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**Natural Products – Review**

# Small molecule inducers of actinobacteria natural product biosynthesis

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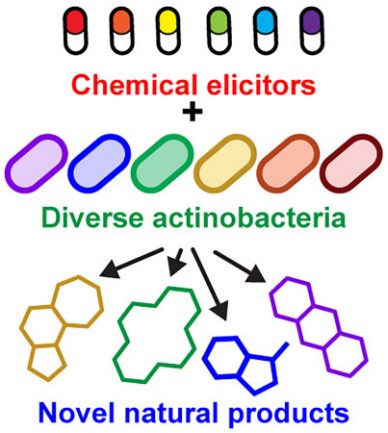
**Abstract:** Actinobacteria are a large and diverse group of bacteria that are known to produce a wide range of secondary metabolites, many of which have important biological activities, including antibiotics, anti-cancer agents, and immunosuppressants. The biosynthesis of these compounds is often highly regulated with many natural products (NPs) being produced at very low levels in laboratory settings. Environmental factors, such as small molecule elicitors, can induce the production of secondary metabolites. Specifically, they can increase titers of known NPs as well as enabling discovery of novel NPs typically produced at undetectable levels. These elicitors can be NPs, including antibiotics or hormones, or synthetic compounds. In recent years, there has been a growing interest in the use of small molecule elicitors to induce the production of secondary metabolites from actinobacteria, especially for the discovery of NPs from "silent" biosynthetic gene clusters. This review aims to highlight classes of molecules that induce secondary metabolite production in actinobacteria and to describe the potential mechanisms of induction.

**One-Sentence Summary:** This review describes chemical elicitors of actinobacteria natural products described to date and the proposed mechanisms of induction.

**Keywords:** Natural products, Natural product biosynthesis, Natural product elicitors, Hormones, Actinobacteria

### Graphical abstract




Chemicals, including natural product antibiotics and hormones, are capable of inducing production of natural products in actinobacteria.

## Introduction

Natural products (NPs) from the soil-dwelling bacteria *Streptomyces* are a bountiful source of medicines and agricultural products (Bérdy, 2012; Olanrewaju & Babalola, 2019; Newman & Cragg, 2020). Additionally, their unique mechanisms of action make them excellent chemical tools for manipulating and studying biological processes (Carlson, 2010). Genomics data suggest that hundreds of thousands of novel NPs likely to have interesting bioactivities remain to be discovered from *Streptomyces* (Doroghazi & Metcalf, 2013; Doroghazi et al., 2014; Palazzolo et al., 2017; Pye et al., 2017). Many of these NPs are not produced, or are produced at very low levels, when grown in the laboratory. This is likely, at least in part, because the bacteria are not exposed to the same chemical stimuli that they encounter in the soil (Romero et al., 2011). Novel strategies to access these low-abundance NPs are critical to maximizing the NP potential from *Streptomyces* and identifying novel biological probes and leads for drug discovery.

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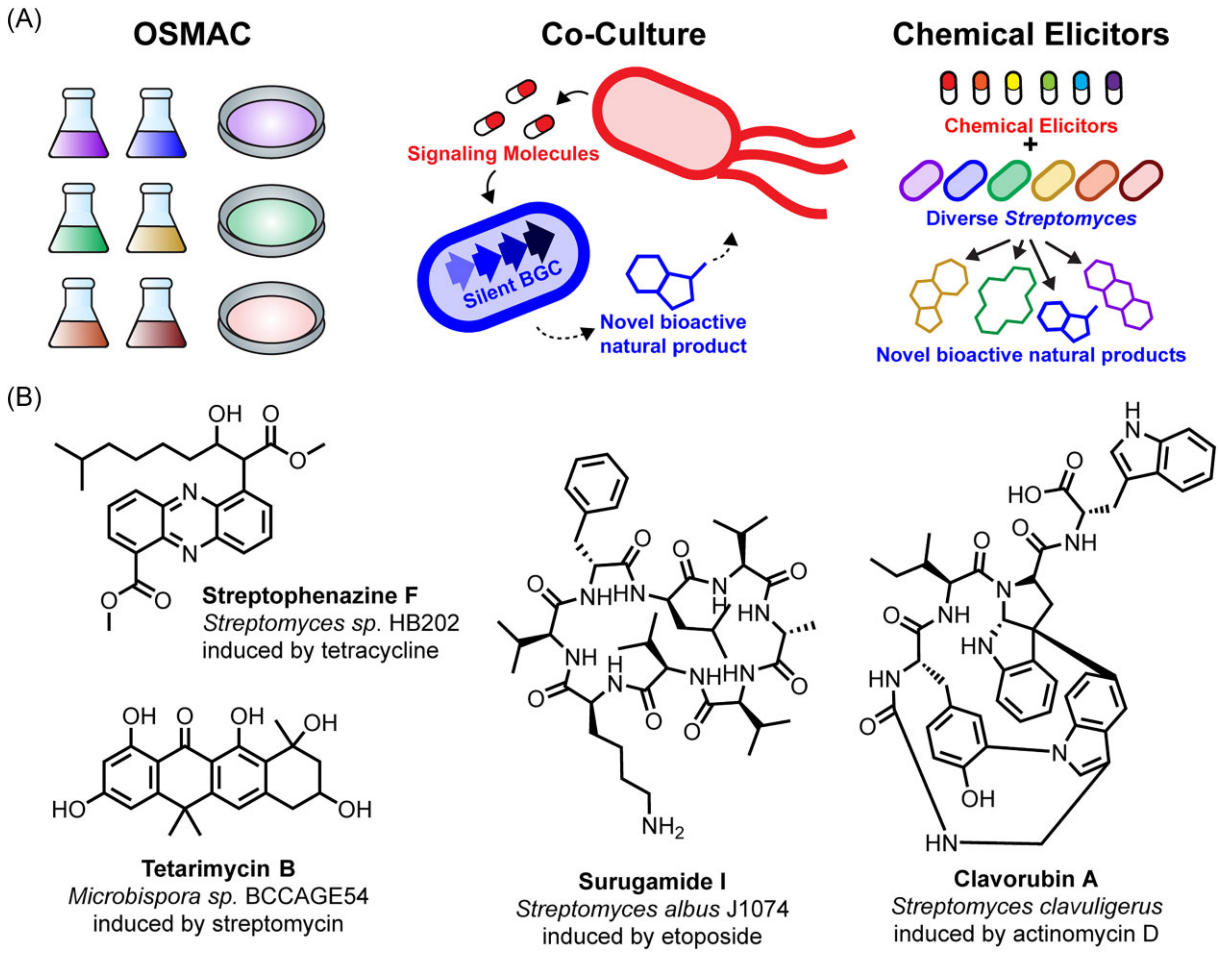


**Fig. 1.** (A) Cultivation methods utilized to induce production of NPs. OSMAC (one strain many compounds, left) focuses on changing media composition to alter NP production. This can include changes in carbon or nitrogen sources, media type (i.e., liquid vs. solid media), as well as changes to pH or temperature. Another method that has been employed is growing bacterial co-cultures (middle). Molecules produced by one of the bacteria can act as chemical signals to elicit the production of NPs. Finally, small molecules can be used as chemical elicitors to activate production of NPs (right). (B) A subset of actinobacteria NPs discovered via chemical elicitation along with the strains from which the molecules were isolated and the chemical elicitors used.

Many strategies have been explored to activate silent NP biosynthetic gene clusters (BGCs). These strategies can generally be split into genetic-based and cultivation-based approaches. Genetic techniques for artificially activating BGCs include engineering transcription and translation machinery, altering levels of regulatory systems, refactoring the BGC or heterologous expression (Abdelmohsen et al., 2015; Rutledge & Challis, 2015; Zarins-Tutt et al., 2015; Liu et al., 2021; El-Hawary et al., 2023). These methods have recently been reviewed elsewhere and will not be discussed further here. While genetic approaches have proven incredibly useful, many are limited due to the need for knowledge *a priori* of the BGC that one wishes to target. Additionally, they can also be time-consuming and low-throughput (Rutledge & Challis, 2015). Cultivation approaches, oftentimes referred to as the one strain many compounds (OSMAC) approach (Bode et al., 2002), have also shown great success in the activation of production of NPs. While the initial OSMAC approach focused on basic growth components such as media composition, pH, culture vessel, and growth temperature (Fig. 1A), it has since been greatly expanded to include many other aspects, including co-cultivation with other microbes as well as chemical elicitors (Rutledge & Challis, 2015). *Streptomyces*' interactions with other soil-dwelling organisms induce production of NPs that are not produced in pure cultures (Watrous et al., 2012; Derewacz et al., 2015; Hiroyasu Onaka, 2017). These interactions are mediated by chemical signals that are often poorly understood (Bibb, 2005; Abdelmohsen et al., 2015; Antoraz et al., 2015). Herein, we describe the recent literature exploring small molecule signals that activate production of silent NPs (Fig. 1B and Supplementary Table S1). Specifically, we focus on two main types of signals that have been demonstrated to induce NPs in Actinobacteria: (1) Antibiotics/Stressors and (2) Hormones/Autoinducers.

## Antibiotic Induction

It has become increasingly clear that BGC activation is influenced by the ecological conditions in which the NP has evolved (van der Meij et al., 2017). Actinobacteria engage in chemical signaling with other members of their community, resulting in alterations in growth and metabolic production. The chemical signals, including NP antibiotics, are believed to prompt the production of NPs to combat threats (i.e., the chemical warfare model) (Traxler & Kolter, 2015). However, it has also been noted that low concentrations of antibiotics have differing effects on a strain compared to lethal concentrations (Davies et al., 2006; Imai et al., 2015). This concept is known as hormesis and provides support for the hypothesis that NP antibiotics may be involved in chemical sig-

naling, in addition to the chemical warfare that has often been proposed (Traxler & Kolter, 2015). As described in the following sections, subinhibitory concentrations of antibiotics have been demonstrated to elicit production of NPs. Interestingly, this effect has been with antibiotics that target a variety of cellular processes, including Deoxyribonucleic acid (DNA) synthesis, protein synthesis, and cell wall integrity. However, the specific NPs that are elicited appear to differ depending on the type of antibiotic used. Understanding the mechanisms by which differing antibiotics induce NP production can provide insights into the regulation of microbial metabolism and offer new strategies for the discovery of novel compounds with potential pharmaceutical applications.

## Protein Synthesis Inhibitors

Historically, ribosome engineering (i.e., developing resistance to antibiotics that target the ribosome) has been widely used to potentiate production of NPs, with streptomycin resistance being most widely explored (Hesketh & Ochi, 1997; Hosaka et al., 2006, 2009; Nishimura et al., 2007; Y. Tanaka et al., 2009; Shentu et al., 2016). Similarly, resistance to the DNA-dependent RNA polymerase inhibitor rifampicin has also been found to induce NP production (Hu et al., 2002; Lai et al., 2002). While these methods have been extremely useful for generating mutants with improved titers, they do require generation of antibiotic resistant mutants. This generation of antibiotic resistance is often time consuming and requires screening of many colonies to identify the mutant with the highest titers (Hesketh & Ochi, 1997; Hu et al., 2002; Hosaka et al., 2009). These methods of induction have been well reviewed elsewhere (Ochi & Hosaka, 2013; Okada & Seyedsayamdost, 2017). Direct addition of protein synthesis inhibitors (rather than generation of resistance) has recently been shown to also be an effective strategy for eliciting NPs, including for the discovery of novel NPs (Fig. 2A). For example, in a study by Hosaka and coworkers a variety of protein synthesis inhibitors, including chloramphenicol, erythromycin, lincomycin, tetracycline, streptomycin, gentamcin, thiostrepton, and tylosin were tested for their abilities to induce NP production in *Streptomyces coelicolor* A3(2) and *Streptomyces lividans* 66 (Imai et al., 2015). Lincomycin, chloramphenicol, and tetracycline all greatly increased production of actinorhodin in *S. coelicolor*. For *S. lividans*, a higher production of actinorhodin and calcium dependent antibiotics (CDAs) were observed only when grown in the presence of lincomycin. Interestingly, this production was greatly enhanced when an *rpoB*-mutant strains of *S. lividans* was exposed to lincomycin, suggesting that a combination of ribosome engineering with addition of ribosome inhibitors may lead to synergistic improvements in production (Mukai et al., 2020).

Given the ability of lincomycin to induce NPs in *S. coelicolor* and *S. lividans*, there is great interest in the mechanism of induction. Lincomycin inhibits protein synthesis by binding to the peptidyl transferase center (Tenson et al., 2003), however, the exact mechanism of NP induction remains under investigation (Fig. 2B). In 2015, Hosaka and co-workers discovered that lincomycin increases transcript levels for genes responsible for production of actinorhodins and CDAs, which provides strong evidence that the induction is caused by more than simply a change in translation (Imai et al., 2015). Interestingly, lincomycin also increases intracellular levels of ATP as well as other NTPs, which could cause global alteration of gene expression (Imai et al., 2015). The global alteration of gene expression was corroborated in another study by Hosaka and co-workers, where RNAseq analysis demonstrated large changes in *S. coelicolor* gene expression profiles after treatment with lincomycin (Ishizuka et al., 2018). They observed increased production of the 23S rRNA methyltransferase gene and the ribosome splitting factor HflX gene, which are both involved in resistance to ribosome targeting antibiotics. It is possible that such mutations have a similar effect to the ribosome engineering discussed above. Additionally, increases in the cytoplasmic stress sensing extra cytoplasmic function sigma factor SigR and reduction in levels of the housekeeping sigma factor HrdB were observed. Alternative sigma factors (i.e., extra-cytoplasmic function, ECF, sigma factors) are very abundant in *Streptomyces,* with 50 being encoded in the *S. coelicolor* genome (Hahn et al., 2003). It is common for ECF sigma factors to regulate NP biosynthesis (Sun et al., 2017), and thus, it is possible that increased levels of SigR are responsible for the induction of NP biosynthesis in *S. coelicolor*. Others have previously observed that translation-inhibiting antibiotics including hygromycin, chloramphenicol, erythromycin, fusidic acid, streptomycin, and tetracycline result in increases in SigR levels via WblC signaling (Yoo et al., 2016). It is also possible that WblC directly affects transcription of biosynthetic genes, but studies on the direct targets of WblC have not confirmed this (Lee et al., 2020). Currently, how protein synthesis inhibitors activate WblC is not fully understood, and further investigation of the relationship between ribosomal stress, SigR, and WblC will aid our understanding of the complexities of secondary metabolite regulation.

Others have also explored the ability of ribosome inhibitors to induce NPs in additional strains. In the marine *Streptomyces* sp. HB202, production of new phenazines (streptophenazines A–H) was induced by tetracycline (Mitova et al., 2008). Specifically, an approximate three-fold increase in total phenazines was observed with approximately ten-fold increases in streptophenazine A/B and streptophenazine E/F. However, inhibition of another streptophenazine was observed, suggesting that tetracycline does not improve production of all phenazines in this strain and highlights the complexity of NP induction. Bachmann and co-workers explored the effect of subinhibitory concentrations of ribosome targeting antibiotic streptomycin on 405 actinobacteria isolated from cave environments (Covington et al., 2018). Of those explored, generally between 1% and 3% of molecules observed were present at ten-fold or higher abundance after streptomycin treatment. In particular, the polyketide tetarimycin B (*Microbispora* sp. BCCAGE54) was induced over thirty-fold.

Ochi and coworkers found that chloramphenicol, erythromycin, and tetracycline all induced production of the nonribosomal peptide actinomycin D by *Streptomyces antibioticus* KO-1164, an *rpoB* mutant strain, with even greater induction observed in nutritionally poor media (Yukinori Tanaka et al., 2017). Interestingly, lincomycin and streptomycin had no effect, suggesting that the mechanism of induction might differ depending on the ribosome inhibitor. A similar pattern of induction was observed for the nonribosomal peptide calcium-dependent antibiotics (CDAs, *S. coelicolor*) and piperidamycin (*Streptomyces mauvecolor*). The authors found that the amino acid pool was increased after exposure to ribosome inhibitors and proposed the increase in the starting materials of nonribosomal peptides is likely at least partly responsible for the induction that is observed. The fact that production of the ribosomally synthesized peptide thiostrepton (*Streptomyces azureus*) was inhibited by chloramphenicol and lincomycin provides support for this hypothesis. However, additional mechanisms (perhaps SigR activation) are needed to explain the transcription level changes that were observed and the activation of other types of NPs such as polyketides.

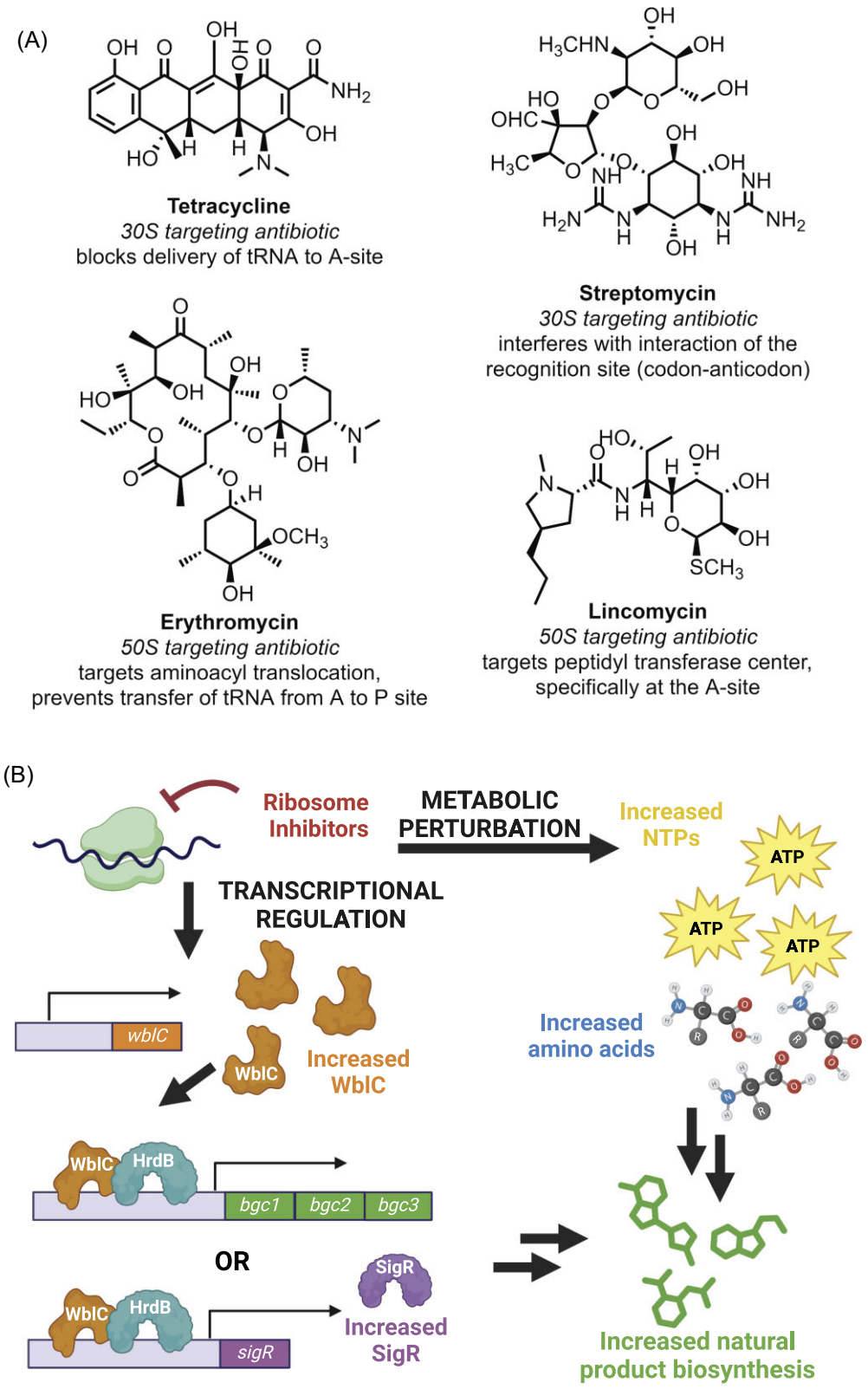


**Fig. 2.** (A) Protein synthesis inhibitors that induce NP biosynthesis along with details of their mechanism of protein synthesis inhibition. (B) Potential mechanisms for NP induction by protein synthesis inhibitors. Specifically, two major mechanisms are proposed. The first is induction via metabolic perturbation. Ribosome inhibition has been shown to cause increases in both NTPs and amino acids, which could provide the necessary energy and starting materials for NPs. A second potential mechanism is changes in transcriptional regulation. Ribosome inhibition results in increased levels of the transcription factor WblC. While all of the targets of WblC are not known, it is likely that it increases transcription of biosynthetic gene cluster, the sigma factor SigR, or both, which would result in increased NP production.

## DNA Damaging Agents

Several DNA damaging agents have been found to induce production of NPs (Fig. 3A and Supplementary Table S1). Utilizing their initial GFP-based high-throughput screening approach (HiTES), Seyedsayamdost and co-workers discovered that etoposide induces production of the cyclic peptides surugamides and the albucyclones in *Streptomyces albus* J1074 (F. Xu et al., 2017). Etoposide is typically used as an anticancer agent due to its ability to inhibit human topoisomerase II. However, it has also been shown to inhibit bacterial topoisomerase II, specifically DNA gyrase, and stabilize both single-stranded and double-stranded breaks (Chan et al., 2015; Chatterji et al., 2001). These breaks result in activation of the SOS-response, which is controlled by LexA and RecA. Prior to DNA damage, LexA represses gene expression. Upon singled-stranded DNA damage, RecA binds to sites of damage and serves as a co-protease of LexA. Degradation of LexA results in its release from the DNA and ultimately transcription of downstream genes (Save Our Soul (SOS) response genes, Fig. 3B). Seyedsayamdost and co-workers were able to demonstrate that etoposide does activate the SOS response, suggesting that the SOS response may be involved in activation of transcription of NP biosynthetic genes.

At least two additional studies provide support for the SOS-response being involved in NP regulation. First, Burroughs and co-workers developed a method to predict transcription factor binding sites (Iqbal et al., 2012). In this work, they predicted that ActII-ORF4, the actinorhodin cluster activator protein found in *S. coelicolor*, is under control of LexA. Unfortunately, they did not provide experimental evidence for this claim. Second, Schlimpert and co-workers used a *lexA* deletion mutant of *Streptomyces venezuelae* to determine the genes under the control of the SOS response (Stratton et al., 2022). Interestingly, they found that secondary metabolism genes were among the SOS-regulon, in addition to the expected DNA damage repair genes. They further validated these genes by treating wild type *S. venezuelae* with the DNA damaging agent mitomycin C and found similar patterns.

More recently, Seyedsayamdost and co-workers used HiTES in combination with anticancer screening and found that treatment of *Streptomyces clavuligerus* with the intercalator/topoisomerase II inhibitor pyronaridine (Bailly, 2020) induced production of several NPs (Han et al., 2022). Specifically, pyronaridine resulted in increased production of the tunicamycins. Interestingly, actinomycin D, a GC-specific DNA intercalator that inhibits topoisomerase I, also resulted in induction of NPs. However, the molecules that it induced are distinct from those discovered in the pyronaridine treated cells. Specifically, actinomycin D treatment resulted in production of the siderophore nocardamine, the clavamates, and the novel tryptorubin-like NP clavorubin A. The different induction profile between pyronaridine and actinomycin D suggests different specific mechanisms of induction, not simply an activation of the SOS-response. One possible explanation for this was recently explored by Jakimowicz and colleagues (Szafran et al., 2019). They hypothesize that changes in supercoiling of the DNA will impact global gene expression in *Streptomyces* strains (Fig. 3C) (Szafran et al., 2019). To test this, they utilize novobiocin, a topoisomerase II/DNA gyrase inhibitor that causes relaxation of negatively supercoiled DNA. Upon treatment of *S. coelicolor* with novobiocin and subsequent RNA-seq, they found that transcript levels of 1.5% of orfs were affected. Several of the induced genes were NP biosynthetic genes, including those responsible for production of actinorhodin. Interestingly, some transcript levels increased while others decreased. The authors proposed this may relate to whether the promoters are more AT rich (induced) versus

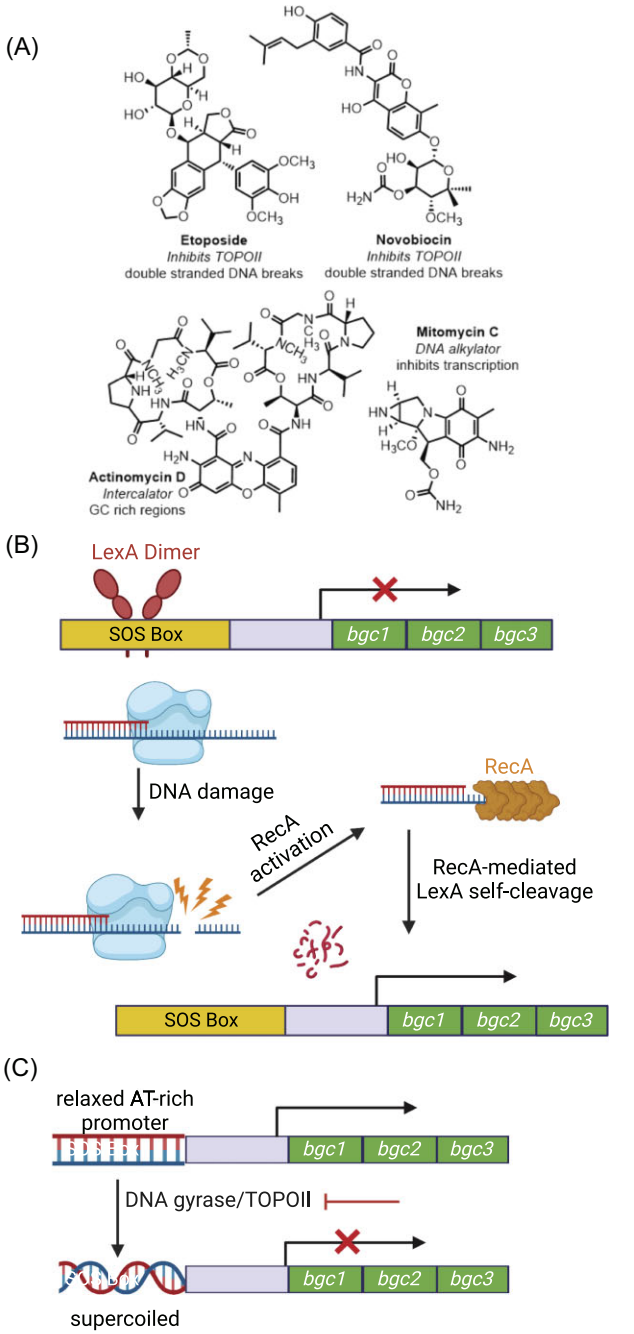


**Fig. 3.** (A) Deoxyribonucleic acid (DNA) synthesis inhibitors that induce NP biosynthesis along with details of their mechanism of DNA synthesis inhibition. (B) One proposed mechanism for induction by DNA synthesis inhibitors is that biosynthetic gene clusters may be under the control of the SOS response. Prior to treatment with a DNA damaging agent, the LexA repressor binds to the SOS box, preventing transcription of downstream genes. Upon DNA damage, RecA is recruited to the single stranded DNA that is formed, mediating LexA self-cleavage and ultimately transcription of downstream genes. Whether the transcribed genes are the biosynthetic genes themselves or signals that then activate biosynthetic gene transcription remains unclear. (C) A second potential mechanism relates to the chromosome relaxation in the promoter region. Inhibition of DNA gyrase/topoisomerase II (TOPOII) results in reduced supercoiling of the DNA. This has been shown to correlate with increased gene transcription in *Streptomyces*, especially for genes with AT-rich promoters.

GC rich (repressed). The authors also explored the effect of genetic knockdown of topoisomerase I, which causes the buildup of negatively supercoiled DNA. Downregulation of the topoisomerase I resulted in induction of 264 genes that were not observed in the upregulation sample. Overall, this suggests that topoisomerase I and II inhibitors likely induce transcription of differing biosynthetic genes and that this may relate to the AT/GC content of the promoters. This provides a potential explanation for the differences observed in the case of pyronaridine and actinomycin D. However, more work is necessary to confirm this hypothesis.

## Fatty-Acid Synthesis Inhibitors

The primary example of fatty-acid synthesis inhibitors that induce NPs are the antibiotic-remodeling compounds (ARCs) discovered by Nodwell and colleagues in 2012 (Craney et al., 2012). The ARCs were discovered in a screen focused on molecules that could alter pigmentation in *S. coelicolor* (Craney et al., 2012). They have a relatively wide scope, also inducing production of desferrioxamine B in *Streptomyces pristinaespiralis* ATCC 25486, doxorubicin and baumycin in *Streptomyces peucetius* 27 952, and an unknown metabolite in *Kutzneria* sp. 744. More recently, Ochi and co-workers found that triclosan (a fatty-acid synthesis inhibitor with close structural similarity to the ARCs) enhanced production of the polyketide salinomycin in *Streptomyces albus* KO-606 by 40% (Yukinori Tanaka et al., 2017).

The mechanism of the ARCs was originally proposed to be fatty acid synthesis inhibition, thus increasing the free pool of acetyl-CoA that could be utilized in polyketide biosynthesis (Craney et al., 2012). However, further examination revealed that it is likely more complicated (Ahmed et al., 2013). ARC2 was found to have little-to-no effect on absolute levels of fatty acids. Instead, it increased levels of unsaturated fatty acids. ARC6, on the other hand, elevated branched fatty acids while reducing levels of linear and unsaturated fatty acids. This helps to explain the difference in NP induction observed between ARC2 and ARC6. More recently, Nodwell and co-workers delved deeper into the mechanism of ARC2 activation (Calvelo et al., 2021). To explore this, RNA-seq analysis of *S. coelicolor* treated with ARC2 was performed. In addition to altered transcription of genes involved in the biosynthesis of 16 NPs, they also found that *afsS* and *afsR* were upregulated. These genes encode for the pleiotropic regulator AfsS and its regulator AfsR. AfsS is known to be involved in the regulation of both NP biosynthetic genes as well as genes involved in the nutritional stress response (Lian et al., 2008). Therefore, it is likely that ARC2 induction of NPs is through activation of AfsS via a global stress response (Fig. 4A–B) (Calvelo et al., 2021).

## Disruptors of Cell Wall Integrity

Disruptors of cell wall integrity, including cell wall synthesis inhibitors and ionophores, have both been shown to induce production of NPs. However, the mechanisms remain understudied. In the same study that evaluated the effect of the ribosome inhibitor tetracycline on streptophenazine production, Imhoff and co-workers also explored the ability of bacitracin to induce streptophenazine production by *Streptomyces* sp. Strain HB202 (Mitova et al., 2008). Bacitracin, a mixture of cyclic dodecapeptides from *Bacillus licheniformis*, inhibits dephosphorylation of C55-isoprenyl pyrophosphate, which is required for construction of peptidoglycan (Stone & Strominger, 1971). Bacitracin has also been shown to cause potassium efflux at high concentrations, but the relevance to its antibiotic activity is unclear (Economou et al., 2013). Interestingly, bacitracin's stimulation of streptophenazine differs dramatically from the stimulation by tetracycline. Specifically, bacitracin greatly stimulated the production of streptophenazine H, which was reduced upon exposure to tetracycline. Alternatively, bacitracin reduced production of the other streptophenazines, including streptophenazine A/B and streptophenazine E/F, which were both highly induced by tetracycline. These differences suggest that cell wall disruptors have differing mechanisms of induction compared to protein synthesis inhibitors. Unfortunately, little else is known about how or why bacitracin may have these effects.

The majority of other cell wall disruptors that have been explored for NP induction are ionophores. Ueda and coworkers found that the polyether promomycin induces antibiotic production in several strains of *Streptomyces* (Amano et al., 2010, 2011). This was discovered using a cross-feeding assay where two strains were grown together and then screened for antibiotic activity against *Bacillus subtilus*. Strain 574 was found to produce an antibiotic when grown with strain 153. Further studies revealed that an ethyl acetate extract of strain 153 was also able to stimulate antibiotic production by strain 574, suggesting that this induction was via chemical elicitation. Isolation of the inducing molecule (named promomycin) revealed it to be highly structurally similar to the polyether ionophores, including salinomycin, monensin, and nigericin. Interestingly, these polyether ionophores also induced antibiotic induction in strain 574, suggesting that they may all be inducing via their ionophore activity. It has been shown that high salt conditions stimulate undecylprodigiosin production and inhibit actinorhodin production in *S. coelicolor*, providing further support that the ionophore activity may affect NP induction (Sevcikova & Kormanec, 2004). Promomycin was also shown to induce antibiotic production in three other strains, suggesting it may be a general inducer (Amano et al., 2010). The authors suggest that the potassium efflux may affect a currently unidentified pleiotropic regulator (Fig. 4A and C) (Amano et al., 2010). Alternatively, the osmotic stress that results from the potassium efflux may cause changes in activity of two-component systems and/or in utilization of alternative sigma factors (Bhowmick et al., 2023), which could affect NP production.

## Other Small Molecule Elicitors

In addition to antibiotics with known mechanisms, other less explored molecules with antibiotic activity have been found to induce production of NPs. Below are described a few such examples with a more extensive list provided in Supplementary Table S1. Onaka and co-workers hypothesized that molecules produced by actinobacteria are likely to induce production of NPs in other actinobacteria (H Onaka et al., 2001). To test this, they screened fermentation broths of 405 actinobacteria for their ability to induce pigment production in *S. lividans*. An extract from *Streptomyces* sp. TP-A0584 was found to be active, and purification of the inducing molecule revealed that it is a ribosomally synthesized and post-translationally modified peptide (RiPP) which they named goadsporin. Goadsporin was then tested for its ability to affect sporulation and induce NPs in 42 *Streptomyces* strains. Goadsporin caused sporulation in 32 of the strains and pigment production in 20, suggesting that it is a general inducer of NPs. Goadsporin was also found to have antibiotic activity against *Streptomyces* but not other bacteria. Analysis of the BGC of goadsporin revealed a self-resistance gene *godI*, which is highly similar to the signal recognition particle *ffh* (Hiroyasu Onaka et al., 2005). The signal recognition particle is responsible for localizing proteins to the membrane, suggesting that goadsporin may inhibit translocation of proteins to the membranes in *Streptomyces* strains

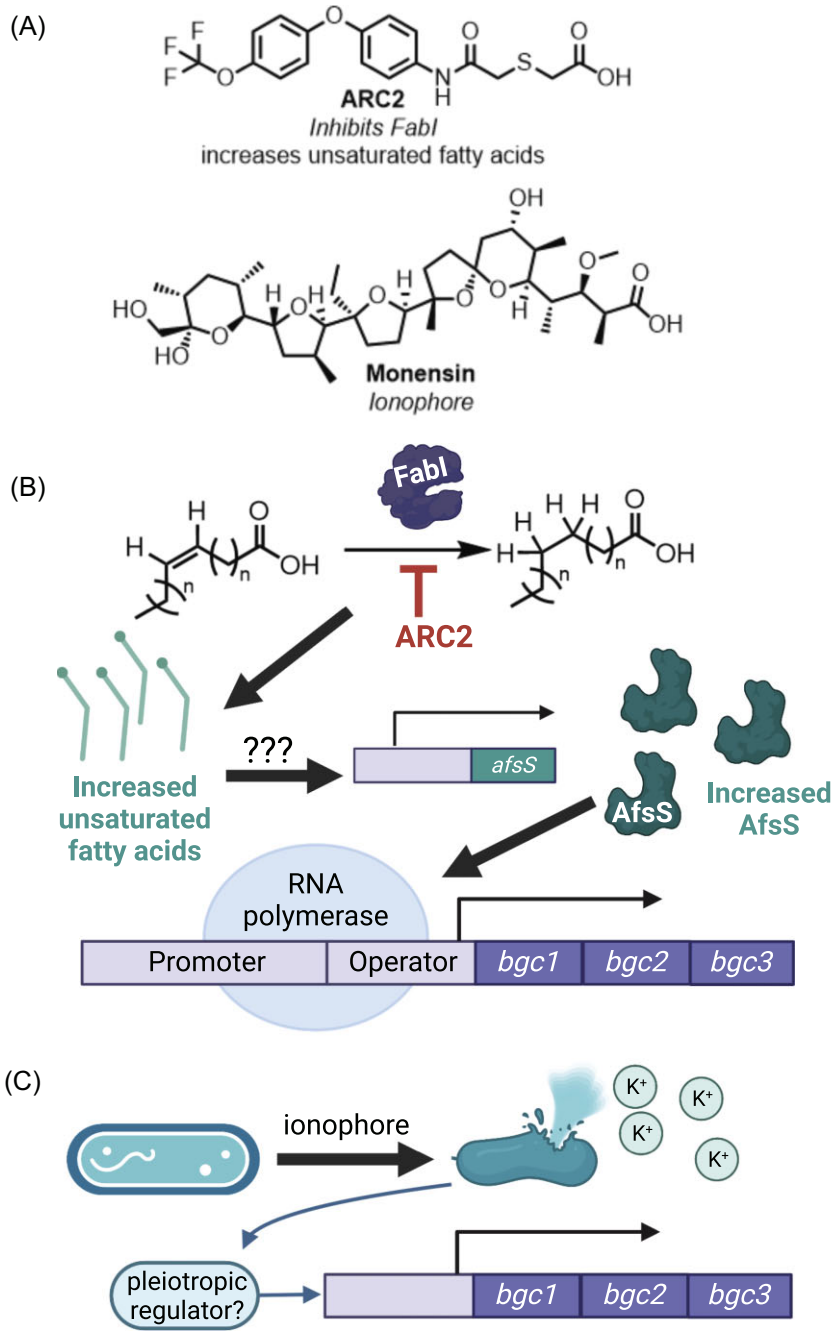


**Fig. 4.** (A) Structures of other antibiotics that induce NP biosynthesis and their mechanisms of action. (B) A proposed mechanism for how inhibition of FabI leads to induction of biosynthetic gene clusters. Inhibition leads to a buildup of unsaturated fatty acids that likely result in a signaling cascade that ultimately activates transcription of AfsS, which is a protein that shares homology with sigma factors and is known to be a positive regulator of antibiotic production. (C) A potential mechanism for how ionophores such as monensin promote NP biosynthesis. Ionophores result in release of ions such as potassium, resulting in reduction in osmotic pressure. While it is unclear whether the actual release of ions or the osmotic pressure acts as the signal, it is likely that one, or both, of these result in activation of pleiotropic regulators, which can induce NP biosynthesis.

(Hiroyasu Onaka et al., 2005; Ongpipattanakul et al., 2022). However, it remains unclear how this results in induction of sporulation and NPs.

Using HiTES, Seyedsayamdost and colleagues have discovered many other inducers of NPs in addition to the DNA damaging agents described earlier. In their study of surugamide production by *S. albus*, they found that ivermectin efficiently induced production of several surugamides (F. Xu et al., 2017). Ivermectin is a synthetic analogue of the avermectins, which are NPs produced by *Streptomyces avermitilis* (Burg et al., 1979). They both are anthelmintic agents and act via opening of the invertebrate glutamate-gated chloride channels and ultimately hyperpolarization (Batiha et al., 2020). Given bacteria do not have analogous channels, it is likely that ivermectin is inducing through an alternative mechanism. Ivermectin has been shown to have antibiotic activity, including activity against *Mycobacteria tuberculosis* (Lim et al., 2013; Crump, 2017). Unfortunately, the mechanism of antibiosis, as well as the mechanism of NP induction, remain unknown.

MALDI-MS-guided HiTES, which combines matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

with high-throughput elicitor screening (HiTES), revealed that amygdalin elicits production of a novel non-ribosomal peptide (cinnapeptin) from *Streptomyces ghanaensis* (C. Zhang & Seyedsayamdost, 2020; Li et al., 2022). Amygdalin is a cyanogenic glycoside naturally found in many seeds, including almonds, apricots, and apples (Barakat et al., 2022). It has been explored for a variety of applications, including as an antioxidant, prebiotic, anti-fibrotic, and anticancer agent. More recently, it has also been explored as an antibacterial (Mhawesh et al., 2018; Barakat et al., 2022), but these studies are preliminary and give no potential insight into mechanism.

Using bioactivity-HiTES, which combines bioactivity testing with traditional HiTES, it was found that atenolol (33 μM) induced production of NP antibiotics from *Streptomyces hiroshimensis* (Moon et al., 2019). Mass spectrometry analysis revealed that atenolol induces multiple NPs. Activity-guided fractionation allowed for identification of toxoflavin analogues which were named as the taylorflavins. Atenolol is a clinically utilized $\beta$-blocker and has no antibiotic activity against *S. hiroshimensis*. Recently, atenolol has been shown to inhibit biofilm formation and quorum sensing activity in Gram-negative bacteria, albeit at high concentrations (~1.5 mM) (Cavalu et al., 2022). The mechanism by which atenolol induces *Streptomyces* NP biosynthesis remains unclear (Moon et al., 2019).

Van Wezel and co-workers have taken a different approach, specifically focusing on ecological context to find inducers of bacterial NPs (van der Meij et al., 2018; van Bergeijk et al., 2022). Specifically, they explored endophytic actinobacteria from *Arabidopsis* and their production of antibiotics when exposed to plant hormones, including salicylic acid, indole acetic acid, and jasmonic acid (van der Meij et al., 2018). They hypothesized that plant hormones may act as "cry for help" signal, activating their microbiome to produce antibiotic molecules to protect them from a pathogenic attack. Interestingly, the percentage of bacteria that produced antibiotics approximately doubled when exposed to plant hormones, with indole acetic acid showing the largest impact. Additionally, many of the strains that showed activity without hormones had increased activity with hormones, suggesting that the titers of the antibiotic NP were likely increased. More recently, van Wezel and colleagues explored the animal stress hormone epinephrine for its ability to induce NPs in *Streptomyces*.(van Bergeijk et al., 2022) They found that epinephrine can cause either promotion or inhibition of antibiotic production, depending on the strain of *Streptomyces* tested. Exposure of *Streptomyces* sp. MBT42 to epinephrine resulted in increased production of an antibiotic with activity against *Bacillus subtilis*. To further explore the mechanism, they tested other catecholamines and found that catechol containing molecules, including catechol, dopamine, levodopa, and norepinephrine all had similar effects. They also found that catechol induced production of aquayamycin, urdamycinone, and other structurally related angucyclines in *Strepomyces* MBT84. However, other molecules, including epinephrine did not have this effect. Catechols chelate iron, and it was hypothesized that lower iron availability might, in part, be responsible for the induction of NPs. However, iron addition did not affect changes in bioactivity in *Streptomyces* MBT84, suggesting that there is a more complex mechanism involved.

## Hormones/Autoinducers

### *Gamma-Butyrolactone Type Hormones*

Quorum sensing molecules have also been extensively studied for their ability to induce NPs. In actinobacteria, gamma-butyrolactones (GBLs, e.g. A-factor, SCBs, and VB) (Khokhlov, 1980; Yamada et al., 1987; Hashimoto et al., 1992; Creamer et al., 2021; E. Takano et al., 2000; Eriko Takano et al., 2001; Hsiao et al., 2009; Kudo et al., 2020; Wilbanks et al., 2023), gamma-butenolides (e.g., avenolide, SABs, and SRBs) (Kitani et al., 2011; Arakawa et al., 2012; Nguyen et al., 2018; Wenxi Wang et al., 2018), and methylenomycin furans (Corre et al., 2008; Zhou et al., 2021) are the most well studied (Fig. 5A). These molecules serve as ligands for TetR type repressors (also referred to as GBL receptors, GBLRs), releasing the repressors from the DNA and allowing for the genes downstream to be transcribed (Fig. 5B) (Cuthbertson & Nodwell, 2013). While many of these repressors are cluster-situated, many others have also been found to modulate transcription of distal BGCs, suggesting they have more pleiotropic effects. For example,

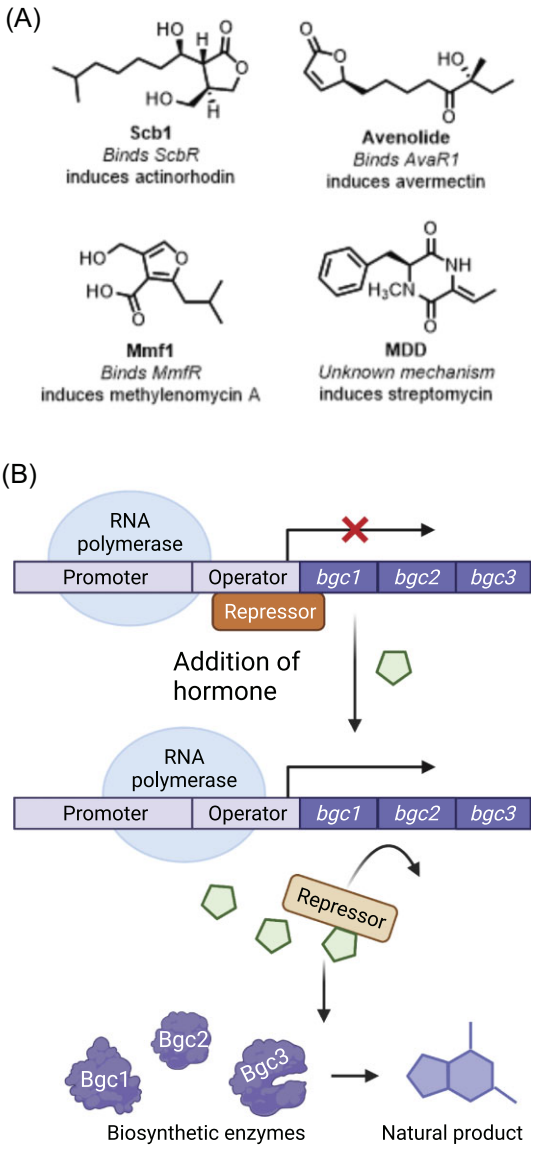


**Fig. 5.** (A) Hormones that induce NP biosynthesis along with the repressors that they bind and the NPs they induce. (B) Mechanism for GBL induction of NP biosynthesis. A TetR type repressor often binds upstream of biosynthetic gene clusters, preventing their transcription. As the bacterial community grows, hormones (aka quorum sensing molecules) are produced at higher levels. Once they reach a threshold, they bind to the repressor, causing it to change conformation and release from the DNA. This allows for transcription of the biosynthetic gene cluster and subsequent NP production.

A-factor is known to bind ArpA resulting in production of AdpA, a regulator involved in transcription of multiple genes involved in a variety of functions, including morphological development and NP biosynthesis (Ohnishi et al., 2005).

While the mechanisms of many of these hormones have been well studied and previously reviewed (Polkade et al., 2016; Daniel-Ivad et al., 2018), surprisingly little has been done using GBLs to chemically induce the production of NPs in wild-type *Streptomyces* strains. This is likely because of the challenge of accessing the GBLs combined with the need to determine the best timing for addition (Hashimoto et al., 1992; Yang et al., 1995). One of the few examples utilized chemically synthesized VB-C to induce production of virginiamycins M and S (Yang et al., 1995). Addition of VB-C (23 nM) to *S. virginiae* at 8 hr resulted in a two-fold increase in production of virginiamycin M and a four-fold increase in virginiamycin S as well as earlier production (Yang et al., 1995). Interestingly, addition of VB-C at the start of culture completely inhibited production of the virginiamycin, suggesting the importance of timing of addition. Further studies into timing of hormone addition are needed to understand this effect. Others have attempted to use the unsubstituted 1,4-butyrolactone since it is commercially available (Tan et al., 2013; Tan et al., 2013). However, very high concentrations had to be used (10 mM) and only modest increases in titers were observed (~30%). More recently, introduction of the genes that produce SCBs into *S. albus* 1074 resulted in increased production of paulomycin, suggesting that addition of external SCBs should also increase production. However, addition of racemic SCB2 did not result in similar improvements in titers. The reasons for this might be the utilization of racemic compound, the timing or concentration of addition, or that other SCB molecules with differing side chains are responsible for the activity observed (Y. Zhang et al., 2020).

### *Pseudo-GBLRs*

In addition to the hormone binding GBLRs, many pseudo-GBLRs have been discovered. Based on their phylogenetic differences from GBLRs, these were hypothesized to bind antibiotics and/or NP intermediates rather than the GBL-type hormones (Cuthbertson & Nodwell, 2013). While many pseudo-GBLRs have been proposed based on sequence similarity (Cuthbertson & Nodwell, 2013; G. Xu & Yang, 2019), only a handful have been experimentally validated with even fewer having identified ligands. The two best studied examples are ScbR2 from *S. coelicolor*, which controls the production of coelimycin, and JadR2 from *S. venezuelae*, which controls production of jadomycin. Both ScbR2 and JadR2 have been shown to bind NPs produced by their native organism (actinorhodin and undecylprodigiosin for ScbR2 and jadomycin and chloramphenicol for JadR2). These activities require relatively high concentrations of molecule (between 100 and 750 µM) (G. Xu et al., 2010), causing slight speculation about the biological relevance of these results. In the case of ScbR2, it has also been shown to respond to jadomycin at much lower concentrations (1–5 µM) (Weishan Wang et al., 2014), suggesting potential crosstalk with *S. venezuelae*. One important thing to note about these studies is that they were all electrophoretic mobility shift assays (EMSAs) demonstrating the ability of these ligands to promote release of the repressors from the DNA. While this is highly suggestive that addition of these molecules to culture will increase production of NPs, to the best of our knowledge, this has not been experimentally validated.

AvaR2 is a pseudo-GBLR found in *S. avermitilis*, and it negatively regulates production of the anthelmintic avermectin (Zhu et al., 2016). Electrophoretic mobility shift assays revealed that exogenous jadomycin B as well as aminoglycosides such as apramycin, hygromycin B, kanamycin, and streptomycin were are able to disrupt its binding to DNA at 1 mM. A Lux reporter system was then used to determine the relevance of the activity *in vivo*. Jadomycin B, hygromycin B, and apramycin all showed significant induction of bioluminescence at more biologically relevant concentrations (12.5–50 µg/mL), suggesting that these may actually serve as ligands for AvaR2. Exposure of *S. avermitilis* to 10 µM jadomycin resulted in increases in GBL-associated genes, providing strong evidence for its relevance for activation of NP biosynthesis. More recently, Aparicio and co-workers discovered the pseudo-GBLR SfbR2 in *Streptomyces filipinensis* (Barreales et al., 2020). SfbR2 was found to inhibit production of the antifungal NP filipin. Screening of a variety of NPs revealed that antimycin was capable of releasing SfbR2 from the DNA in an EMSA assay, albeit at 5 mM. Interestingly, when *S. filipinensis* was exposed to much lower concentrations of antimycin (10–50 µM), three-to-eight-fold increases in transcription of GBL-associated genes were observed. The response to these more biologically relevant concentrations suggests that exogenous antimycin might be, at least in part, responsible for controlling filipin production.

### *Diketopiperazines*

While the GBL hormones remain the best studied, several other classes of Actinobacteria hormones exist (Daniel-Ivad et al., 2018). One of particular interest are the diketopiperazines (DKPs). Diketopiperazines have been shown to be signals for many different microorganisms, including Gram-negative bacteria such as *Pseudomonas aeruginosa* (Holden et al., 1999; Daniel-Ivad et al., 2018). More recently, the DKP N-methylphenylalanyl-dehydrobutyrine diketopiperazine (MDD) has been shown to modulate production of landomycin E in the MDD producing strain *S. globisporus* as well as the production of streptomycin from *Streptomyces griseus*, a strain that does not naturally produce MDD (Matselyukh et al., 2014). While the mechanism by which this occurs is currently unknown, DKPs from Gram-negative bacteria have been shown to interrupt their acyl-homoserine lactone signaling (Holden et al., 1999). It is possible that DKPs compete with GBL binding to GBLRs, given the structural similarity of GBLs to the acyl-homoserine lactones. However, this remains to be explored. Additionally, the widespread nature of DKPs in *Streptomyces* strains (Gondry et al., 2018) suggests that this could be a general mechanism of NP induction. Further investigations into the mechanism as well as the ability of other DKPs to induce NPs are needed to confirm this.

## Conclusions and Perspectives

Natural products from actinobacteria have played a significant role in industrial and medical fields (Bérdy, 2012; Olanrewaju & Babalola, 2019; Newman & Cragg, 2020). In particular, the soil dwelling bacteria *Streptomyces* has played a pivotal role in the production of antibiotics. Genomics data suggest that a plethora of the novel NPs likely have interesting bioactivities remain to be discovered from *Streptomyces* (Doroghazi & Metcalf, 2013; Doroghazi et al., 2014; Palazzolo et al., 2017; Pye et al., 2017). Many of these NP BGCs are considered to be silent because their associated NPs are undetectable in laboratory grown cultures. The poor production of these NPs is likely due to low transcription/translation of their BGCs when grown under standard laboratory conditions. It is hypothesized that this is due, at least in part, to the lack of chemical stimuli that they would encounter in their native environments (van der Meij et al., 2017). To enhance the production of known antibiotics and to elicit the induction

of silent BGCs, numerous strategies have been employed. In recent years, the use of small molecule elicitors has gained traction as an effective way to access both known and novel NPs. In this review, we describe the elicitors that have successfully induced BGCs and their potential mechanisms of induction. Interestingly, many of the elicitors are antibiotics, with different classes of antibiotics resulting in induction of unique groups of NPs. This implies distinct mechanisms of induction for different antibiotics. Unfortunately, many of the mechanisms are poorly understood making predictions of elicitors difficult. Better understanding of the mechanisms of induction will enable more targeted application of small molecule elicitors. In addition to the need to understand the mechanisms, there is also a great need to better understand the timing of addition. While the addition of elicitors to culture is oftentimes easier than co-culture or genetic manipulation, it does require extensive evaluation for the best concentration and timing of addition. This is particularly clear with hormones, which if added at the wrong time or concentration can have inhibitory effects on NPs rather than the desired stimulatory effects. Overall, the continued exploration of elicitors combined with the evolution of bioinformatics and genetics have great potential to enable the community to unravel the intricacies involved in the regulation of secondary metabolites and ultimately allow for a new era in NP discovery.

## Supplementary Material

Supplementary material is available online at *JIMB* (*www.academic.oup.com/jimb*).

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## Conflicts of Interest

The authors declare no conflicts of interest.

## References

Abdelmohsen, U. R., Grkovic, T., Balasubramanian, S., Kamel, M. S., Quinn, R. J., & Hentschel, U. (2015). Elicitation of secondary metabolism in actinomycetes. *Biotechnology Advances*, 33(6), 798–811. https://doi.org/10.1016/j.biotechadv.2015.06.003

Ahmed, S., Craney, A., Pimentel-Elardo, S. M., & Nodwell, J. R. (2013). A synthetic, species-specific activator of secondary metabolism and sporulation in streptomyces coelicolor. *Chembiochem*, 14(1), 83–91. https://doi.org/10.1002/CBIC.201200619

Amano, S., Morota, T., Kano, Y., Narita, H., Hashidzume, T., Yamamoto, S., Mizutani, K., Sakuda, S., Furihata, K., Takano-Shiratori, H., Takano, H., Beppu, T., & Ueda, K. (2010). Promomycin, a polyether promoting antibiotic production in Streptomyces spp. *The Journal of Antibiotics*, 63(8), 486–491. https://doi.org/10.1038/ja.2010.68

Amano, S., Sakurai, T., Endo, K., Takano, H., Beppu, T., Furihata, K., Sakuda, S., & Ueda, K. (2011). A cryptic antibiotic triggered by monensin. *The Journal of Antibiotics*, 64(10), 703. https://doi.org/10.1038/ja.2011.69

Antoraz, S., Santamaría, R. I., Díaz, M., Sanz, D., & Rodríguez, H. (2015). Toward a new focus in antibiotic and drug discovery from the Streptomyces arsenal. *Frontiers in Microbiology*, 6, 461. https://doi.org/10.3389/fmicb.2015.00461

Arakawa, K., Tsuda, N., Taniguchi, A., & Kinashi, H. (2012). The butenolide signaling molecules SRB1 and SRB2 induce lankacidin and lankamycin production in *Streptomyces rochei*. *Chembiochem*, 13(10), 1447–1457. https://doi.org/10.1002/cbic.201200149

Bailly, C. (2020). Pyronaridine: An update of its pharmacological activities and mechanisms of action. *Biopolymers*, 112(4), e23398. https://doi.org/10.1002/bip.23398

Barakat, H., Aljutaily, T., Almujaydil, M. S., Algheshairy, R. M., Alhomaid, R. M., Almutairi, A. S., Alshimali, S. I., & Abdellatif, A. A. H. (2022). Amygdalin: a review on its characteristics, antioxidant potential, gastrointestinal microbiota intervention, anticancer therapeutic and mechanisms, toxicity, and encapsulation. *Biomolecules*, 12(10), 1514. https://doi.org/10.3390/BIOM12101514

Barreales, E. G., Payero, T. D., Jambrina, E., & Aparicio, J. F. (2020). The *Streptomyces filipinensis* gamma-butyrolactone system reveals novel clues for understanding the control of secondary metabolism. *Applied and Environmental Microbiology*, 86(18), e00443–20. https://doi.org/10.1128/AEM.00443-20%0A

Batiha, G. E. S., Alqahtani, A., Ilesanmi, O. B., Saati, A. A., El-Mleeh, A., Hetta, H. F., & Beshbishy, A. M. (2020). Avermectin derivatives, pharmacokinetics, therapeutic and toxic dosages, mechanism of action, and their biological effects. *Pharmaceuticals*, 13(8), 1–37. https://doi.org/10.3390/PH13080196

Bérdy, J. (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. *The Journal of Antibiotics*, 65(8), 385–395. https://doi.org/10.1038/ja.2012.27

Bhowmick, S., Shenouda, M. L., & Tschowri, N. (2023). Osmotic stress responses and the biology of the second messenger c-di-AMP in Streptomyces. *MicroLife*, 4, uqad020. https://doi.org/10.1093/femsml/uqad020

Bibb, M. J. (2005). Regulation of secondary metabolism in streptomycetes. *Current Opinion in Microbiology*, 8(2), 208–215. https://doi.org/10.1016/j.mib.2005.02.016

Bode, H. B., Bethe, B., Höfs, R., & Zeeck, A. (2002). Big effects from small changes: possible ways to explore nature's chemical diversity. *Chembiochem*, 3(7), 619. https://doi.org/10.1002/1439-7633(20020703)3:7⟨619::AID-CBIC619⟩3.0.CO;2-9

Burg, R. W., Miller, B. M., Baker, E. E., Birnbaum, J., Currie, S. A., Hartman, R., Kong, Y. L., Monaghan, R. L., Olson, G., Putter, I., Tunac, J. B., Wallick, H., Stapley, E. O., Oiwa, R., & Omura, S. (1979). Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. *Antimicrobial Agents and Chemotherapy*, 15(3), 361. https://doi.org/10.1128/AAC.15.3.361

Calvelo, V. Y., Crisante, D., Elliot, M., & Nodwell, J. R. (2021). The arc2 response in *Streptomcyes coelicolor* requires the global regulatory genes afsr and afss. *Microbiology*, 167(5), 001047. https://doi.org/10.1099/mic.0.001047

Carlson, E. E. (2010). Natural products as chemical probes. In *ACS Chemical Biology* (Vol. 5, Issue 7, pp. 639–653). American Chemical Society. https://doi.org/10.1021/cb100105c

Cavalu, S., Elbaramawi, S. S., Eissa, A. G., Radwan, M. F., Ibrahim, T. S., Khafagy, E.-S., Lopes, B. S., Ali, M. A. M., Hegazy, W. A. H., & Elfaky, M. A. (2022). Characterization of the anti-biofilm and anti-quorum sensing activities of the β-adrenoreceptor antagonist atenolol against Gram-negative bacterial pathogens. *International Journal of Molecular Sciences*, 21(23), 13088. https://doi.org/10.3390/ijms232113088

Chan, P. F., Srikannathasan, V., Huang, J., Cui, H., Fosberry, A. P., Gu, M., Hann, M. M., Hibbs, M., Homes, P., Ingraham, K., Pizzollo, J.,

Shen, C., Shillings, A. J., Spitzfaden, C. E., Tanner, R., Theobald, A. J., Stavenger, R. A., Bax, B. D., & Gwynn, M. N. (2015). Structural basis of DNA gyrase inhibition by antibacterial QPT-1, anticancer drug etoposide and moxifloxacin. *Nature Communications*, 6(1), 1–13. https://doi.org/10.1038/ncomms10048

Chatterji, M., Unniraman, S., Mahadevan, S., & Nagaraja, V. (2001);. Effect of different classes of inhibitors on DNA gyrase from Mycobacterium smegmatis. *Journal of Antimicrobial Chemotherapy*, 48(4), 479–485. https://doi.org/10.1093/JAC/48.4.479

Corre, C., Song, L., O'Rourke, S., Chater, K. F., & Challis, G. L. (2008). 2-Alkyl-4-hydroxymethylfuran-3-carboxylic acids, antibiotic production inducers discovered by *Streptomyces coelicolor* genome mining. *Proceedings of the National Academy of Sciences of the United States of America*, 105(45), 17510–17515. https://doi.org/10.1073/pnas.080553010

Covington, B. C., Spraggins, J. M., Ynigez-Gutierrez, A. E., Hylton, Z. B., & Bachmann, B. O. (2018). Response of secondary metabolism of hypogean actinobacterial genera to chemical and biological stimuli. *Applied and Environmental Microbiology*, 84(19), e01125–18. https://doi.org/10.1128/AEM.01125-18

Craney, A., Ozimok, C., Pimentel-Elardo, S. M., Capretta, A., & Nodwell, J. R. (2012). Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism. *Chemistry & Biology*, 19(8), 1020–1027. https://doi.org/10.1016/j.chembiol.2012.06.013

Creamer, K. E., Kudo, Y., Moore, B. S., & Jensen, P. R. (2021). Phylogenetic analysis of the salinipostin $\gamma$-butyrolactone gene cluster uncovers new potential for bacterial signalling-molecule diversity. *Microbial Genomics*, 7(5), 000568. https://doi.org/10.1099/mgen.0.000568

Crump, A. (2017). Ivermectin: enigmatic multifaceted 'wonder' drug continues to surprise and exceed expectations. *The Journal of Antibiotics*, , 70(5), 495–505. https://doi.org/10.1038/ja.2017.11

Cuthbertson, L. & Nodwell, J. R. (2013). The TetR family of regulators. *Microbiology and Molecular Biology Reviews*, 77(3), 440–475. https://doi.org/10.1128/mmbr.00018-13

Daniel-Ivad, M., Pimentel-Elardo, S., & Nodwell, J. R. (2018). Control of specialized metabolism by signaling and transcriptional regulation: opportunities for new platforms for drug discovery? *Annual Review of Microbiology*, 72(1), 25–48. https://doi.org/10.1146/annurev-micro-022618-042458

Davies, J., Spiegelman, G. B., & Yim, G. (2006). The world of subinhibitory antibiotic concentrations. *Current Opinion in Microbiology*, 9(5), 445–453. https://doi.org/10.1016/J.MIB.2006.08.006

Derewacz, D. K., Covington, B. C., McLean, J. a., & Bachmann, B. O. (2015). Mapping microbial response metabolomes for induced natural product discovery. *ACS Chemical Biology*, 10(9), 1998–2006. https://doi.org/10.1021/acschembio.5b00001

Doroghazi, J. R., Albright, J. C., Goering, A. W., Ju, K.-S., Haines, R. R., Tchalukov, K. A., Labeda, D. P., Kelleher, N. L., & Metcalf, W. W. (2014). A roadmap for natural product discovery based on large-scale genomics and metabolomics. *Nature Chemical Biology*, 10(11), 963–968. https://doi.org/10.1038/nchembio.1659

Doroghazi, J. R. & Metcalf, W. W. (2013). Comparative genomics of actinomycetes with a focus on natural product biosynthetic genes. *BMC Genomics [Electronic Resource]*, 14(1), 611. https://doi.org/10.1186/1471-2164-14-611

Economou, N. J., Cocklin, S., & Loll, P. J. (2013). High-resolution crystal structure reveals molecular details of target recognition by bacitracin. *Proceedings of the National Academy of Sciences of the United States of America*, 110(35), 14207–14212. https://doi.org/10.1073/pnas.1308268110

El-Hawary, S. S., Hassan, M. H. A., Hudhud, A. O., Abdelmohsen, U. R., & Mohammed, R. (2023). Elicitation for activation of the actinomycete genome's cryptic secondary metabolite gene clusters. *RSC Advances*, 13, 5778–5795. https://doi.org/10.1039/D2RA08222E

Gao, X., Wang, Y., & Chu, J. (2019). A preliminary study on the impact of exogenous A-Factor analogue 1,4-butyrolactone on stimulating bitespiramycin biosynthesis. *Bioprocess and Biosystems Engineering*, 42(12), 1903–1913. https://doi.org/10.1007/s00449-019-02184-9

Gondry, M., Jacques, I. B., Thai, R., Babin, M., Canu, N., Seguin, J., Belin, P., Pernodet, J. L., & Moutiez, M. (2018). A comprehensive overview of the cyclodipeptide synthase family enriched with the characterization of 32 new enzymes. *Frontiers in Microbiology*, 9(FEB), 46. https://doi.org/10.3389/fmicb.2018.00046

Hahn, M. Y., Bae, J. B., Park, J. H., & Roe, J. H. (2003). Isolation and characterization of *Streptomyces coelicolor* RNA polymerase, its sigma, and antisigma factors. *Methods in Enzymology*, 370, 73–82. https://doi.org/10.1016/S0076-6879(03)70007-X

Han, E. J., Lee, S. R., Hoshino, S., & Seyedsayamdost, M. R. (2022). Targeted discovery of cryptic metabolites with antiproliferative activity. *ACS Chemical Biology*, 17(11), 3121–3130. https://doi.org/10.1021/acschembio.2c00588

Hashimoto, K., Nihira, T., Sakuda, S., & Yamada, Y. (1992). IM-2, a butyrolactone autoregulator, induces production of several nucleoside antibiotics in Streptomyces sp. FRI-5. *Journal of Fermentation and Bioengineering*, 73(6), 449–455. https://doi.org/10.1016/0922-338X(92)90136-I

Hesketh, A. & Ochi, K. (1997). A novel method for improving *Streptomyces coelicolor* A3(2) for production of actinorhodin by introduction of rpsL (encoding ribosomal protein S12) mutations conferring resistance to streptomycin. *The Journal of Antibiotics*, 50(6), 532–535. https://doi.org/10.7164/ANTIBIOTICS.50.532

Holden, M. T. G., Chhabra, S. R., De Nys, R., Stead, P., Bainton, N. J., Hill, P. J., Manefield, M., Kumar, N., Labatte, M., England, D., Rice, S., Givskov, M., Salmond, G. P. C., Stewart, G. S. A. B., Bycroft, B. W., Kjelleberg, S., & Williams, P. (1999). Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other Gram-negative bacteria. *Molecular Microbiology*, 33(6), 1254–1266. https://doi.org/10.1046/J.1365-2958.1999.01577.X

Hosaka, T., Ohnishi-Kameyama, M., Muramatsu, H., Murakami, K., Tsurumi, Y., Kodani, S., Yoshida, M., Fujie, A., & Ochi, K. (2009). Antibacterial discovery in actinomycetes strains with mutations in RNA polymerase or ribosomal protein S12. *Nature Biotechnology*, 27(5), 462–464. https://doi.org/10.1038/nbt.1538

Hosaka, T., Xu, J., & Ochi, K. (2006). Increased expression of ribosome recycling factor is responsible for the enhanced protein synthesis during the late growth phase in an antibiotic-overproducing *Streptomyces coelicolor* ribosomal rpsL mutant. *Molecular Microbiology*, 61(4), 883–897. https://doi.org/10.1111/J.1365-2958.2006.05285.X

Hsiao, N.-H., Nakayama, S., Merlo, M. E., de Vries, M., Bunet, R., Kitani, S., Nihira, T., & Takano, E. (2009). Analysis of two additional signaling molecules in *Streptomyces coelicolor* and the development of a butyrolactone-specific reporter system. *Chemistry & Biology*, 16(9), 951–960. https://doi.org/10.1016/j.chembiol.2009.08.010

Hu, H., Zhang, Q., & Ochi, K. (2002). Activation of antibiotic biosynthesis by specified mutations in the rpoB gene (encoding the RNA polymerase $\beta$ subunit) of *Streptomyces lividans*. *Journal of Bacteriology*, 184(14), 3984–3991. https://doi.org/10.1128/jb.184.14.3984-3991.2002

Imai, Y., Sato, S., Tanaka, Y., Ochi, K., & Hosaka, T. (2015). Lincomycin at subinhibitory concentrations potentiates secondary

metabolite production by *Streptomyces* spp. *Applied and Environmental Microbiology*, 81(11), 3869–3879. https://doi.org/10.1128/AEM.04214-14

Iqbal, M., Mast, Y., Amin, R., Hodgson, D. A., Wohlleben, W., & Burroughs, N. J. (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 Research*, 40(12), 5227–5239. https://doi.org/10.1093/NAR/GKS205

Ishizuka, M., Imai, Y., Mukai, K., Shimono, K., Hamauzu, R., Ochi, K., & Hosaka, T. (2018). A possible mechanism for lincomycin induction of secondary metabolism in Streptomyces coelicolor A3(2). *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 111(5), 705–716. https://doi.org/10.1007/s10482-018-1021-0

Khokhlov, A. S. (1980). Problems of studies of specific cell autoregulators (on the example of substances produced by some Actinomycetes). In *Frontiers of Bioorganic Chemistry and Molecular Biology* (pp. 201–210). Elsevier. https://doi.org/10.1016/B978-0-08-023967-5.50030-9

Kitani, S., Miyamoto, K. T., Takamatsu, S., Herawati, E., Iguchi, H., Nishitomi, K., Uchida, M., Nagamitsu, T., Omura, S., Ikeda, H., & Nihira, T. (2011). Avenolide, a Streptomyces hormone controlling antibiotic production in *Streptomyces avermitilis*. *Proceedings of the National Academy of Sciences of the United States of America*, 108(39), 16410–16415. https://doi.org/10.1073/pnas.1113908108

Kudo, Y., Awakawa, T., Du, Y. L., Jordan, P. A., Creamer, K. E., Jensen, P. R., Linington, R. G., Ryan, K. S., & Moore, B. S. (2020). Expansion of gamma-butyrolactone signaling molecule biosynthesis to phosphotriester natural products. *ACS Chemical Biology*, 15(12), 3253–3261. https://doi.org/10.1021/acschembio.0c00824

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(11), 3365–3373. https://doi.org/10.1099/00221287-148-11-3365

Lee, J. H., Yoo, J. S., Kim, Y., Kim, J. S., Lee, E. J., & Roe, J. H. (2020). The WBLC/WHIB7 transcription factor controls intrinsic resistance to translation-targeting antibiotics by altering ribosome composition. *MBio*, 11(2). https://doi.org/10.1128/mbio.00625-20[CrossRef]

Li, Y., Lee, S. R., Han, E. J., & Seyedsayamdost, M. R. (2022). Momomycin, an antiproliferative cryptic metabolite from the oxytetracycline producer *Streptomyces rimosus*. *Angewandte Chemie International Edition*, 61(39), 1–10. https://doi.org/10.1002/ANIE.202208573

Lian, W., Jayapal, K. P., Charaniya, S., Mehra, S., Glod, F., Kyung, Y. S., Sherman, D. H., & Hu, W. S. (2008). Genome-wide transcriptome analysis reveals that a pleiotropic antibiotic regulator, AfsS, modulates nutritional stress response in *Streptomyces coelicolor* A3(2). *BMC Genomics [Electronic Resource]*, 9(1), 1–14. https://doi.org/10.1186/1471-2164-9-56

Lim, L. E., Vilchèze, C., Ng, C., Jacobs, W. R., Ramón-García, S., & Thompson, C. J. (2013). Anthelmintic avermectins kill mycobacterium tuberculosis, including multidrug-resistant clinical strains. *Antimicrobial Agents and Chemotherapy*, 57(2), 1040–1046. https://doi.org/10.1128/aac.01696-12

Liu, Z., Zhao, Y., Huang, C., & Luo, Y. (2021). Recent advances in silent gene cluster activation in Streptomyces. *Frontiers in Bioengineering and Biotechnology*, 9, 1–10. https://doi.org/10.3389/fbioe.2021.632230CrossRef

Matselyukh, B., Mohammadipanah, F., Laatsch, H., Rohr, J., Efremenkova, O., & Khilya, V. (2014). N-methylphenylalanyl-dehydrobutyrine diketopiperazine, an A-factor mimic that restores antibiotic biosynthesis and morphogenesis in Streptomyces globisporus 1912-B2 and Streptomyces griseus 1439. *The Journal of Antibiotics*, 68(1), 9–14. https://doi.org/10.1038/ja.2014.86

Mhawesh, A. A., Al-Ugaili, D. N., & Olaiwy, Y. M. (2018). Investigate the antibacterial activity of amygdalin against some types of antibiotic resistant pathogenic bacteria. *Basrah Journal of Veterinary Research*, 17, 640–651. https://doi.org/10.23975/bjvetr.2018.174007

Mitova, M. I., Lang, G., Wiese, J., & Imhoff, J. F. (2008). Subinhibitory concentrations of antibiotics induce phenazine production in a marine Streptomyces sp. *Journal of Natural Products*, 71(5), 824–827. https://doi.org/10.1021/np800032a

Moon, K., Xu, F., Zhang, C., & Seyedsayamdost, M. R. (2019). Bioactivity-HiTES unveils cryptic antibiotics encoded in actinomycete bacteria. *ACS Chemical Biology*, 4(14), 767–774. https://doi.org/10.1021/acschembio.9b00049

Mukai, K., Kobayashi, M., Hoshino, K., Maruyama, T., Hayashi, D., Hamauzu, R., & Hosaka, T. (2020). Lincomycin-induced secondary metabolism in *Streptomyces lividans* 66 with a mutation in the gene encoding the RNA polymerase beta subunit. *Current Microbiology*, 77(10), 2933–2939. https://doi.org/10.1007/s00284-020-02126-9

Newman, D. J. & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Nguyen, T. B., Kitani, S., Shimma, S., & Nihira, T. (2018). Butenolides from *Streptomyces albus* J1074 act as external signals to stimulate avermectin production in *Streptomyces avermitilis*. *Applied and Environmental Microbiology*, 84(9), 1–11. https://doi.org/10.1128/AEM.02791-17[CrossRef]

Nishimura, K., Hosaka, T., Tokuyama, S., Okamoto, S., & Ochi, K. (2007). Mutations in rsmG, encoding a 16S rRNA methyltransferase, result in low-level streptomycin resistance and antibiotic overproduction in *Streptomyces coelicolor* A3(2). *Journal of Bacteriology*, 189(10), 3876–3883. https://doi.org/10.1128/jb.01776-06

Ochi, K. & Hosaka, T. (2013). New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. *Applied Microbiology and Biotechnology*, 97(1), 87–98. https://doi.org/10.1007/s00253-012-4551-9

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*, 3(69), 431–439. https://doi.org/10.1271/bbb.69.431

Okada, B. K. & Seyedsayamdost, M. R. (2017). Antibiotic dialogues: induction of silent biosynthetic gene clusters by exogenous small molecules. *FEMS Microbiology Reviews*, 41(1), 19–33. https://doi.org/10.1093/femsre/fuw035

Olanrewaju, O. S. & Babalola, O. O. (2019). Streptomyces: implications and interactions in plant growth promotion. *Applied Microbiology and Biotechnology*, 103(3), 1179–1188. https://doi.org/10.1007/s00253-018-09577-y

Onaka, H. (2017). Novel antibiotic screening methods to awaken silent or cryptic secondary metabolic pathways in actinomycetes. *The Journal of Antibiotics*, 70(8), 865–870. https://doi.org/10.1038/ja.2017.51

Onaka, H., Nakaho, M., Hayashi, K., Igarashi, Y., & Furumai, T. (2005). Cloning and characterization of the goadsporin biosynthetic gene cluster from Streptomyces sp. TP-A0584. *Microbiology*, 151(12), 3923–3933. https://doi.org/10.1099/mic.0.28420-0

Onaka, H., Tabata, H., Igarashi, Y., Sato, Y., & Furumai, T. (2001). Goadsporin, a chemical substance which promotes secondary

metabolism and morphogenesis in streptomycetes. I. Purification and characterization. *The Journal of Antibiotics*, 54(12), 1036–1044. https://doi.org/10.7164/antibiotics.54.1036

Ongpipattanakul, C., Desormeaux, E. K., DiCaprio, A., Donk, W. A. v. d., Mitchell, D. A., & Nair, S. K. (2022). Mechanism of action of ribosomally synthesized and post-translationally modified peptides. *Chemical Reviews*, 18(122), 14722–14814. https://doi.org/10.1021/acs.chemrev.2c00210

Palazzolo, A. M. E., Simons, C. L. W., & Burke, M. D. (2017). The natural productome. *Proceedings of the National Academy of Sciences of the United States of America*, 114(22), 5564–5566. https://doi.org/10.1073/pnas.1706266114

Polkade, A. V., Mantri, S. S., Patwekar, U. J., & Jangid, K. (2016). Quorum sensing: an under-explored phenomenon in the phylum actinobacteria. *Frontiers in Microbiology*, 7, 131. https://doi.org/10.3389/fmicb.2016.00131

Pye, C. R., Bertin, M. J., Lokey, R. S., Gerwick, W. H., & Linington, R. G. (2017). Retrospective analysis of natural products provides insights for future discovery trends. *Proceedings of the National Academy of Sciences of the United States of America*, 114(22), 5601–5606. https://doi.org/10.1073/pnas.1614680114

Romero, D., Traxler, M. F., López, D., & Kolter, R. (2011). Antibiotics as signal molecules. *Chemical Reviews*, 111(9), 5492–5505. https://doi.org/10.1021/cr2000509

Rutledge, P. J. & Challis, G. L. (2015). Discovery of microbial natural products by activation of silent biosynthetic gene clusters. *Nature Reviews Microbiology*, 13(8), 509–523. https://doi.org/10.1038/nrmicro3496

Sevcikova, B. & Kormanec, J. (2004). Differential production of two antibiotics of *Streptomyces coelicolor* A3(2), actinorhodin and undecylprodigiosin, upon salt stress conditions. *Archives of Microbiology*, 181(5), 384–389. https://doi.org/10.1007/s00203-004-0669-1

Shentu, X., Liu, N., Tang, G., Tanaka, Y., Ochi, K., Xu, J., & Yu, X. (2016). Improved antibiotic production and silent gene activation in Streptomyces diastatochromogenes by ribosome engineering. *The Journal of Antibiotics*, 69, 406–410. https://doi.org/10.1038/ja.2015.123

Stone, K. J. & Strominger, J. L. (1971). Mechanism of action of bacitracin: complexation with metal ion and C55-isoprenyl Pyrophosphate. *Proceedings of the National Academy of Sciences*, 12(68), 3223–3227. https://doi.org/10.1073/pnas.68.12.3223

Stratton, K. J., Bush, M. J., Chandra, G., Stevenson, C. E. M., Findlay, K. C., & Schlimpert, S. (2022). Genome-wide identification of the LexA-mediated DNA damage response in *Streptomyces venezuelae*. *Journal of Bacteriology*, 204(8), 1–15. https://doi.org/10.1128/jb.00108-22[CrossRef]

Sun, D., Liu, C., Zhu, J., & Liu, W. (2017). Connecting metabolic pathways: sigma factors in *Streptomyces* spp. *Frontiers in Microbiology*, 8(DEC), 2546. https://doi.org/10.3389/fmicb.2017.02546

Szafran, M. J., Gongerowska, M., Małecki, T., Elliot, M., & Jakimowicz, D. (2019). Transcriptional response of *Streptomyces coelicolor* to rapid chromosome relaxation or long-term supercoiling imbalance. *Frontiers in Microbiology*, 10, 1605. https://doi.org/10.3389/fmicb.2019.01605

Takano, E., Chakraburtty, R., Nihira, T., Yamada, Y., & Bibb, M. J. (2001). A complex role for the $\gamma$-butyrolactone SCB1 in regulating antibiotic production in *Streptomyces coelicolor* A3(2). *Molecular Microbiology*, 41(5), 1015–1028. https://doi.org/10.1046/j.1365-2958.2001.02562.x

Takano, E., Nihira, T., Hara, Y., Jones, J. J., Gershater, C. J., Yamada, Y., & Bibb, M. (2000). Purification and structural determination of SCB1, a gamma-butyrolactone that elicits antibiotic production in *Streptomyces coelicolor* A3(2). *The Journal of Biological Chemistry*, 275(15), 11010–11016. https://doi.org/10.1074/jbc.275.15.11010

Tan, G.-Y., Bai, L., & Zhong, J.-J. (2013). Exogenous 1,4-butyrolactone stimulates A-factor-like cascade and validamycin biosynthesis in *Streptomyces hygroscopicus* 5008. *Biotechnology and Bioengineering*, 110(11), 2984–2993. https://doi.org/10.1002/bit.24965

Tanaka, Y., Izawa, M., Hiraga, Y., Misaki, Y., Watanabe, T., & Ochi, K. (2017). Metabolic perturbation to enhance polyketide and nonribosomal peptide antibiotic production using triclosan and ribosome-targeting drugs. *Applied Microbiology and Biotechnology*, 101(11), 4417–4431. https://doi.org/10.1007/s00253-017-8216-6

Tanaka, Y., Komatsu, M., Okamoto, S., Tokuyama, S., Kaji, A., Ikeda, H., & Ochi, K. (2009). Antibiotic overproduction by rpsL and rsmG mutants of various actinomycetes. *Applied and Environmental Microbiology*, 75(14), 4919–4922. https://doi.org/10.1128/AEM.00681-09

Tenson, T., Lovmar, M., & Ehrenberg, M. (2003). The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *Journal of Molecular Biology*, 330(5), 1005–1014. https://doi.org/10.1016/S0022-2836(03)00662-4

Traxler, M. F. & Kolter, R. (2015). Natural products in soil microbe interactions and evolution. *Natural Product Reports*, 32(7), 956–970. https://doi.org/10.1039/c5np00013k

van Bergeijk, D. A., Elsayed, S. S., Du, C., Santiago, I. N., Roseboom, A. M., Zhang, L., Carrión, V. J., Spaink, H. P., & van Wezel, G. P. (2022). The ubiquitous catechol moiety elicits siderophore and angucycline production in *Streptomyces*. *Communications Chemistry*, 5(1), 1–12. https://doi.org/10.1038/s42004-022-00632-4

van der Meij, A., Willemse, J., Schneijderberg, M. A., Geurts, R., Raaijmakers, J. M., & van Wezel, G. P. (2018). Inter- and intracellular colonization of Arabidopsis roots by endophytic actinobacteria and the impact of plant hormones on their antimicrobial activity. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 111(5), 679–690. https://doi.org/10.1007/s10482-018-1014-z

van der Meij, A., Worsley, S. F., Hutchings, M. I., & van Wezel, G. P. (2017). Chemical ecology of antibiotic production by actinomycetes. *FEMS Microbiology Reviews*, 41(3), 392–416. https://doi.org/10.1093/FEMSRE/FUX005

Wang, W., Ji, J., Li, X., Wang, J., Li, S., Pan, G., Fan, K., & Yang, K. (2014). Angucyclines as signals modulate the behaviors of *Streptomyces coelicolor*. *Proceedings of the National Academy of Sciences*, 111(15), 5688–5693. https://doi.org/10.1073/PNAS.1324253111

Wang, W., Zhang, J., Liu, X., Li, D., Li, Y., Tian, Y., & Tan, H. (2018). Identification of a butenolide signaling system that regulates nikkomycin biosynthesis in Streptomyces. *Journal of Biological Chemistry*, 293(52), 20029–20040. https://doi.org/10.1074/jbc.RA118.005667

Watrous, J., Roach, P., Alexandrov, T., Heath, B. S., Yang, J. Y., Kersten, R. D., van der Voort, M., Pogliano, K., Gross, H., Raaijmakers, J. M., Moore, B. S., Laskin, J., Bandeira, N., & Dorrestein, P. C. (2012). Mass spectral molecular networking of living microbial colonies. *Proceedings of the National Academy of Sciences of the United States of America*, 109(26), E1743–E1752. https://doi.org/10.1073/pnas.1203689109

Wilbanks, L. E., Hennigan, H. E., Martinez-Brokaw, C. D., Lakkis, H., Thormann, S., Eggly, A. S., Buechel, G., & Parkinson, E. I. (2023). Synthesis of gamma-butyrolactone hormones enables understanding of natural product induction. *ACS Chemical Biology*,18(7), 1624–1631. https://doi.org/10.1021/acschembio.3c00241

Xu, F., Nazari, B., Moon, K., Bushin, L. B., & Seyedsayamdost, M. R. (2017). Discovery of a cryptic antifungal compound from *Strepto-*

*myces albus* J1074 using high-throughput elicitor screens. *Journal of the American Chemical Society*, 139(27), 9203–9212. https://doi.org/10.1021/jacs.7b02716

Xu, G., Wang, J., Wang, L., Tian, X., Yang, H., Fan, K., Yang, K., & Tan, H. (2010). "Pseudo" γ-butyrolactone receptors respond to antibiotic signals to coordinate antibiotic biosynthesis. *Journal of Biological Chemistry*, 285(35), 27440–27448. https://doi.org/10.1074/jbc.M110.143081

Xu, G. & Yang, S. (2019). Regulatory and evolutionary roles of pseudo γ-butyrolactone receptors in antibiotic biosynthesis and resistance. In *Applied Microbiology and Biotechnology* (Vol. 103, Issues 23–24, pp. 9373–9378). Springer. https://doi.org/10.1007/s00253-019-10219-0

Yamada, Y., Sugamura, K., Kondo, K., Okada, H., & Yanagimoto, M. (1987). The structure of inducing factors for virginiamycin production in *Streptomyces virginias*. *The Journal of Antibiotics*, 40(4), 496–504. https://doi.org/10.7164/ANTIBIOTICS.40.496

Yang, Y. K., Shimizu, H., Shioya, S., Suga, K. -i., Nihira, T., & Yamada, Y. (1995). Optimum autoregulator addition strategy for maximum virginiamycin production in batch culture of *Streptomyces virginiae*. *Biotechnology and Bioengineering*, 46(5), 437–442. https://doi.org/10.1002/bit.260460507

Yoo, J. S., Oh, G. S., Ryoo, S., & Roe, J. H. (2016). Induction of a stable sigma factor SigR by translation-inhibiting antibiotics confers resistance to antibiotics. *Scientific Reports*, 6(1), 1–10. https://doi.org/10.1038/srep28628

Zarins-Tutt, J. S., Barberi, T. T., Gao, H., Mearns-Spragg, A., Zhang, L., Newman, D. J., & Goss, R. J. M. (2015). Prospecting for new bacterial metabolites: a glossary of approaches for inducing, activating and upregulating the biosynthesis of bacterial cryptic or silent natural products. *Natural Product Reports*, 33(1), 54–72. https://doi.org/10.1039/C5NP00111K

Zhang, C. & Seyedsayamdost, M. R. (2020). Discovery of a cryptic depsipeptide from *Streptomyces ghanaensis* via MALDI-MS-Guided high-throughput elicitor screening. *Angewandte Chemie International Edition*, 51(59), 22824. https://doi.org/10.1002/anie.202009611

Zhang, Y., Wang, M., Tian, J., Liu, J., Guo, Z., Tang, W., & Chen, Y. (2020). Activation of paulomycin production by exogenous γ-butyrolactone signaling molecules in *Streptomyces albidoflavus* J1074. *Applied Microbiology and Biotechnology*, 104(4), 1695–1705. https://doi.org/10.1007/s00253-019-10329-9

Zhou, S., Bhukya, H., Malet, N., Harrison, P. J., Rea, D., Belousoff, M. J., Venugopal, H., Sydor, P. K., Styles, K. M., Song, L., Cryle, M. J., Alkhalaf, L. M., Fülöp, V., Challis, G. L., & Corre, C. (2021). Molecular basis for control of antibiotic production by a bacterial hormone. *Nature*, 590(7846), 463–467. https://doi.org/10.1038/s41586-021-03195-x

Zhu, J., Sun, D., Liu, W., Chen, Z., Li, J., & Wen, Y. (2016). AvaR2, a pseudo γ-butyrolactone receptor homologue from *Streptomyces avermitilis*, is a pleiotropic repressor of avermectin and avenolide biosynthesis and cell growth. *Molecular Microbiology*, 102(4), 562–578. https://doi.org/10.1111/mmi.13479



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