Chapter 7

# Production of Antibacterial Compounds from Actinomycetes

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Additional information is available at the end of the chapter

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**Abstract**

Actinomycetes are soil-dwelling Gram-positive bacteria, industrially relevant as producers of a wide range of bioactive secondary metabolites, including many antibiotics of clinical and commercial importance.

The understanding of actinomycete biology has been based on extensive studies on the model organism *Streptomyces coelicolor* over many years and on the availability of its complete genome sequence. This bacterium has an unusual complex developmental cycle that includes programmed cell death phenomena that make this bacterium a multicellular prokaryotic model.

Morphological differentiation in *S. coelicolor* is strictly related to physiological differentiation: indeed the onset of morphological differentiation generally coincides with the production of secondary metabolites. During cell death, degradative proteins are synthesized and involved in an extensive degradation of some cellular constituents (proteins and lipids) used for a second growth phase, that is accompanied by antibiotic production.

If on one hand, many factors with pleiotropic activity have been identified as key players to control both morphological and physiological differentiation in *S. coelicolor*, on the other hand, for most actinomycetes, mechanisms and factors governing morphological and physiological processes have not been deeply investigated.

This chapter reviews the regulatory mechanisms known to control antibiotic production in actinomycetes and both genetic and physiological methods adopted to improve antibiotic yields.

**Keywords:** Antibiotic production, Actinomycetes, genetic engineering, regulation, heterologous expression

## 1. Introduction

The discovery by Alexander Fleming of penicillin opened up a completely new era of chemotherapy. Antibiotics have saved a large number of lives and also contributed to the increase in life expectancy. They are mainly produced by the fermentation of fungi (e.g., *Penicillium*) and bacteria (e.g., *Actinomycetes*). In particular, 80% of antibiotics are sourced from the genus *Streptomyces* and rare actinomycetes, such as *Actinomadura*, and only the 20% is produced by fungal species.

Actinomycetes are soil-dwelling Gram-positive bacteria that have extensive arsenals of secondary metabolites, metabolism products that, differently from primary metabolites such as vitamins, amino acids, nucleotides, etc., are not essential for the bacterial growth, at least in laboratory conditions; indeed, many mutants in antibiotic biosynthesis have been generated revealing that they are still vital and were used as models to understand molecular mechanisms governing antibiotic production.

Secondary metabolites include antitumorals (e.g., doxorubicin and bleomycin), antifungals (e.g., amphotericin B and nystatin), immunosuppressives (e.g., FK-506 and rapamycin), insecticides (e.g., spinosyn A and avermectin B), herbicides (e.g., phosphinotricin) and many antibiotics of clinical and commercial importance.

This chapter reviews common regulatory mechanisms that control antibiotic production in actinomycetes and both genetic and physiological methods to improve antibiotic yields.

## 2. Antibiotics and their targets

Antibiotics are molecules that selectively inhibit bacterial growth without damaging the eukaryotic organisms. The selectivity of action of these substances is given by the fact that they interfere with processes essential for the bacterial cell and absent or different in the eukaryotic cell.

Antibiotics essentially target bacterial structures or functions, such as cell wall biosynthesis (e.g., vancomycin), translation (e.g., streptomycin), RNA transcription (e.g., rifampicin), DNA replication and synthesis (e.g., novobiocin and metronidazole), membrane (polimyxins), and in general they inhibit bacterial growth (Figure 1).

Among the antibiotics that target the cell wall, glycopeptides are a class of drugs produced by Actinomycetes and are composed of glycosylated cyclic or polycyclic non-ribosomal peptides. Glycopeptides bind to the dipeptide D-alanyl--D-alanine (D-Ala-D-Ala) within the cell wall of Gram-positive bacteria preventing the addition of new units to the peptidoglycan and inhibiting the peptidoglycan synthesis. Significant glycopeptide antibiotics include the anti-infective antibiotics vancomycin, teicoplanin, telavancin, ramoplanin, decaplanin, and the antitumor antibiotic bleomycin. Vancomycin is used as antibiotic of last resort for infections with methicillin-resistant *Staphylococcus aureus*. Toxigenic *S. aureus* strains can be isolated from food making it an actual problem for public health [1].

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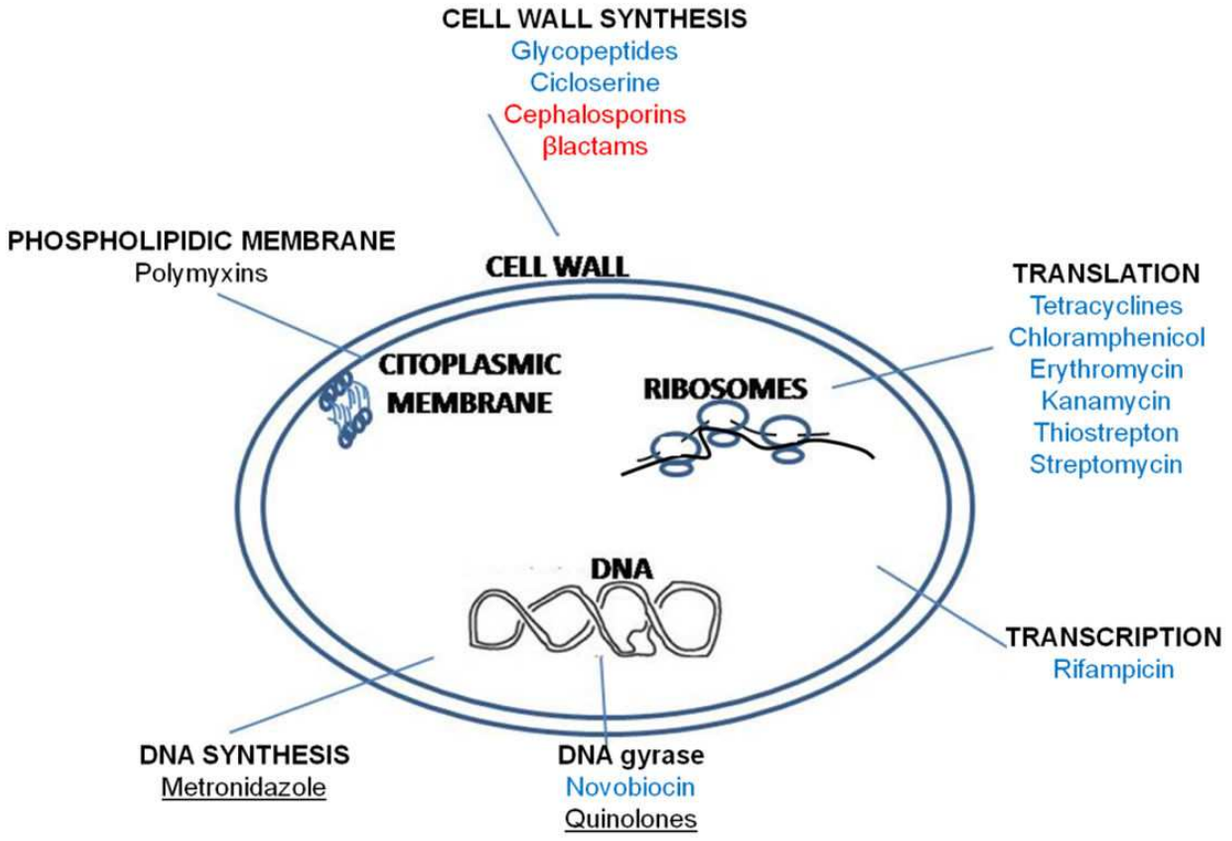


**Figure 1.** Principal targets of antibiotics. The antibiotics produced by Actinomycetes and by fungi are indicated in blue and red, respectively. The synthetic antibiotics are underlined.

Cycloserin, produced by *Streptomyces orchidaceus*, is a cyclic analogue of D-alanine that acts against two crucial enzymes important in the cytosolic stages of peptidoglycan synthesis: alanine racemase and D-Ala-D-Ala ligase. When both of these enzymes are inhibited, D-alanine residues cannot form and previously formed D-alanine molecules cannot be joined together.

Penicillins and cephalosporins are the most important antibiotics obtained from fungi P*enicillium notatum* and *P. chrysogenum*, respectively. Penicillins and cephalosporins mimic the D-alanyl-D-alanine groups found at the terminus of the pentapeptide in most newly synthesized peptidoglycan monomers. Binding of the drug to the transpeptidase inhibits the formation of cross-links between the rows and layers of peptidoglycan in the cell wall. Collectively, this results in degradation of the peptidoglycan and osmotic lysis of the bacterium [2].

Many different classes of antibiotics block protein synthesis. Tetracycline (produced by *Streptomyces aureofaciens*), for example, inhibits aminoacyl-tRNA binding, while chloramphenicol (*Streptomyces venezuelae*) and erythromycin (produced by *Saccaropolyspora erythraea*) bind to 50S subunit, blocking the peptidyl transferase activity; kanamycin (produced by *Streptomyces kanamyceticus*) binds to 30S subunit; thiostrepton (produced by *Streptomyces laurentii*) inhibits ribosome-dependent EF-Tu and EF-G GTPase, while streptomycin (produced by

*Streptomyces griseus*) prevents formation of initiation complex by insertion of improper amino acids.

Rifampicin is a semisynthetic antibiotic produced by the fermentation of a strain of *Amycolatopsis mediterranei* and it is a key component of anti-tuberculosis therapy. It inhibits the bacterial RNA polymerase by binding a pocket of the RNAP β subunit within the DNA/RNA channel and destabilizing the DNA-RNA polymerase-oligonucleotide-complexes [3].

Novobiocin, also known as albamycin or cathomycin, is an aminocoumarin antibiotic that is produced by the actinomycete *Streptomyces niveus*. Novobiocin is a very potent inhibitor of bacterial DNA gyrase and functions by targeting the GyrB subunit of the enzyme involved in energy transduction [4–5].

Polymyxins are antibiotics produced by nonribosomal peptide synthetase systems in Gram-positive bacteria, such as *Paenibacillus polymyxa*. Their structure consists of a cyclic peptide with a long hydrophobic tail. They act by disrupting the structure of the bacterial cell membrane and interacting with its phospholipids [6].

A few antibiotics are produced by chemical synthesis (e.g., quinolone and metronidazole). Quinolones are synthetic, bactericidal agents that inhibit the enzyme topoisomerase II, a DNA gyrase necessary for the replication of the microorganism. Topoisomerase II enzyme produces a negative supercoil on DNA, permitting transcription or replication; thus, by inhibiting this enzyme, DNA replication and transcription are blocked.

Metronidazole is a synthetic compound used in the treatment of infections caused by Gram-negative anaerobic bacteria and protozoa. It was shown to induce base-pair substitutions [7] and to be a potent mutagen in bacteria and low eukaryotic systems [8].

## 3. Genetic organization of antibiotic biosynthesis

Genes involved in the biosynthesis of antibiotics and other secondary metabolites are typically clustered within the respective genome or, rarely, in circular plasmid. A biosynthetic gene cluster contains many genes, often located within a few thousand base pairs of each other that encode for proteins participating in a metabolic pathway that leads to the production of a particular secondary metabolite (Figure 2).


**Figure 2.** Schematic example of a gene cluster for antibiotic production. Different colors indicate different functions.

The size of gene clusters can vary significantly, from a few to several hundred genes. Commonly, 10–50 genes are required for the synthesis of an antibiotic. A gene cluster usually contains genes involved in the precursor biosynthesis, tailoring steps, export, resistance, and regulation.

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Some peptide antibiotics are formed by amino acidic precursors that are then assembled by non-ribosomal machinery. In the case of non-ribosomal peptide synthesis, non-proteinogenic amino acids, such as 3,5-dihydroxyphenylglycine (DPG) and 4-hydroxyphenylglycine (HPG), can be found. In many cases, the backbone of the antibiotic is modified by the so-called tailoring steps, i.e., chlorination, methylation, glycosylation, N-acylation, and so on.

The polyketides are another class of natural antibiotics synthesized through the decarboxylative condensation of malonyl-CoA-derived extender units in a process similar to the fatty acid synthesis. The polyketide chains produced by a minimal polyketide synthase are often further modified (e.g., glycosylated) into bioactive natural products.

Actinorhodin and undecylprodiginines are two of the antibiotics produced by *S. coelicolor*. Actinorhodin is a red/blue pH-indicating benzoisochromanequinone made by a type II polyketide synthase-based pathway, while undecylprodiginines are red hydrophobic tripyrroles made by a fatty acid synthase-like.

Usually, a gene cluster for antibiotic production encodes for regulatory genes, named pathway specific, with positive or negative control on the cluster. Moreover, there could be some pleiotropic regulators that affect antibiotic production, morphological development, and primary metabolism of the bacteria. As examples, actinorhodin biosynthesis is regulated by the transcriptional activator ActII-ORF4 [9–10], while the undecylprodigiosin pathway is regulated via a minicascade of two cluster specific regulators, with RedZ activating the expression of *redD*, an aberrant orphan response regulator, direct activator gene for the biosynthetic genes [11].

In the bacteria producers of antibiotics, resistance genes are necessary to avoid the suicide, while transport genes are used to export the antibiotic outside the cell. Resistance to antibiotics can be caused by several general mechanisms (Figure 3): increased efflux or decreased influx of the antibiotic, target site alteration, target amplification, or antibiotic inactivation/modification [12].

The production of β-lactamase is a common mechanism found in many pathogens. This enzyme is capable of hydrolyzing and destroying the β-lactam ring of the antibiotic avoiding its antibacterial activity.

As example of alteration of the target site, the methylation of an adenine of the ribosomal RNA prevents the interaction between macrolides and ribosome.

Resistance to glycopeptides is frequently due to the presence of genes encoding for enzymes involved in the synthesis of alternative forms of peptidoglycan, with low affinity for glycopeptides. For example, the C terminal D-Ala-D-Ala is replaced by D-Ala-D-Lac or D-Ala-D-Ser [13]. Glycopeptide resistance has been explored in three different actinomycetes: *Streptomyces coelicolor*, which does not produce glycopeptide, *Streptomyces toyocaensis* and *Actinoplanes teichomyceticus*, which produce the glycopeptides A47934 and teicoplanin, respectively. For the producers of glycopeptides, activation of the resistance genes by the endogenously produced antibiotic prevents suicide, while in non-producing bacteria resistance may be due to genetic changes, such as mutation or acquisition of resistance genes through

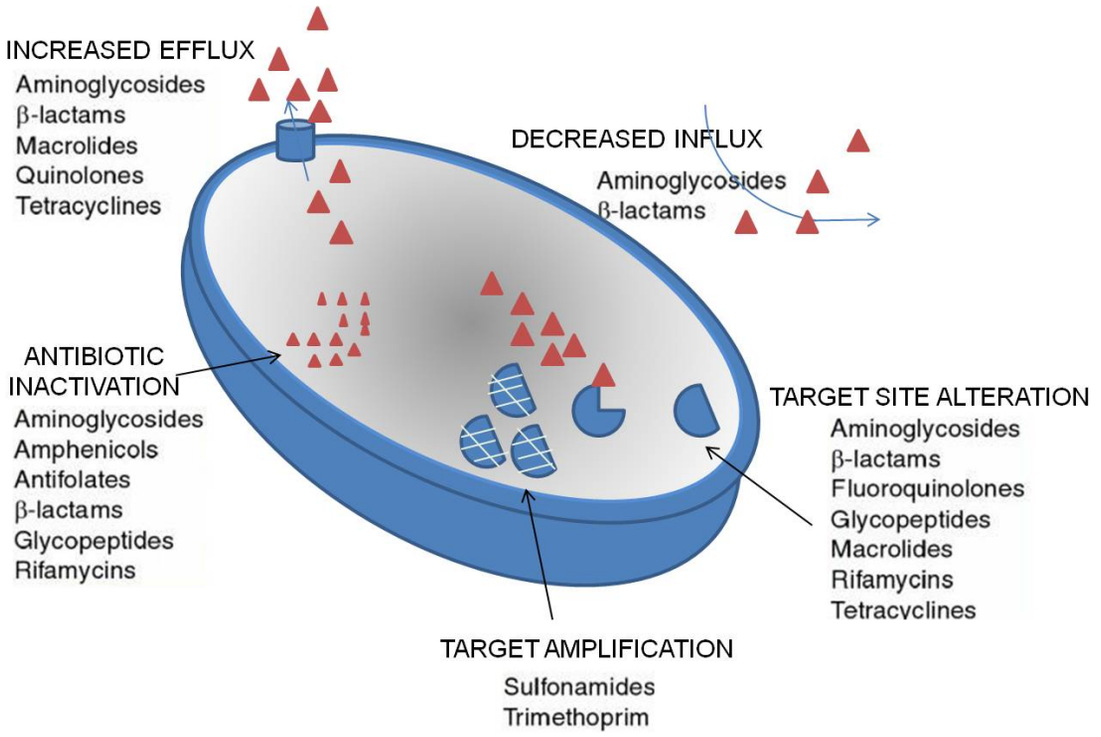


**Figure 3.** Mechanisms of antibiotic resistance.

horizontal transfer. In other systems, the antibiotic is acetylated by specific acetyl transferase inactivating its antibacterial property [14–15].

The best known efflux system regards the tetracycline, the gene *tetL,* carried by a transposon, codifies for a protein that transports the antibiotic outside the cell.

## 4. Morphological and physiological differentiation

Actinomycetes represent an important model of bacterial development; they display an unusual complex life cycle with different cell types (spores, vegetative and reproductive mycelium) and with the morphological changes strictly connected to the physiological differentiation. The understanding of Actinomycetes biology has been based on extensive studies on the model organism *Streptomyces coelicolor* over many years and on the availability of its complete genome sequence [16]. *S. coelicolor* is considered a ''multicellular'' prokaryotic model that includes programmed cell death and sporulation. After spore germination, vegetative growth leads to formation of a mycelium consisting of a ramifying network of syncytial hyphae that penetrate a moist substrate by extension of hyphal tips and subapical branching (vegetative mycelium). Subsequent reproductive growth often proceeds with the formation of filamentous aerial hyphae that eventually undergo differentiation into chains of unigenomic spores (Figure 4). This complex developmental cycle includes programmed cell death phenomena that make this bacterium a multicellular prokaryotic model [17–19].

Genetic studies of *Streptomyces* development regulation focused largely on analyses of *S. coelicolor* mutant strains defective in different stages of development. The main studied strains are the so-called "*bald*" mutants that are defective in aerial growth and the "*white*" mutants that are defective in the formation of mature grey spores on the tips of the white aerial mycelium. The *bald* mutants are affected in genes that regulate the ''sky-pathway'' that activates the expression of genes encoding proteins forming hydrophobic cover, such as eight chaplin proteins, RdlA, and Rdl rodlin proteins; the *white* genes are responsible of the formation of grey spores [20–21].

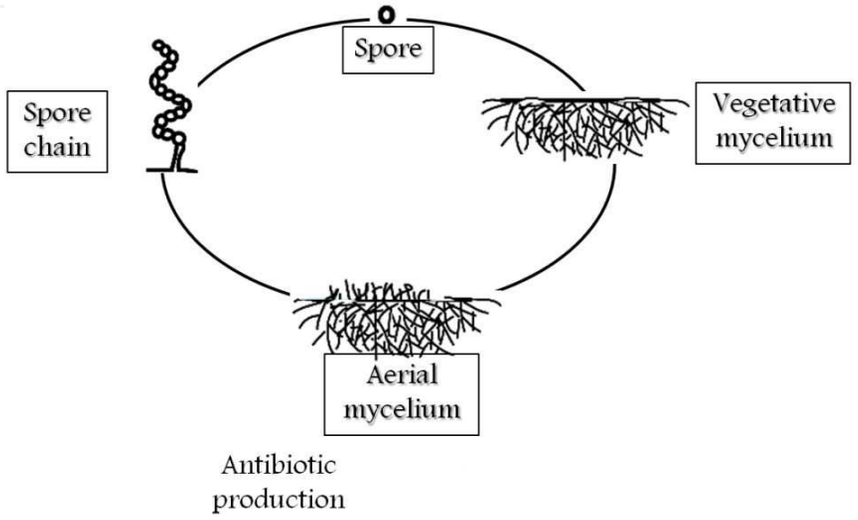


**Figure 4.** The *Streptomyces coelicolor* life cycle.

Morphological differentiation in Actinomycetes is strictly related to physiological differentiation: indeed the onset of morphological differentiation generally coincides with the production of secondary metabolites.

If on one hand, many factors with pleiotropic activity were identified as key players to control both morphological and physiological differentiation in *S. coelicolor*, on the other hand, for most actinomycetes, mechanisms and factors governing morphological and physiological processes were not deeply investigated yet.

## 5. General approaches to overproduce natural antibiotic

Natural bacterial strains often produce only small amounts of antibiotic (μg/l), while production rates in the range of g/l are needed to set up a cost-effective production process. In order to increase the industrial yield of products, different strategies can be adopted.

Random mutagenesis for the selection of overproducing mutants remains the preferred method when molecular genetic tools have not been developed for the producer microorganism. Although random mutagenesis and screening procedures have been widely used for

genetic improvement of antibiotic production, there are certain disadvantages, such as the time necessary to obtain a favorable mutation. The knowledge-driven genetic manipulation can make the optimization of strains and conditions more efficient.

In general, many approaches have been used to improve antibiotic production as schematically represented in Figure 5.

The tuning of media composition and fermentation conditions (carbon source, phosphate and nitrogen concentrations, pH, temperature) and the supply of specific precursors are the first approaches used in order to increase the yield in fermentation. Moreover, genetic manipulation of primary or secondary metabolism can be applied. Regarding primary metabolism, mutations in pathways for amino acids or other molecules that are used as precursors in antibiotic biosynthesis or mutations in the ribosome can improve indirectly the yield of secondary metabolites.

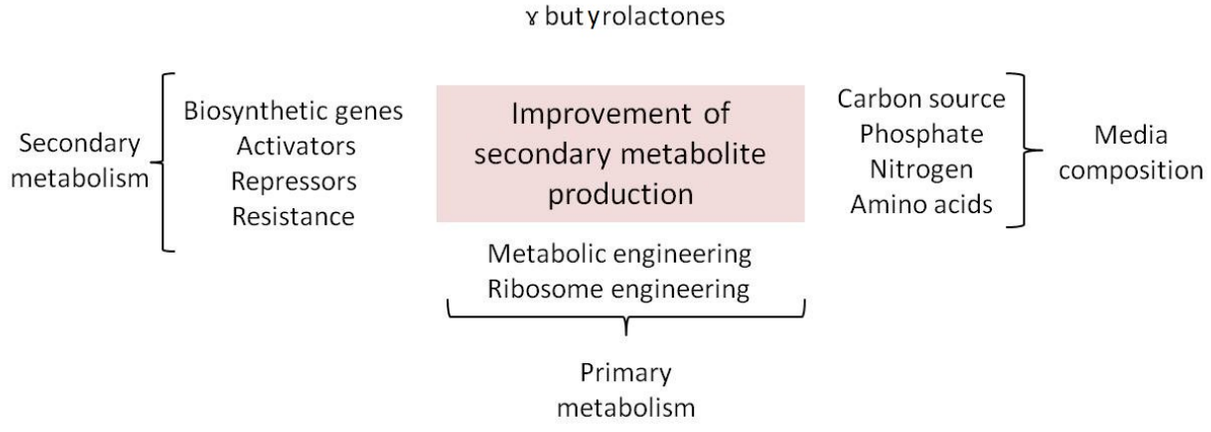


**Figure 5.** Approaches used to improve the antibiotic production.

Regarding secondary metabolism, the over-expression of biosynthetic genes, such as the genes that codify for antibiotic specific precursors, the over-expression of pathway-specific positive regulators or the inactivation of pathway-specific negative regulators can result in an increase of antibiotic yield. Increasing self-resistance levels in producing organisms has been also used for improving production yields. Manipulation of pleiotropic regulators, involved in both primary and secondary metabolisms, was also successfully used to improve antibiotic yields.

The production of antibiotics in some *Streptomyces spp*. depends upon diffusible butyrolactones structurally similar to homoserine lactones. γ butyrolactones have been applied for improvement of secondary metabolite production.

## 6. Media composition effect on antibiotic production

In bacteria, several sugars can be used as carbon sources. Although glucose is often an excellent carbon and energy source for microbial growth, it is infrequently utilized as the major carbon

and energy source in secondary metabolite fermentation. When incubated in media containing glucose and another carbon source, bacteria metabolize first glucose that represses the transcription of genes required for the utilization of the secondary carbon sources. When glucose is exhausted, the metabolism of the second carbon source is activated, and generally this correlates with the onset of antibiotic production. This phenomenon is referred to as carbon catabolite repression and is mediated via components of the phosphoenolpyruvate:carbohydrate phosphotransferase system, which transports and phosphorylates carbohydrates.

Glucose repression in *S. coelicolor* was demonstrated to be dependent upon the formation of intermediates of carbohydrate catabolism, for example, fructose 1,6-diphosphate and glucose 6-phosphate [22–23] or enzymes of the glucose catabolic pathway, such as glucose kinase, by exerting transcriptional repression of enzymes involved in the use of glycerol, arabinose, fructose, and galactose [24].

Glucose and other carbon sources have been found to suppress production of many secondary metabolites, e.g., actinorhodin in *S. lividans* [25]. In fact, it was reported that glucose inhibits actinorhodin production by repressing the transcription of *afsR2* that encodes a global regulatory protein involved in the stimulation of secondary metabolite biosynthesis [25] (Figure 6).

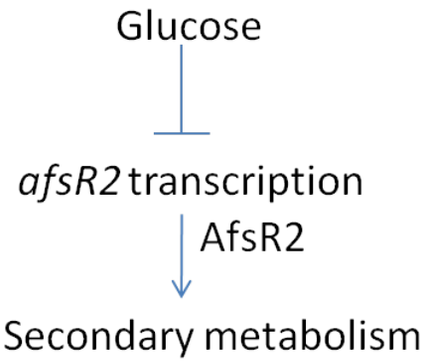


**Figure 6.** Effect of glucose on the secondary metabolism in *S. lividans*.

cAMP, ATP, and adenosine were reported to regulate antibiotic production [26]. When glucose is the carbon source, inhibition of the cAMP-producing enzyme, adenylate cyclase, occurs and cAMP levels are low (Figure 7). cAMP is important to activate the transcription factor cAMP receptor protein (CRP). In the absence of cAMP, CRP does not activate the transcription of target genes. When glucose is absent, cAMP is accumulated and it forms a complex with CRP, thereby activating the expression of a large number of genes, including some encoding enzymes that can supply energy independently from glucose and trigger spore germination, aerial mycelium formation, and actinorhodin production [27–30]. Extracellular ATP (exATP) was reported to massively increase actinorhodin and lightly increase undecylprodigiosin yields in *S. coelicolor* [31]. The nucleoside adenosine was reported to enhance production of undecylprodigiosin and conversely to suppress the actinorhodin production [32].

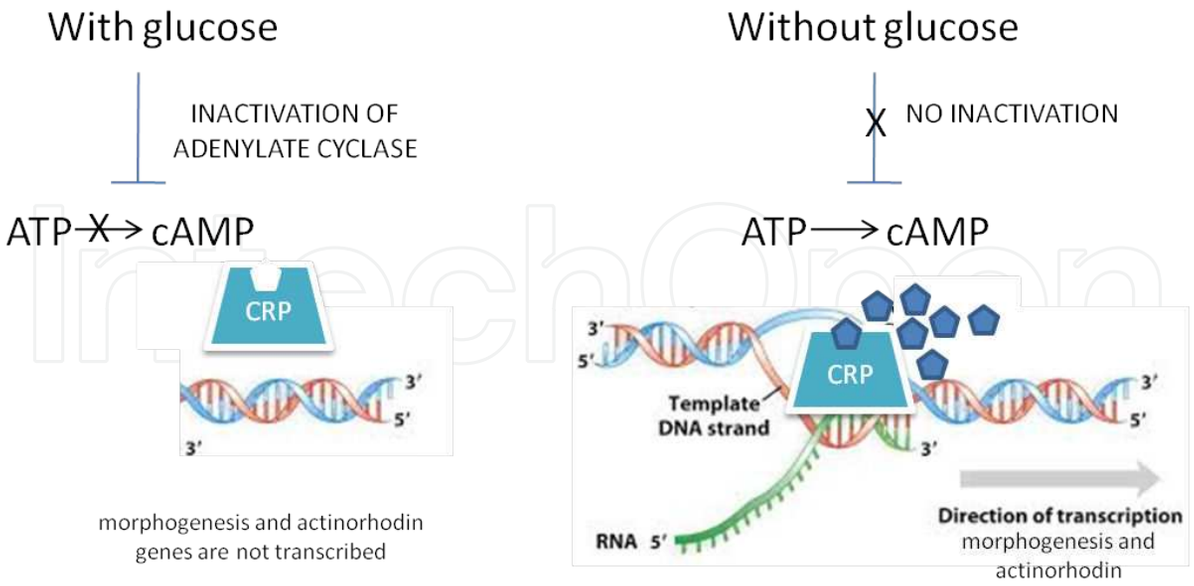


**Figure 7.** Effect of glucose on cAMP (blue pentagon) accumulation and antibiotic production in *S. coelicolor*.

Several microorganisms' nutrients, such as phosphate and nitrogen compounds, affect the production of antibiotics and other secondary metabolites. The lack of specific nutrients is perceived by microorganisms through complex signaling mechanisms. The study of these pathways is often the key in the understanding of regulatory processes underlying the synthesis of secondary metabolites.

Streptomycetes sense and respond to the stress of phosphate starvation via the two-component PhoR-PhoP signal transduction system (Figure 8). In *Streptomyces coelicolor*, phosphate negatively controls antibiotic biosynthesis by the two-component PhoR-PhoP system. The PhoR protein is a membrane sensor kinase, whereas PhoP is a DNA-binding response regulator (OmpR family). Primary and secondary metabolisms are interconnected. In fact, when *S. coelicolor* encounters phosphate limitation in its environment, PhoP activates the transcription of *afsS* that positively regulates secondary metabolism biosynthesis through the transcription of pathway-specific activators, such as *actII-ORF4* and *redD* for actinorhodin and undecylprodigiosin biosynthesis, respectively [33].

The biosynthesis of many antibiotics is very sensitive to phosphate. In *Nonomuraea*, A40926 production is negatively influenced by phosphate. In particular, phosphate depletion induced *dbv4* transcription that encodes an StrR-like protein, positive regulator of A40926 cluster genes [34]. In a few cases, phosphate has been reported to have a positive control, i.e., on lantibiotic production in Firmicutes strains and in *Microbispora sp*. ATCC-PTA-5024. It was surmised that phosphate has a different effect on ribosomal and non-ribosomal peptide biosynthesis [35].

A simple strategy to improve antibiotic production is to alter the PhoP concentration, by disrupting the *phoP* gene or the *phoP-R* cluster. In *S nataliensis*, the mutants obtained showed an increase of 80% in antibiotic production [36]. However, for some *Streptomyces* strains, the

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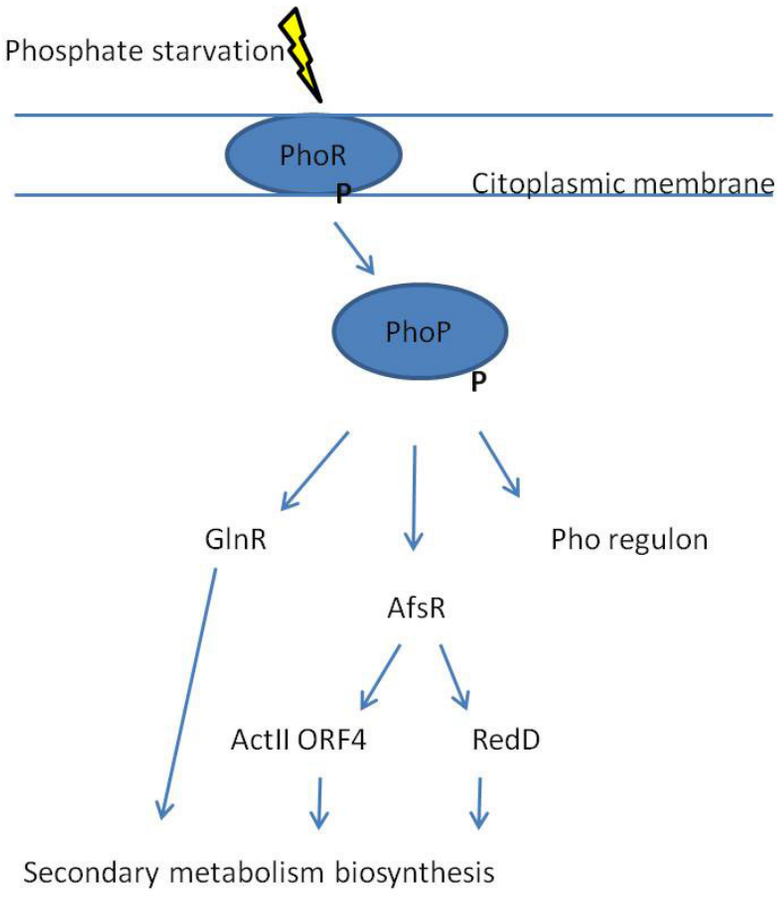


**Figure 8.** Cascade mechanism involved in phosphate control of actinorhodin and undecylprodigiosin biosynthesis in *S. coelicolor*.

*phoP* null mutant did not show an antibiotic overproduction, probably because of the complexity of the network [37].

High concentration of nitrogen sources (such as ammonium or amino acids) also suppresses the secondary metabolism. Complex fermentation media include proteins as nitrogen sources. For example, production of streptomycin antibiotic in *S. griseus* occurs in soybean meal with L-proline and low concentration of ammonium salt. Aminoglycoside antibiotic production is repressed by ammonium salt, while nitrate and certain amino acids stimulate their production [38].

In *S. coelicolor*, it was demonstrated that nitrogen assimilation is transcriptionally regulated by GlnR (Figure 8), which is an orphan response regulator with no coupled sensor kinase [39]. The Δ*glnR* mutant strain did not grow on nitrate as the sole nitrogen source and showed reduced growth on ammonium. Furthermore, no production of the pigmented antibiotics actinorhodin and undecylprodigiosin was observed [40].

Nitrogen metabolism under phosphate control exerted by the binding of PhoP to the promoter region of *glnR* reveals that crosstalk between global regulators, such as PhoP and GlnR, controls the expression of secondary metabolites [41].

In some bacteria of the phylum Actinobacteria, such as *S. lividans* and *S. coelicolor*, a shortage of nitrogen compounds is reflected in the increased concentration of tRNA discharges, a consequence of the limited availability of amino acids; this phenomenon leads to the activation of the RelA protein that binds ribosome stalling and allows the synthesis of the nucleotide pppGpp (guanine 5'- triphosphate 3'-diphosphate). In conditions of nutrient deficiency, there is a decrease of the GTP pool and the accumulation of pppGpp. pppGpp binds to the RNA polymerase subunit encoded by the *rpoB* (RNA polymerase subunit) gene, directing the transcription of genes important for the production of secondary metabolites. This mechanism of adaptation to changes in environmental conditions is called "stringent response" [42–43].

## 7. Genetic engineering

Antibiotic production can be improved by metabolic engineering in several ways. A flux increase in the biosynthetic pathway can be improved by directed mutagenesis or by elevated precursor availability. As an example, acetyl-CoA carboxylase was cloned into an expression vector and introduced into *S. coelicolor*; the conversion of acetyl-CoA into malonyl-CoA was enhanced and channeled to actinorhodin production [44]. Similarly, deletion of the *pfkA2* gene that encodes one of the three reported homologs of phosphofructokinase in *S. coelicolor* and led to an increased carbon flux through the pentose phosphate pathway increased the actinorhodin and undecylprodigiosin production four times [24].

In *Streptomyces clavuligerus*, deletion of the glyceraldehyde 3-phosphate dehydrogenase encoding gene *gap1* improved clavulanic acid production because of increased supply of precursor glyceraldehyde 3-phosphate [45]. Furthermore, *S. coelicolor* over-expression of acetyl-CoA carboxylase improved the yield of actinorhodin, which uses malonyl-CoA as a precursor for actinorhodin biosynthesis [44]. In *S. coelicolor*, deletion of citrate synthase or aconitase led to overproduction of organic acids, as well as changes in secondary metabolite production and morphological differentiation [46–47]. In *Streptomyces lividans*, polyphosphate kinase gene inactivation resulted in the accumulation of polyphosphates and activation of actinorhodin production, which is normally silenced in this species [48–49].

A promising method to increase antibiotic production is the ribosome engineering developed by Ochi and colleagues [50]. This method consists of the isolation of spontaneous mutants that are resistant to sub-lethal levels of antibiotic that targets the ribosomal proteins (such as streptomycin, gentamicin, kanamicin, cloramphenicol) or the RNA polymerase. Rifampicin resistance mutation in *rpoB* and streptomycin resistance mutation in *rpsL* for the ribosomal protein S12 led to an increased antibiotic production in *S. coelicolor* and *S. lividans* [51].

Some actinomycetes possess two *rpoB* genes (e.g., *Nocardia species*), in contrast to the widely accepted consensus of the existence of a single RNA polymerase in bacteria. In the *Nonomuraea sp.* strain ATCC 39727, two alleles of RNA polymerase B subunit gene *rpoB* ($rpoB^{(S)}$ and $rpoB^{(R)}$) provide the microorganism with two functionally distinct and developmentally regulated RNA polymerases [52]. $rpoB^{(R)}$ is characterized by an 18-bp in-frame deletion and mutations causing five amino acid substitutions located within or close to the so-called *rif*

cluster that play a key role in fundamental activities of RNA polymerase. *rpoB*$^{(R)}$ transcription is tightly regulated during *Nonomuraea* growth. Indeed, the expression of the *rpoB*$^{(R)}$ allele is growth phase-dependent in the wild type strain: the allele is silent during the pseudo-exponential phase of growth and begins to be expressed during the transition to the stationary phase. The merodiploidy might contribute to the developmental strategy of this actinomycete and the two isoforms may contribute to assemble growth phase-specific variants of RNA polymerase with different functional properties.

The constitutive expression of *rpoB*$^{(R)}$ gene in *Nonomuraea* increased the production of the glycopeptide antibiotic A40926 in this organism, while the heterologous expression in both the wild-type *Streptomyces lividans* strain 1326 and in strain $K_O$-421 (a relaxed mutant unable to produce ppGpp) markedly activated antibiotic biosynthesis of actinorhodin and undecylprodigiosin [43].

The basic knowledge of phosphate and nitrogen metabolic pathway can be used for rational manipulation. For example, amplification of *asfR*/*asfS* on a high copy number plasmid led to overproduction of actinorhodin and undecylprodigiosin in both *S. coelicolor* and *S lividans* [53–54]. In the same way, RelA overexpression leads to an enhanced antibiotic production in *S. coelicolor* [55].

## 8. Secondary metabolism to control antibiotic production

Antibiotic production is controlled at two main levels: pleiotropic regulators controlling the production of more than one antibiotic and cluster-situated regulator modulating the antibiotic biosynthetic genes of the cluster in which they are included.

The complex gene cluster for the biosynthesis of each antibiotic usually contains regulatory and resistance genes. Typically, there may be more than one such pathway-specific regulatory gene per cluster. Overexpression of positive regulators or deletion of genes that codify for repressors can be a strategy to improve antibiotic production.

Among the regulatory genes, two-component systems are the most important transduction signal mechanism in bacteria. Typically, the two-component system comprises a membrane-bound histidine kinase and a cognate response regulator. The receptor senses specific environmental stimuli, it auto-phosphorylates and activates by phosphorylation the response regulator that mediates the cellular response, mainly through the transcriptional regulation of target genes in the cluster for antibiotic [56].

The AbrC1 protein is a histidine kinase part of a two-component system in *Streptomyces coelicolor* M145. It is a negative regulator of antibiotic production and morphological differentiation. Indeed, the deletion of this repressor led to a clear increase in actinorhodin, undecylprodigiosin, and calcium-dependent antibiotic yields [57].

Other examples of regulators are those of the StrR and LuxR families. StrR was initially identified in *Streptomyces griseus* as a pathway specific positive regulator of the expression

streptomycin biosynthetic gene [58]. LuxR was initially identified in the marine Gram-negative bacteria *Vibrio fisheri,* where it regulates the quorum sensing phenomena of bioluminescence in a population density dependent manner [59]. Proteins of the LuxR family have been identified among Actinomycetes and two LAL proteins were identified as pleiotropic regulators affecting various cellular processes in S*treptomyces coelicolor* [60].

The filamentous actinomycete *Nonomuraea sp.* strain ATCC39727 codifies for *dbv4* and *dbv3*, two pathway-specific regulatory genes that code for StrR- and LuxR-type transcriptional positive regulators, respectively [61]. The over-expression of *dbv*3 led to a two-fold increase in the glycopeptide antibiotic A40926 productivity.

The increase of self-resistance levels in producing organisms was used for improving production yields. This strategy was used for *S. kanamyceticus* and *S. fradiae*, kanamycin and neomycin producers, respectively. The 6'-N-acetyltransferase derived from *Streptomyces kanamyceticus* strain M1164 was cloned into the high copy plasmid vector pIJ702 and transferred in both strains. In both cases, transformants containing the recombinant plasmid showed increased resistance to a number of aminoglycoside antibiotics and substantially increased production of kanamycin and neomycin [62]. Similar results were described in several antibiotic overproducing organisms, such as *S. aureofaciens*, producer of the chlortetracycline 6-demethylchlortetracycline, through the overexpression of a self-defense gene involved in drug efflux [63].

To increase the balhimycin production by *Amycolatopsis balhimycina*, the *dahp* and *pdh* genes, from the biosynthetic cluster that codify for key steps of the shikimate pathway, were overexpressed both individually and together. The constructed strains expressing an additional copy of the *dahp* gene and the strain carrying an extra copy of both *dahp* and *pdh* showed improved specific glycopeptide productivities by approximately a factor of three [64].

## 9. γ butyrolactones to control the onset of antibiotic biosynthesis

The production of antibiotics in some *Streptomyces spp*. depends upon diffusible butyrolactones structurally similar to homoserine lactones. The most intensively studied is the A factor that positively controls the pleiotropic regulator AdpA (Figure 9). A factor is gradually produced by AfsA and it is accumulated in a growth dependent manner [65]. When the concentration of A-factor reaches a critical level, it binds ArpA, which is released from the promoter of *adpA*, thus leading to *adpA* transcription. The transcriptional activator AdpA then activates a variety of genes that are required for the biosynthesis of secondary metabolites, including streptomycin, and morphological differentiation in *Streptomyces griseus*. Overexpression of *adpA* caused *S. griseus* to produce streptomycin earlier and with a ten-fold higher yield than the wild-type strain [66].

So far, various γ-butyrolactone molecules, synthases, and receptors have been identified [67–68].

γ butyrolactones have been applied for improvement of secondary metabolite production. A factor from *S. griseus* stimulated antibiotic production in *S. natalensis* showing an efficient cross-

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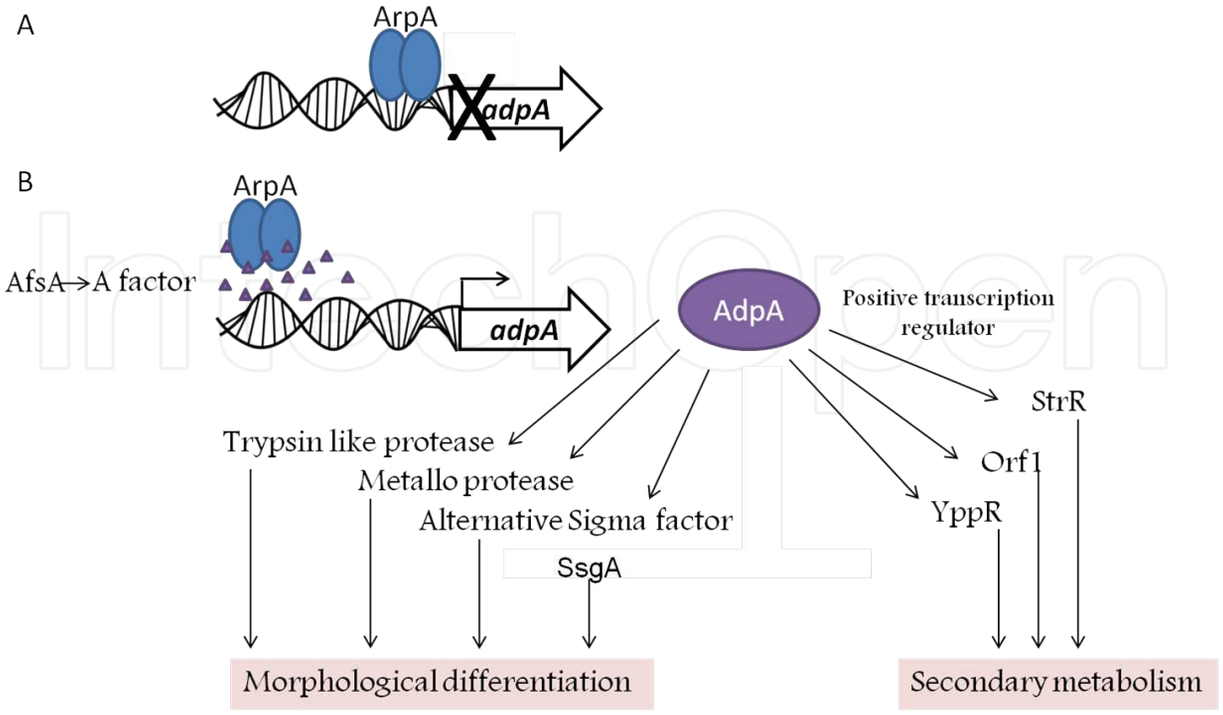


**Figure 9.** The A-factor regulatory cascade in *S. griseus*. (A) In the absence of A factor, ArpA binds to the *adpA* promoter and represses its transcription. (B) When A factor accumulates and reaches a threshold level, it binds to ArpA that is detached from the *adpA* promoter, leading to its expression.

talk among different species [69]. Little is known about the use of these small molecules as global elicitors of antibiotic production, but considering the effect on antibiotic production even at low concentration, this could be an interesting approach to follow.

## 10. Heterologous expression of actinomycetes biosynthetic gene clusters

Many antibiotic producing actinomycetes are recalcitrant to manipulation and suitable protocols for their genetic manipulation are not always available. The transfer of the genetic information for secondary metabolite production from the original producer to a model host represents a successful strategy to manipulate biosynthetic gene clusters. Heterologous expression of large biosynthetic pathways could be also useful in all those cases in which bacteria are not cultivable or to produce cryptic metabolites, revealed by genome sequencing and mining. Actinomycetes are characterized by large genomes that are GC-rich [70] and their genes are not easily expressed in *Escherichia coli*. To date, several shuttle vectors that can be maintained in model streptomycetes (*Streptomyces coelicolor*, *S. avermitilis*, *S. lividans*) have been described, and several libraries have been constructed in bacterial and P1-derived artificial chromosomes [71–74].

In some cases, a successful heterologous expression of actinomycetes biosynthetic gene clusters was obtained after changing fermentation conditions, that is, feeding with a biosynthetic

precursor, minimizing background endogenous activities, or after cloning strong promoters upstream of production genes weakly transcribed (for a review see [75]). Heterologous expression in amenable hosts can be useful to exploit and to explore the genetic potential of actinomycetes.

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## References

[1] Vitale M, Scatassa ML, Cardamone C, Oliveri G, Piraino C, Alduina R, Napoli C. (2015) Staphylococcal food poisoning case and molecular analysis of toxin genes in Staphylococcus aureus strains isolated from food in Sicily, Italy. Foodborne Pathog Dis. 12(1):21-3.

[2] Van Bambeke F. (2004) Glycopeptides in clinical development: pharmacological profile and clinical perspectives. Curr Opin Pharmacol. Oct;4(5):471-8.

[3] Schulz W, Zillig W. (1981) Rifampicin inhibition of RNA synthesis by destabilization of DNA-RNA polymerase-oligonucleotide complexes. Nucleic Acids Res. 9:6889-6906.

[4] Walsh TJ, Standiford HC, Reboli AC, John JF, Mulligan ME, Ribner BS, Montgomerie JZ, Goetz MB, Mayhall CG, Rimland D, et al. (1993) Randomized Double-Blinded Trial of Rifampin with Either Novobiocin or Trimethoprim-Sulfamethoxazole against Methicillin-Resistant *Staphylococcus aureus* Colonization: Prevention of Antimicrobial Resistance and Effect of Host Factors on Outcome. Antimicrobial agents and chemotherapy. 37(6):1334-1342.

[5] Maxwell A. (1999) DNA gyrase as a drug target. Biochem Soc Trans. 27(2):48-53.

[6] Dixon RA, Chopra I. (1986) Leakage of periplasmic proteins from *Escherichia coli* mediated by polymyxin B nonapeptide. Antimicrob Agents Chemother. 29(5): 781-788.

[7] Menéndez D, Rojas E, Herrera LA, López MC, Sordo M, Elizondo G, Ostrosky-Wegman P. (2001) DNA breakage due to metronidazole treatment. Mutat Res. 478(1-2): 153-8.

[8] Voogd CE. (1981) On the mutagenicity of nitroimidazoles. Mutat Res. 86(3):243-77.

[9] Iqbal M, Mast Y, Amin R, Hodgson DA, Wohlleben W, Burroughs NJ. (2012) Extracting regulator activity profiles by integration of de novo motifs and expression data: Characterizing key regulators of nutrient depletion responses in *Streptomyces coelicolor*. Nucleic Acids Res. 40:5227-5239. 29.

[10] 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. 25:1181-1184.

[11] White J, Bibb M. (1997) bldA dependence of undecylprodigiosin production in *Streptomyces coelicolor* A3(2) involves a pathway-specific regulatory cascade. J. Bacteriol. 179:627-633.

[12] Tenover FC. (2006) Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control. Jun;34(5 Suppl 1):S3-10;discussion S64-73.

[13] Arthur M, Reynolds PE, Depardieu F, Evers S, Dutka-Malen S, Quintiliani R Jr, Courvalin P. (1996) Mechanisms of glycopeptide resistance in *enterococci*. J Infect. 32(1): 11-6.

[14] Perry JA, Westman EL, Wright GD. (2014) The antibiotic resistome: What's new? Curr Opin Microbiol. 21:45-50.

[15] Ramirez MS, Nikolaidis N, Tolmasky ME. (2003) Rise and dissemination of aminoglycoside resistance: The aac(6')-Ib paradigm. Front Microbiol. 4:121.

[16] Bentley SD, Chater KF, Cerdeño-Tárraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA. (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature. 417(6885):141-7.

[17] Manteca A, Jung HR, Schwämmle V, Jensen ON, Sanchez J. (2010) Quantitative proteome analysis of *Streptomyces coelicolor* Non sporulating liquid cultures demonstrates a complex differentiation process comparable to that occurring in sporulating solid cultures. J Proteome Res. 9(9):4801-11.

[18] Yagüe P, Rodríguez-García A, López-García MT, Martín JF, Rioseras B, Sánchez J, Manteca A. (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.

[19] Rioseras B, López-García MT, Yagüe P, Sánchez J, Manteca A. (2014) Mycelium differentiation and development of *Streptomyces coelicolor* in lab-scale bioreactors: Pro-

grammed cell death, differentiation, and lysis are closely linked to undecylprodigiosin and actinorhodin production. Bioresour Technol. Jan;151:191-8.

[20] Flärdh K, Buttner MJ. (2009) *Streptomyces* morphogenetics: Dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol. 7(1):36-49.

[21] Claessen D, de Jong W, Dijkhuizen L, Wösten HA. (2006) Regulation of *Streptomyces* development, reach for the sky! Trends Microbiol. 14:313-319.

[22] Ramos I, Guzmán S, Escalante L, Imriskova I, Rodríguez-Sanoja R, Sanchez S, Langley E. (2004) Glucose kinase alone cannot be responsible for carbon source regulation in *Streptomyces peucetius* var. *caesius*. Res Microbiol. 155(4):267-74.

[23] Borodina I, Siebring J, Zhang J, Smith CP, van Keulen G, Dijkhuizen L, Nielsen J. (2008) Antibiotic overproduction in *Streptomyces coelicolor* A3 2 mediated by phosphofructokinase deletion. J Biol Chem. 283(37):25186-99. doi: 10.1074/jbc.M803105200.

[24] Hodgson DA. (1982) Glucose Repression of Carbon Source Uptake and Metabolism in *Streptomyces coelicolor* A3(2) and its Perturbation in Mutants Resistant to 2-Deoxyglucose. Microbiology. 2417-2430.

[25] Kim ES, Hong H, Choi CY, Cohen SN. (2001) Modulation of actinorodin biosynthesis in *Streptomyces lividans* by glucose repression of afsR2 gene transcription. J. Bacteriol. 183:2198-2203.

[26] Sanchez S, Chavez A, Forero A, Garcıa-Huante Y, Romero A, Sanchez M, Rocha D, Sanchez B, Avalos M, Guzman-Trampe S, Rodrıguez-Sanoja R, Langley E, Ruiz B. (2010) Carbon source regulation of antibiotic production. The Journal of Antibiotics. 63(8):442-59.

[27] Satoh H, Satoh Y, Notsu Y, Honda F. (1976) Adenosine 3',5'-cyclic monophosphate as a possible mediator of rotational behaviour induced by dopaminergic receptor stimulation in rats lesioned unilaterally in the substantia nigra. Eur J Pharmacol. 39(2): 365-77.

[28] Tata M, Menawat AS. (1994) Cyclic AMP regulation of tylosin biosynthesis and secondary metabolism in *Streptomyces fradiae*. Biotechnol Bioeng. 44(3):283-90.

[29] Süsstrunk U, Pidoux J, Taubert S, Ullmann A, Thompson CJ. (1998) Pleiotropic effects of cAMP on germination, antibiotic biosynthesis and morphological development in *Streptomyces coelicolor*. Mol Microbiol. 30(1):33-46.

[30] Derouaux A, Halici S, Nothaft H, Neutelings T, Moutzourelis G, Dusart J, Titgemeyer F, Rigali S. (2004) Deletion of a cyclic AMP receptor protein homologue diminishes germination and affects morphological development of *Streptomyces coelicolor.* J Bacteriol. 186(6):1893-7.

[31] Li M, Kim TJ, Kwon HJ, Suh JW. (2008) Effects of extracellular ATP on the physiology of *Streptomyces coelicolor* A3(2). FEMS Microbiol Lett. 286(1):24-31.

[32] Rajkarnikar A, Kwon HJ, Suh JW. (2007) Role of adenosine kinase in the control of *Streptomyces* differentiations: Loss of adenosine kinase suppresses sporulation and actinorhodin biosynthesis while inducing hyperproduction of undecylprodigiosin in *Streptomyces lividans*. Biochem Biophys Res Commun. 363(2):322-8.

[33] Martín 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.

[34] Alduina R, Lo Piccolo L, D'Alia D, Ferraro C, Gunnarsson N, Donadio S, Puglia AM. (2007) Phosphate-controlled regulator for the biosynthesis of the dalbavancin precursor A40926. J Bacteriol. 189(22):8120-9.

[35] Giardina A, Alduina R, Gallo G, Monciardini P, Sosio M, Puglia AM. (2014) Inorganic phosphate is a trigger factor for *Microbispora* sp. ATCC-PTA-5024 growth and NAI-107 production. Microb Cell Fact. 13:133.

[36] Mendes MV, Tunca S, Antón N, Recio E, Sola-Landa A, Aparicio JF, Martín JF. (2007) The two-component *phoR-phoP system* of *Streptomyces natalensis*: Inactivation or deletion of phoP reduces the negative phosphate regulation of pimaricin biosynthesis. Metab Eng. 9(2):217-27. Epub 2006 Oct 24.

[37] Santos-Beneit F, Rodríguez-García A, Apel AK, Martín JF. (2009) Phosphate and carbon source regulation of two PhoP-dependent glycerophosphodiester phosphodiesterase genes of *Streptomyces coelicolor.* Microbiology. 155(Pt 6):1800-11.

[38] Shapiro S. (1989) Nitrogen assimilation in actinomycetes and the influence of nitrogen nutrition on actinomycete secondary metabolism. In: Shapiro S (ed) Regulation of secondary metabolism in actinomycetes. CRC Press, Boca Raton, pp 135–211.

[39] Wray LV, Fisher SH. (1993) The *Streptomyces coelicolor* glnR gene encodes a protein similar to other bacterial response regulators. Gene. 130:145-150.

[40] Tiffert Y, Franz-Wachtel M, Fladerer C, Nordheim A, Reuther J, Wohlleben W, Mast Y (2011) Proteomic analysis of the GlnR-mediated response to nitrogen limitation in *Streptomyces coelicolor M145*. Appl Microbiol Biotechnol

[41] Martín JF, Sola-Landa A, Santos-Beneit F, Fernández-Martínez LT, Prieto C, Rodríguez-García A. (2011) Cross-talk of global nutritional regulators in the control of primary and secondary metabolism in *Streptomyces*. Microb Biotechnol. 4(2):165-74.

[42] Cassels R, Oliva B, Knowles D. (1995) Occurrence of the regulatory nucleotides ppGpp and pppGpp following induction of the stringent response in *staphylococci*. J Bacteriol. 177(17):5161-5.

[43] Talà A, Wang G, Zemanova M, Okamoto S, Ochi K, Alifano P. (2009) Activation of dormant bacterial genes by *Nonomuraea sp*. strain ATCC 39727 mutant-type RNA polymerase. J Bacteriol. 191(3):805-14.

[44] Ryu YG, Butler MJ, Chater KF, Lee KJ. (2006) Engineering of primary carbohydrate metabolism for increased production of actinorhodin in *Streptomyces coelicolor*. Appl Environ Microbiol. 72(11):7132-9.

[45] Li R, Townsend CA. (2006) Rational strain improvement for enhanced clavulanic acid production by genetic engineering of the glycolytic pathway in *Streptomyces clavuligerus*. Metab Eng. 8(3):240-52.

[46] Viollier PH, Nguyen KT, Minas W, Folcher M, Dale GE, Thompson CJ. (2001) Roles of aconitase in growth, metabolism, and morphological differentiation of *Streptomyces coelicolor*. J. Bacteriol. 183:3193-3203.

[47] Viollier PH, Minas W, Dale GE, Folcher M, Thompson CJ. (2001) Role of acid metabolism in *Streptomyces coelicolor* morphological differentiation and antibiotic biosynthesis J. Bacteriol. 183:3184-3192.

[48] Chouayekh H, Virolle MJ. (2002) The polyphosphate kinase plays a negative role in the control of antibiotic production in *Streptomyces lividans.* Mol. Microbiol. 43:919-930.

[49] Ghorbel S, Kormanec J, Artus A, Virolle MJ. (2006) Transcriptional studies and regulatory interactions between the *phoR-phoP* operon and the *phoU, mtpA,* and *ppk* genes of *Streptomyces lividans* TK24. J. Bacteriol. 188:677-686

[50] Ochi K, Tanaka Y, Tojo S. (2014) Activating the expression of bacterial cryptic genes by *rpoB* mutations in RNA polymerase or by rare earth elements. J Ind Microbiol Biotechnol. 41(2):403-14.

[51] Lai C, Xu J, Tozawa Y, Okamoto-Hosoya Y, Yao X, Ochi K. (2002) Genetic and physiological characterization of rpoB mutations that activate antibiotic production in *Streptomyces lividans.* Microbiology. 148(Pt 11):3365-73.

[52] Vigliotta G, Tredici SM, Damiano F, Montinaro MR, Pulimeno R, di Summa R, Massardo DR, Gnoni GV, Alifano P. (2005) Natural merodiploidy involving duplicated *rpoB* alleles affects secondary metabolism in a producer actinomycete. Mol Microbiol. 55(2):396-412.

[53] Floriano B, Bibb M. (1996) *afsR* is a pleiotropic but conditionally required regulatory gene for antibiotic production in *Streptomyces coelicolor* A3(2). Mol Microbiol. 21(2): 385-96.

[54] Vögtli M, Chang PC, Cohen SN. (1994) *afsR2*: A previously undetected gene encoding a 63-amino-acid protein that stimulates antibiotic production in *Streptomyces lividans.* Mol Microbiol. 14(4):643-53.

[55] 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):5854-61.

[56] Krell T, Lacal J, Busch A, Silva-Jimenez H, Guazzaroni ME, Ramos JL. (2010) Bacterial sensor kinases: diversity in the recognition of environmental signals. Annu Rev Microbiol. 12:539-559.

[57] Rodríguez H, Rico S, Yepes A, Franco-Echevarría E, Antoraz S, Santamaría RI, Díaz M. (2015) The two kinases, AbrC1 and AbrC2, of the atypical two-component system AbrC are needed to regulate antibiotic production and differentiation in *Streptomyces coelicolor*. Front Microbiol. 6:450.

[58] Retzlaff L, Distler J. (1995) The regulator of streptomycin gene expression, StrR, of *Streptomyces griseus* is a DNA binding activator protein with multiple recognition sites. Mol Microbiol. 18(1):151-62.

[59] Kaplan HB, Greenberg EP. (1987) Overproduction and purification of the luxR gene product: Transcriptional activator of the *Vibrio fischeri* luminescence system. Proc Natl Acad Sci USA. 84(19):6639-43.

[60] Guerra SM, Rodríguez-García A, Santos-Aberturas J, Vicente CM, Payero TD, Martín JF, Aparicio JF. (2012) LAL regulators SCO0877 and SCO7173 as pleiotropic modulators of phosphate starvation response and actinorhodin biosynthesis in *Streptomyces coelicolor*. PLoS One. 7(2):e31475.

[61] Lo Grasso L, Maffioli S, Sosio M, Bibb M, Puglia AM, Alduina R. (2015) Two master switch regulators trigger A40926 biosynthesis in Nonomuraea sp. ATCC 39727. J Bacteriol. pii: JB.00262-15.

[62] Crameri R, Davies JE. (1986) Increased production of aminoglycosides associated with amplified antibiotic resistance genes. J Antibiot (Tokyo). 39(1):128-35.

[63] Dairi T, Aisaka K, Katsumata R, Hasegawa M. (1995) A self-defense gene homologous to tetracycline effluxing gene essential for antibiotic production in *Streptomyces aureofaciens*. Biosci Biotechnol Biochem. 59(10):1835-41.

[64] Thykaer J, Nielsen J, Wohlleben W, Weber T, Gutknecht M, Lantz AE, Stegmann E. (2010) Increased glycopeptide production after overexpression of shikimate pathway genes being part of the balhimycin biosynthetic gene cluster. Metab Eng. Sep;12(5): 455-61.

[65] Horinouchi S, Beppu T. (1994) A-factor as a microbial hormone that controls cellular differentiation and secondary metabolism in *Streptomyces griseus*. Mol Microbiol. 12(6):859-64.

[66] 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. Mol Microbiol. 34(1):102-11.

[67] Mehra S, Charaniya S, Takano E, Hu WS. (2008) A bistable gene switch for antibiotic biosynthesis: the butyrolactone regulon in *Streptomyces coelicolor*. PLoS One. 3(7):e2724.

[68] Yang YH, Song E, Kim JN, Lee BR, Kim EJ, Park SH, Kim WS, Park HY, Jeon JM, Rajesh T, Kim YG, Kim BG. (2012) Characterization of a new ScbR-like γ-butyrolactone binding regulator (SlbR) in *Streptomyces coelicolor*. Appl Microbiol Biotechnol. Oct; 96(1):113-21.

[69] Recio E, Colinas A, Rumbero A, Aparicio JF, Martín JF. (2004) PI factor, a novel type quorum-sensing inducer elicits pimaricin production in *Streptomyces natalensis*. J Biol Chem. 279(40):41586-93.

[70] Alduina R, Pisciotta A. (2015) Pulsed field gel electrophoresis and genome size estimates. Methods Mol Biol. 1231:1-14.

[71] Donadio S, Monciardini P, Alduina R, Mazza P, Chiocchini C, Cavaletti L, Sosio M, Puglia AM. (2002) Microbial technologies for the discovery of novel bioactive metabolites. J Biotechnol. 99(3):187-98.

[72] Alduina R, De Grazia S, Dolce L, Salerno P, Sosio M, Donadio S, Puglia AM. (2003) Artificial chromosome libraries of Streptomyces coelicolor A3(2) and Planobispora rosea. FEMS Microbiol Lett. 2003 Jan 21;218(1):181-6.

[73] Alduina R, Giardina A, Gallo G, Renzone G, Ferraro C, Contino A, Scaloni A, Donadio S, Puglia AM. (2005) Expression in Streptomyces lividans of Nonomuraea genes cloned in an artificial chromosome. Appl Microbiol Biotechnol. 68(5):656-62.

[74] Giardina A, Alduina R, Gottardi E, Di Caro V, Süssmuth RD, Puglia AM. (2010) Two heterologously expressed Planobispora rosea proteins cooperatively induce Streptomyces lividans thiostrepton uptake and storage from the extracellular medium. Microb Cell Fact. 9:44.

[75] Alduina R, Gallo G. (2012) Artificial chromosomes to explore and to exploit biosynthetic capabilities of actinomycetes. J Biomed Biotechnol. 2012:462049.