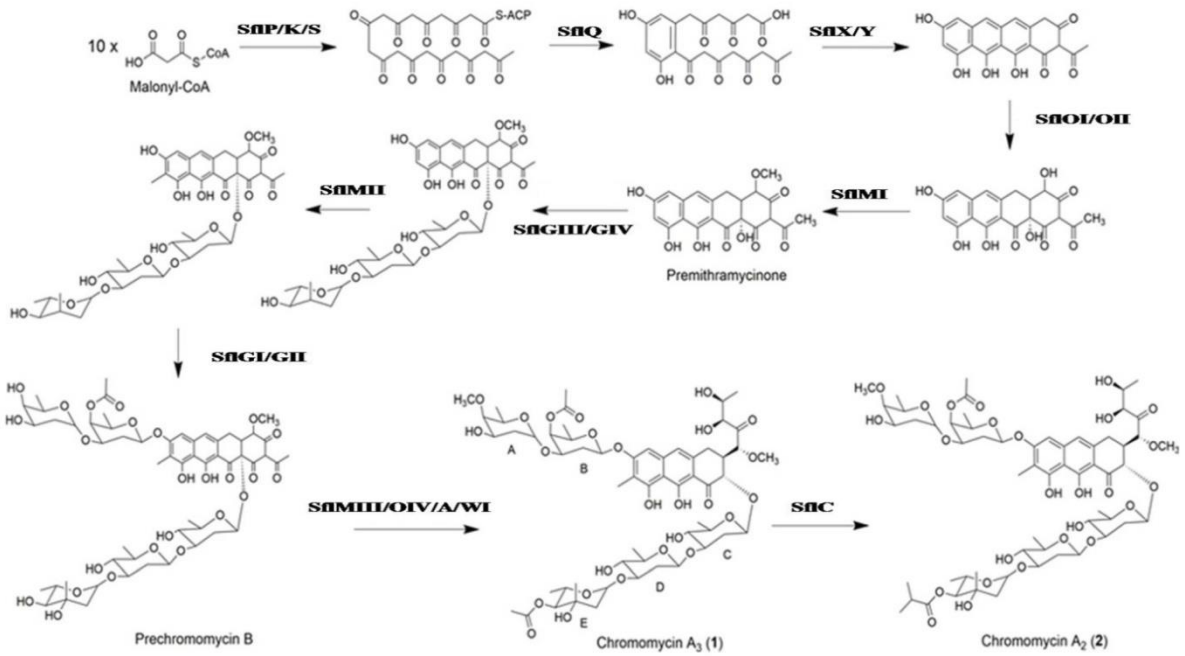

Fig. 1.4 Proposed pathway for biosynthesis of chromomycin A3 and A2 [reproduced from (135)].

1.6 References

1. Servin JA, Herbold CW, Skophammer RG, & Lake JA (2008) Evidence excluding the root of the tree of life from the actinobacteria. *Mol Biol Evol* 25(1):1-4.
2. Hugenholtz P (2002) Exploring prokaryotic diversity in the genomic era. *Genome Biol* 3(2):REVIEWS0003.
3. Stackebrandt E, Rainey FA, & Ward-Rainey NL (1997) Proposal for a new hierarchic classification system, Actinobacteria classis nov. *International Journal of Systematic and Evolutionary Microbiology* 47(2):479-491.
4. Ventura M*, et al.* (2007) Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiol Mol Biol Rev* 71(3):495-548.
5. Berdy J (2005) Bioactive microbial metabolites. *J Antibiot (Tokyo)* 58(1):1-26.
6. Hodgson DA (2000) Primary metabolism and its control in streptomycetes: a most unusual group of bacteria. *Adv Microb Physiol* 42:47-238.
7. Lechevalier H (1989) A practical guide to generic identification of actinomycetes. *Bergey's manual of systematic bacteriology* 4:2344-2347.
8. Williams S (1989) Genus Streptomyces waksman and henrici 1943. *BERGEY's Manual of Syntematic Bacteriology* 4:2452-2492.
9. Anderson AS & Wellington E (2001) The taxonomy of Streptomyces and related genera. *International Journal of Systematic and Evolutionary Microbiology* 51(3):797-814.
10. Bentley SD*, et al.* (2002) Complete genome sequence of the model actinomycete Streptomyces coelicolor A3 (2). *Nature* 417(6885):141-147.
11. Wright F & Bibb MJ (1992) Codon usage in the G+ C-rich Streptomyces genome. *Gene* 113(1):55-65.
12. Chen CW (1996) Complications and implications of linear bacterial chromosomes. *Trends in Genetics* 12(5):192-196.
13. Ochi K & Hosaka T (2013) New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. *Appl Microbiol Biotechnol* 97(1):87-98.

14. Ikeda H*, et al.* (2003) Complete genome sequence and comparative analysis of the industrial microorganism Streptomyces avermitilis. *Nature biotechnology* 21(5):526-531.
15. Chen CW, Huang C-H, Lee H-H, Tsai H-H, & Kirby R (2002) Once the circle has been broken: dynamics and evolution of Streptomyces chromosomes. *TRENDS in Genetics* 18(10):522-529.
16. Keiser T, Bibb M, Buttner M, Chater K, & Hopwood D (2000) Practical streptomyces genetics. *The John Innes Foundation, Norwich*.
17. Te Poele EM, Bolhuis H, & Dijkhuizen L (2008) Actinomycete integrative and conjugative elements. *Antonie Van Leeuwenhoek* 94(1):127-143.
18. te Poele EM*, et al.* (2008) Actinomycete integrative and conjugative pMEA-like elements of Amycolatopsis and Saccharopolyspora decoded. *Plasmid* 59(3):202-216.
19. Claessen D, De Jong W, Dijkhuizen L, & Wösten HA (2006) Regulation of Streptomyces development: reach for the sky! *Trends in microbiology* 14(7):313-319.
20. Chater KF (2001) Regulation of sporulation in Streptomyces coelicolor A3 (2): a checkpoint multiplex? *Current opinion in microbiology* 4(6):667-673.
21. Flärdh K & Buttner MJ (2009) Streptomyces morphogenetics: dissecting differentiation in a filamentous bacterium. *Nature Reviews Microbiology* 7(1):36.
22. Manteca A, Fernandez M, & Sanchez J (2005) A death round affecting a young compartmentalized mycelium precedes aerial mycelium dismantling in confluent surface cultures of Streptomyces antibioticus. *Microbiology* 151(11):3689-3697.
23. Manteca A, Mäder U, Connolly BA, & Sanchez J (2006) A proteomic analysis of Streptomyces coelicolor programmed cell death. *Proteomics* 6(22):6008-6022.
24. Yague P, Lopez-Garcia MT, Rioseras B, Sanchez J, & Manteca A (2012) New insights on the development of Streptomyces and their relationships with secondary metabolite production. *Curr Trends Microbiol* 8:65-73.
25. Chouayekh H*, et al.* (2007) Phosphoinositides are involved in control of the glucose-dependent growth resumption that follows the transition phase in Streptomyces lividans. *Journal of bacteriology* 189(3):741-749.
26. Granozzi C, Billetta R, Passantino R, Sollazzo M, & Puglia A (1990) A breakdown in macromolecular synthesis preceding differentiation in Streptomyces coelicolor A3 (2). *Microbiology* 136(4):713-716.
27. Neumann T, Piepersberg W, & Distler J (1996) Decision phase regulation of streptomycin production in Streptomyces griseus. *Microbiology* 142(8):1953-1963.
28. Novotna J*, et al.* (2003) Proteomic studies of diauxic lag in the differentiating prokaryote Streptomyces coelicolor reveal a regulatory network of stress-induced proteins and central metabolic enzymes. *Molecular microbiology* 48(5):1289-1303.
29. Zhou L-h, Li Y-q, Li Y-q, & Wu D (2005) Spatio-temporal expression of the pathway–specific regulatory gene redD in S. coelicolor. *Journal of Zhejiang University. Science. B* 6(6):464.
30. Glazebrook MA, Doull JL, Stuttard C, & Vining LC (1990) Sporulation of Streptomyces venezuelae in submerged cultures. *Microbiology* 136(3):581-588.
31. Kendrick KE & Ensign JC (1983) Sporulation of Streptomyces griseus in submerged culture. *Journal of Bacteriology* 155(1):357-366.
32. Kuimova T & Soina V (1981) A submerged sporulation and ultrastructural changes in the mycelium of Streptomyces chrysomallus. *Hindustan antibiotics bulletin* 23:1.
33. Novella IS, Barbés C, & Sánchez J (1992) Sporulation of Streptomyces antibioticus ETHZ 7451 in submerged culture. *Canadian journal of microbiology* 38(8):769-773.
34. Rho YT & Lee KJ (1994) Kinetic characterization of sporulation in Streptomyces albidoflavus SMF301 during submerged culture. *Microbiology* 140(8):2061-2065.

35. Rueda B, Miguélez EM, Hardisson C, & Manzanal MB (2001) Mycelial differentiation and spore formation by Streptomyces brasiliensis in submerged culture. *Canadian journal of microbiology* 47(11):1042-1047.
36. Daza A, Martin JF, Dominguez A, & Gil JA (1989) Sporulation of several species of Streptomyces in submerged cultures after nutritional downshift. *Microbiology* 135(9):2483-2491.
37. Koepsel R & Ensign J (1984) Microcycle sporulation of Streptomyces viridochromogenes. *Archives of microbiology* 140(1):9-14.
38. Manteca A, Alvarez R, Salazar N, Yagüe P, & Sanchez J (2008) Mycelium differentiation and antibiotic production in submerged cultures of Streptomyces coelicolor. *Applied and environmental microbiology* 74(12):3877-3886.
39. Manteca A, Sanchez J, Jung HR, Schwämmle V, & Jensen ON (2010) Quantitative proteomic analysis of Streptomyces coelicolor development demonstrates that onset of secondary metabolism coincides with hyphae differentiation. *Molecular & Cellular Proteomics*:mcp. M900449-MCP900200.
40. Yagüe P*, et al.* (2013) Transcriptomic analysis of Streptomyces coelicolor differentiation in solid sporulating cultures: first compartmentalized and second multinucleated mycelia have different and distinctive transcriptomes. *PLoS One* 8(3):e60665.
41. Miguélez EM, Hardisson C, & Manzanal MB (1999) Hyphal death during colony development in Streptomyces antibioticus: morphological evidence for the existence of a process of cell deletion in a multicellular prokaryote. *The Journal of cell biology* 145(3):515-525.
42. Manteca A, Fernandez M, & Sanchez J (2006) Cytological and biochemical evidence for an early cell dismantling event in surface cultures of Streptomyces antibioticus. *Research in microbiology* 157(2):143-152.
43. Sexton DL*, et al.* (2015) Resuscitation-promoting factors are cell wall-lytic enzymes with important roles in the germination and growth of Streptomyces coelicolor. *J Bacteriol* 197(5):848-860.
44. St-Onge RJ*, et al.* (2015) Nucleotide second messenger-mediated regulation of a muralytic enzyme in Streptomyces. *Mol Microbiol* 96(4):779-795.
45. Chandra G & Chater KF (2014) Developmental biology of Streptomyces from the perspective of 100 actinobacterial genome sequences. *FEMS Microbiol Rev* 38(3):345-379.
46. Flardh K, Richards DM, Hempel AM, Howard M, & Buttner MJ (2012) Regulation of apical growth and hyphal branching in Streptomyces. *Curr Opin Microbiol* 15(6):737-743.
47. Chaplin AK*, et al.* (2015) GlxA is a new structural member of the radical copper oxidase family and is required for glycan deposition at hyphal tips and morphogenesis of Streptomyces lividans. *Biochem J* 469(3):433-444.
48. Liman R, Facey PD, van Keulen G, Dyson PJ, & Del Sol R (2013) A laterally acquired galactose oxidase-like gene is required for aerial development during osmotic stress in Streptomyces coelicolor. *PLoS One* 8(1):e54112.
49. van Dissel D, Claessen D, Roth M, & van Wezel GP (2015) A novel locus for mycelial aggregation forms a gateway to improved Streptomyces cell factories. *Microb Cell Fact* 14:44.
50. Celler K, Koning RI, Willemse J, Koster AJ, & van Wezel GP (2016) Cross-membranes orchestrate compartmentalization and morphogenesis in Streptomyces. *Nat Commun* 7:ncomms11836.
51. Yague P*, et al.* (2016) Subcompartmentalization by cross-membranes during early growth of Streptomyces hyphae. *Nat Commun* 7:12467.

52. Chandra G & Chater KF (2008) Evolutionary flux of potentially bldA-dependent Streptomyces genes containing the rare leucine codon TTA. *Antonie Van Leeuwenhoek* 94(1):111-126.
53. Champness WC (1988) New loci required for Streptomyces coelicolor morphological and physiological differentiation. *J Bacteriol* 170(3):1168-1174.
54. Merrick MJ (1976) A morphological and genetic mapping study of bald colony mutants of Streptomyces coelicolor. *J Gen Microbiol* 96(2):299-315.
55. Zhang L, Willemse J, Claessen D, & van Wezel GP (2016) SepG coordinates sporulation-specific cell division and nucleoid organization in Streptomyces coelicolor. *Open Biol* 6(4):150164.
56. Donczew M*, et al.* (2016) ParA and ParB coordinate chromosome segregation with cell elongation and division during Streptomyces sporulation. *Open Biol* 6(4):150263.
57. Hopwood DA & Sherman DH (1990) Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. *Annu Rev Genet* 24:37-66.
58. Novotna J*, et al.* (2003) Proteomic studies of diauxic lag in the differentiating prokaryote Streptomyces coelicolor reveal a regulatory network of stress-induced proteins and central metabolic enzymes. *Mol Microbiol* 48(5):1289-1303.
59. Chopra I & Roberts M (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* 65(2):232-260.
60. Ghannoum MA & Rice LB (1999) Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clinical microbiology reviews* 12(4):501-517.
61. Katsuyama Y, Funa N, Miyahisa I, & Horinouchi S (2007) Synthesis of unnatural flavonoids and stilbenes by exploiting the plant biosynthetic pathway in Escherichia coli. *Chemistry & biology* 14(6):613-621.
62. Li J, Kim SG, & Blenis J (2014) Rapamycin: one drug, many effects. *Cell metabolism* 19(3):373-379.
63. Shushni MA, Singh R, Mentel R, & Lindequist U (2011) Balticolid: a new 12-membered macrolide with antiviral activity from an ascomycetous fungus of marine origin. *Marine drugs* 9(5):844-851.
64. Tacar O, Sriamornsak P, & Dass CR (2013) Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *Journal of pharmacy and pharmacology* 65(2):157-170.
65. van de Donk NW, Kamphuis MM, Lokhorst HM, & Bloem AC (2002) The cholesterol lowering drug lovastatin induces cell death in myeloma plasma cells. *Leukemia* 16(7):1362.
66. Hopwood DA & Sherman DH (1990) Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. *Annual review of genetics* 24(1):37-62.
67. Staunton J & Weissman KJ (2001) Polyketide biosynthesis: a millennium review. *Natural product reports* 18(4):380-416.
68. Shen B (2003) Polyketide biosynthesis beyond the type I, II and III polyketide synthase paradigms. *Current opinion in chemical biology* 7(2):285-295.
69. Bisang C*, et al.* (1999) A chain initiation factor common to both modular and aromatic polyketide synthases. *Nature* 401(6752):502-505.
70. Fu H, McDaniel R, Hopwood DA, & Khosla C (1994) Engineered biosynthesis of novel polyketides: stereochemical course of two reactions catalyzed by a polyketide synthase. *Biochemistry* 33(31):9321-9326.
71. Funa N*, et al.* (1999) A new pathway for polyketide synthesis in microorganisms. *Nature* 400(6747):897-899.

72. Moore BS & Hopke JN (2001) Discovery of a new bacterial polyketide biosynthetic pathway. *Chembiochem* 2(1):35-38.
73. Wietzorrek A & Bibb M (1997) A novel family of proteins that regulates antibiotic production in streptomycetes appears to contain an OmpR-like DNA-binding fold. *Mol Microbiol* 25(6):1181-1184.
74. Tahlan K*, et al.* (2007) Initiation of actinorhodin export in Streptomyces coelicolor. *Molecular microbiology* 63(4):951-961.
75. Takano E (2006) γ-Butyrolactones: Streptomyces signalling molecules regulating antibiotic production and differentiation. *Current opinion in microbiology* 9(3):287-294.
76. Wang L*, et al.* (2009) Autoregulation of antibiotic biosynthesis by binding of the end product to an atypical response regulator. *Proceedings of the National Academy of Sciences* 106(21):8617-8622.
77. Willems A*, et al.* (2008) Crystal structures of the Streptomyces coelicolor TetR-like protein ActR alone and in complex with actinorhodin or the actinorhodin biosynthetic precursor (S)-DNPA. *Journal of molecular biology* 376(5):1377-1387.
78. Willey JM & Gaskell AA (2010) Morphogenetic signaling molecules of the streptomycetes. *Chemical reviews* 111(1):174-187.
79. Ohnishi Y, Kameyama S, Onaka H, & Horinouchi S (1999) The A-factor regulatory cascade leading to streptomycin biosynthesis in Streptomyces griseus: identification of a target gene of the A-factor receptor. *Molecular microbiology* 34(1):102-111.
80. Onaka H*, et al.* (1995) Cloning and characterization of the A-factor receptor gene from Streptomyces griseus. *Journal of Bacteriology* 177(21):6083-6092.
81. Onaka H & Horinouchi S (1997) DNA-binding activity of the A-factor receptor protein and its recognition DNA sequences. *Molecular microbiology* 24(5):991-1000.
82. Akanuma G, Hara H, Ohnishi Y, & Horinouchi S (2009) Dynamic changes in the extracellular proteome caused by absence of a pleiotropic regulator AdpA in Streptomyces griseus. *Molecular microbiology* 73(5):898-912.
83. Ohnishi Y, Yamazaki H, Kato J-Y, Tomono A, & Horinouchi S (2005) AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in Streptomyces griseus. *Bioscience, biotechnology, and biochemistry* 69(3):431-439.
84. Uguru GC*, et al.* (2005) Transcriptional activation of the pathway-specific regulator of the actinorhodin biosynthetic genes in Streptomyces coelicolor. *Mol Microbiol* 58(1):131-150.
85. Colson S*, et al.* (2007) Conserved cis-acting elements upstream of genes composing the chitinolytic system of streptomycetes are DasR-responsive elements. *Journal of molecular microbiology and biotechnology* 12(1-2):60-66.
86. Rigali S*, et al.* (2006) The sugar phosphotransferase system of Streptomyces coelicolor is regulated by the GntR-family regulator DasR and links N-acetylglucosamine metabolism to the control of development. *Molecular microbiology* 61(5):1237-1251.
87. Świątek-Połatyńska MA*, et al.* (2015) Genome-wide analysis of in vivo binding of the master regulator DasR in Streptomyces coelicolor identifies novel non-canonical targets. *PLoS One* 10(4):e0122479.
88. Brian P, Riggle PJ, Santos RA, & Champness WC (1996) Global negative regulation of Streptomyces coelicolor antibiotic synthesis mediated by an absA-encoded putative signal transduction system. *Journal of bacteriology* 178(11):3221-3231.
89. Champness W, Riggle P, Adamidis T, & Vandervere P (1992) Identification of Streptomyces coelicolor genes involved in regulation of antibiotic synthesis. *Gene* 115(1-2):55-60.

90. Rico S*, et al.* (2014) Deciphering the regulon of Streptomyces coelicolor AbrC3, a positive response regulator of antibiotic production. *Appl. Environ. Microbiol.* 80(8):2417-2428.
91. Yepes A, Rico S, Rodríguez-García A, Santamaría RI, & Díaz M (2011) Novel two-component systems implied in antibiotic production in Streptomyces coelicolor. *PLoS One* 6(5):e19980.
92. Gao C, Hindra, Mulder D, Yin C, & Elliot MA (2012) Crp is a global regulator of antibiotic production in streptomyces. *MBio* 3(6).
93. Korner H, Sofia HJ, & Zumft WG (2003) Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. *FEMS Microbiol Rev* 27(5):559-592.
94. Piette A*, et al.* (2005) From dormant to germinating spores of Streptomyces coelicolor A3(2): new perspectives from the crp null mutant. *J Proteome Res* 4(5):1699-1708.
95. Chakraburtty R & Bibb M (1997) The ppGpp synthetase gene (relA) of Streptomyces coelicolor A3(2) plays a conditional role in antibiotic production and morphological differentiation. *J Bacteriol* 179(18):5854-5861.
96. Riesenberg D, Bergter F, & Kari C (1984) Effect of serine hydroxamate and methyl aD-glucopyranoside treatment on nucleoside polyphosphate pools, RNA and protein accumulation in Streptomyces hygroscopicus. *Microbiology* 130(10):2549-2558.
97. Ochi K (1987) Metabolic initiation of differentiation and secondary metabolism by Streptomyces griseus: significance of the stringent response (ppGpp) and GTP content in relation to A factor. *J Bacteriol* 169(8):3608-3616.
98. Ochi K (1990) A relaxed (rel) mutant of Streptomyces coelicolor A3(2) with a missing ribosomal protein lacks the ability to accumulate ppGpp, A-factor and prodigiosin. *J Gen Microbiol* 136(12):2405-2412.
99. Sun J, Hesketh A, & Bibb M (2001) Functional analysis of relA and rshA, two relA/spoT homologues of Streptomyces coelicolor A3(2). *J Bacteriol* 183(11):3488-3498.
100. Gomez-Escribano JP, Martin JF, Hesketh A, Bibb MJ, & Liras P (2008) Streptomyces clavuligerus relA-null mutants overproduce clavulanic acid and cephamycin C: negative regulation of secondary metabolism by (p)ppGpp. *Microbiology* 154(Pt 3):744-755.
101. Santos-Beneit F*, et al.* (2011) The RNA polymerase omega factor RpoZ is regulated by PhoP and has an important role in antibiotic biosynthesis and morphological differentiation in Streptomyces coelicolor. *Appl Environ Microbiol* 77(21):7586-7594.
102. Asturias JA, Martín JF, & Liras P (1994) Biosynthesis and phosphate control of candicidin byStreptomyces acrimycini JI2236: effect of amplification of thepabAB gene. *Journal of industrial microbiology* 13(3):183-189.
103. Martin JF (2004) Phosphate control of the biosynthesis of antibiotics and other secondary metabolites is mediated by the PhoR-PhoP system: an unfinished story. *J Bacteriol* 186(16):5197-5201.
104. McDowall KJ, Thamchaipenet A, & Hunter IS (1999) Phosphate control of oxytetracycline production by Streptomyces rimosus is at the level of transcription from promoters overlapped by tandem repeats similar to those of the DNA-binding sites of the OmpR family. *Journal of bacteriology* 181(10):3025-3032.
105. Sola-Landa A, Moura RS, & Martin JF (2003) The two-component PhoR-PhoP system controls both primary metabolism and secondary metabolite biosynthesis in Streptomyces lividans. *Proc Natl Acad Sci U S A* 100(10):6133-6138.

106. Liu G, Chater KF, Chandra G, Niu G, & Tan H (2013) Molecular regulation of antibiotic biosynthesis in Streptomyces. *Microbiology and Molecular Biology Reviews* 77(1):112-143.
107. Tiffert Y*, et al.* (2011) Proteomic analysis of the GlnR-mediated response to nitrogen limitation in Streptomyces coelicolor M145. *Appl Microbiol Biotechnol* 89(4):1149-1159.
108. Tiffert Y*, et al.* (2008) The Streptomyces coelicolor GlnR regulon: identification of new GlnR targets and evidence for a central role of GlnR in nitrogen metabolism in actinomycetes. *Mol Microbiol* 67(4):861-880.
109. Wray LV, Jr., Atkinson MR, & Fisher SH (1991) Identification and cloning of the glnR locus, which is required for transcription of the glnA gene in Streptomyces coelicolor A3(2). *J Bacteriol* 173(22):7351-7360.
110. He JM*, et al.* (2016) Direct Involvement of the Master Nitrogen Metabolism Regulator GlnR in Antibiotic Biosynthesis in Streptomyces. *J Biol Chem* 291(51):26443-26454.
111. Kim E-S, Hong H-J, Choi C-Y, & Cohen SN (2001) Modulation of actinorhodin biosynthesis in Streptomyces lividans by glucose repression of afsR2 gene transcription. *Journal of bacteriology* 183(7):2198-2203.
112. Lee H-N, Im J-H, Lee M-J, Lee SY, & Kim E-S (2009) A putative secreted solute binding protein, SCO6569 is a possible AfsR2-dependent down-regulator of actinorhodin biosynthesis in Streptomyces coelicolor. *Process Biochemistry* 44(3):373-377.
113. Bhatnagar R, Doull J, & Vining L (1988) Role of the carbon source in regulating chloramphenicol production by Streptomyces venezuelae: studies in batch and continuous cultures. *Canadian journal of microbiology* 34(11):1217-1223.
114. Demain AL & Inamine E (1970) Biochemistry and regulation of streptomycin and mannosidostreptomycinase (alpha-D-mannosidase) formation. *Bacteriological reviews* 34(1):1.
115. Bermúdez O, Padilla P, Huitrón C, & Flores ME (1998) Influence of carbon and nitrogen source on synthesis of NADP+-isocitrate dehydrogenase, methylmalonyl-coenzyme A mutase, and methylmalonyl-coenzyme A decarboxylase in Saccharopolyspora erythraea CA340. *FEMS microbiology letters* 164(1):77-82.
116. Escalante L, Lopez H, Mateo RDC, Larac F, & Sanchez S (1982) Transient repression of erythromycin formation in Streptomyces erythraeus. *Microbiology* 128(9):2011-2015.
117. Sanchez S*, et al.* (2010) Carbon source regulation of antibiotic production. *The Journal of antibiotics* 63(8):442.
118. Ramos I*, et al.* (2004) Glucose kinase alone cannot be responsible for carbon source regulation in Streptomyces peucetius var. caesius. *Research in microbiology* 155(4):267-274.
119. Eccleston M, Ali RA, Seyler R, Westpheling J, & Nodwell J (2002) Structural and genetic analysis of the BldB protein of Streptomyces coelicolor. *J Bacteriol* 184(15):4270-4276.
120. Pope MK, Green B, & Westpheling J (1998) The bldB Gene Encodes a Small Protein Required for Morphogenesis, Antibiotic Production, and Catabolite Control inStreptomyces coelicolor. *Journal of bacteriology* 180(6):1556-1562.
121. Pope MK, Green BD, & Westpheling J (1996) The bld mutants of Streptomyces coelicolor are defective in the regulation of carbon utilization, morphogenesis and cell–cell signalling. *Molecular microbiology* 19(4):747-756.
122. Hsiao NH*, et al.* (2007) ScbA from Streptomyces coelicolor A3(2) has homology to fatty acid synthases and is able to synthesize gamma-butyrolactones. *Microbiology* 153(Pt 5):1394-1404.

123. Takano E*, et al.* (2005) A bacterial hormone (the SCB1) directly controls the expression of a pathway-specific regulatory gene in the cryptic type I polyketide biosynthetic gene cluster of Streptomyces coelicolor. *Mol Microbiol* 56(2):465-479.
124. Sidda JD & Corre C (2012) Gamma-butyrolactone and furan signaling systems in Streptomyces. *Methods Enzymol* 517:71-87.
125. Chater KF (2006) Streptomyces inside-out: a new perspective on the bacteria that provide us with antibiotics. *Philos Trans R Soc Lond B Biol Sci* 361(1469):761-768.
126. Nothaft H*, et al.* (2010) The permease gene nagE2 is the key to N-acetylglucosamine sensing and utilization in Streptomyces coelicolor and is subject to multi-level control. *Mol Microbiol* 75(5):1133-1144.
127. Park SS*, et al.* (2009) Mass spectrometric screening of transcriptional regulators involved in antibiotic biosynthesis in Streptomyces coelicolor A3(2). *J Ind Microbiol Biotechnol* 36(8):1073-1083.
128. Rigali S*, et al.* (2008) Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces. *EMBO Rep* 9(7):670-675.
129. Fernández-Moreno MA, Caballero J, Hopwood DA, & Malpartida F (1991) The act cluster contains regulatory and antibiotic export genes, direct targets for translational control by the bldA tRNA gene of Streptomyces. *Cell* 66(4):769-780.
130. Santamarta I*, et al.* (2011) Characterization of DNA-binding sequences for CcaR in the cephamycin–clavulanic acid supercluster of Streptomyces clavuligerus. *Molecular microbiology* 81(4):968-981.
131. Wietzorrek A & Bibb M (1997) A novel family of proteins that regulates antibiotic production in streptomycetes appears to contain an OmpR-like DNA-binding fold. *Molecular microbiology* 25(6):1181-1184.
132. Anderson TB, Brian P, & Champness WC (2001) Genetic and transcriptional analysis of absA, an antibiotic gene cluster-linked two-component system that regulates multiple antibiotics in Streptomyces coelicolor. *Mol Microbiol* 39(3):553-566.
133. Lanoot B, Vancanneyt M, Van Schoor A, Liu Z, & Swings J (2005) Reclassification of Streptomyces nigrifaciens as a later synonym of Streptomyces flavovirens; Streptomyces citreofluorescens, Streptomyces chrysomallus subsp. chrysomallus and Streptomyces fluorescens as later synonyms of Streptomyces anulatus; Streptomyces chibaensis as a later synonym of Streptomyces corchorusii; Streptomyces flaviscleroticus as a later synonym of Streptomyces minutiscleroticus; and Streptomyces lipmanii, Streptomyces griseus subsp. alpha, Streptomyces griseus subsp. cretosus and Streptomyces willmorei as later synonyms of Streptomyces microflavus. *International journal of systematic and evolutionary microbiology* 55(2):729-731.
134. Menendez N*, et al.* (2004) Biosynthesis of the antitumor chromomycin A3 in Streptomyces griseus: analysis of the gene cluster and rational design of novel chromomycin analogs. *Chem Biol* 11(1):21-32.
135. Sun L*, et al.* (2018) Manipulation of two regulatory genes for efficient production of chromomycins in Streptomyces reseiscleroticus. *Journal of biological engineering* 12(1):9.