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Review article

Towards the targeted activation of silent biosynthetic gene clusters by chemical elicitors

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ABSTRACT

Systematic genome mining has revealed that microbes encode numerous uncharacterized secondary metabolite biosynthetic gene clusters (BGCs). The efficient and selective activation of these silent or cryptic BGCs is crucial for the high-throughput discovery of novel natural products. Recent influential studies have demonstrated that using small chemical elicitors is a practical and cost-effective method to unlock the secondary metabolic potential of microbes. However, the current approach mainly relies on high-throughput, non-targeted screening methods to discover chemical elicitors capable of activating these silent BGCs. Therefore, this study comprehensively reviews reported cases of small molecules that activate silent BGCs, covering the chemical structures of elicitors, resulting natural products, and target BGCs, thereby constructing an integrated knowledge graph. We also summarize the underlying activation mechanisms. Leveraging relationships captured in this graph, we outline directions for targeted activation of silent pathways using small molecules, thereby facilitating more efficient natural product discovery.

1. Introduction

The discovery of novel natural products holds significant importance across various scientific fields due to their remarkable structural diversity, which leads to a wide range of biological activities and applications, including pharmaceuticals, food, cosmetics, and nutraceuticals. With the advancement of next-generation sequencing technologies and the development of computational genome mining, the discovery of natural products has entered a genome-driven era (Navarro-Muñoz et al., 2020; Ziemert et al., 2016). In this era, genomic data is leveraged to uncover the secondary metabolic potential of microorganisms, guiding the discovery of novel natural products (Harvey et al., 2015). Numerous genome mining studies have revealed that the majority of the biosynthetic gene clusters (BGCs) encoded by microorganisms remain untapped. For example, Gavriilidou et al. estimated that only about 3% of the natural products encoded in bacterial genomes were experimentally characterized (Gavriilidou et al., 2022). Our recent study indicated that 96.8% of the secondary metabolic potential in marine prokaryotes remains unexplored (Wei et al., 2023). The primary reason for this gap between the natural products that have been characterized and those identified through genome mining is that many BGCs are silent or expressed at low levels under laboratory conditions, thus limiting the production and characterization of these potential natural products (Xu et al., 2019).

Many strategies have been used to activate silent BGCs, leading to the discovery of novel secondary metabolites from diverse microorganisms. These strategies are broadly divided into two categories: stimulation and genetic activation (Baral et al., 2018; El-Hawary et al., 2023; Mao et al., 2018; Zong et al., 2022). Stimulation involves using external factors to activate silent BGCs without altering the organism's genetic composition. This category of strategies can be classified into three types: chemical elicitation, biological elicitation, and environmental perturbation. When microorganisms are grown in pure culture in vitro, many activating signals are absent, leading to the downregulation of specialized metabolite BGCs. To address this, co-culturing with competing species (biological elicitation) or adding chemical elicitors (chemical elicitation) can induce changes in BGC expression (Rutledge and Challis, 2015). Additionally, altering environmental conditions (environmental perturbation), such as nutrient levels, pH, or oxygen availability, can stimulate BGC activity (Romano et al., 2018). Genetic activation, on the other hand, directly manipulates genetic elements to induce BGC expression. Examples include manipulating global or

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pathway-specific regulators (Lu et al., 2017; Mao et al., 2017; Otur and Kurt-Kızıldoğan, 2024), reporter-guided mutant selection (Mao et al., 2018), refactoring (He et al., 2024), and heterologous expression (Kang and Kim, 2021). However, these approaches frequently mandate intricate manual design and often have low success rates, particularly in heterologous expression because compounds may be biosynthesized through the collective contribution of multiple gene clusters (van Bergeijk et al., 2020). By contrast, chemical elicitation offers simplicity, ease of operation, and good reproducibility. In recent years, high-throughput elicitor screening (HiTES) has been successfully employed to activate silent BGCs. HiTES can use various readouts, including transcriptional reporter assays (Xu et al., 2017), as well as genetics-free techniques such as bioactivity assays (bioactivity-HiTES) (Moon et al., 2019b), mass spectrometry detection (MS-coupled HiTES) (Covington and Seyedsayamdost, 2021), and fluorescence resonance energy transfer-based assays (FRET-HiTES) (Han et al., 2023), enabling efficient discovery of elicitors in a multi-well plate format. Over the past decade, chemical elicitation has gained widespread attention and application.

In this review, we define a focused scope on studies in which chemical elicitors were explicitly shown to activate silent or lowly expressed BGCs, leading to the production of cryptic natural products. We note that, in some cases, the addition of small molecules results in the discovery of new structural analogues rather than genuine activation of a silent BGC. In such scenarios, the supplemented compounds often function as metabolic precursors, thereby altering substrate availability within an already active pathway. For example, feeding *Streptomyces chartreusis* NRRL 3882 with 20 different proteinogenic amino acids revealed that calcimycin production and the distribution of its analogs are strongly dependent on amino acid supply, with glutamine yielding the highest calcimycin levels (Arend and Bandow, 2021). Additional examples of precursor-driven modulation of product biosynthesis have been reviewed elsewhere (Yan et al., 2024). These effects, while valuable for expanding chemical diversity, do not constitute activation of a transcriptionally silent BGC and are therefore not discussed further in this review.

While chemical elicitor activation is conceptually simple, the identification of effective elicitors remains a major challenge. Current strategies, such as HiTES, allow for efficient screening of large compound libraries but are largely empirical and lack a strong theoretical foundation. To address this limitation and enable more targeted elicitor selection, we first construct a comprehensive knowledge graph of known elicitors based on data curated from the literature, providing a structured knowledge base. In parallel, we review and generalize experimental paradigms for elucidating the regulatory mechanisms governing BGC expression, with an emphasis on omics-based regulatory networks. Building on these two foundations, we propose a set of guidelines to support the rational selection of small-molecule elicitors. Through this review, we aim to promote the targeted activation of silent BGCs and, ultimately, facilitate the discovery of novel natural products.

2. Overview of chemical elicitors in activating silent BGCs

Dozens of small molecules have been reported to activate silent or poorly expressed gene clusters in microorganisms, leading to the production of new natural products. Here, we construct a comprehensive knowledge graph comprising 32 BGCs, which can be activated or upregulated by 54 small-molecule elicitors, leading to the synthesis or increased production of 87 representative secondary metabolites (**Fig. 1** & Table S1). This knowledge graph was manually curated from published literature and designed as a conceptual integration framework. Specifically, elicitor–BGC–metabolite relationships were systematically extracted from peer-reviewed studies reporting experimentally validated activation or significant upregulation of silent or lowly expressed BGCs. Small-molecule elicitors were classified primarily based on their reported mechanisms of BGC activation. Accordingly, elicitors were grouped into three broad classes, including stress-associated elicitors, signaling-associated elicitors, and others that no clear mechanisms are discussed. Among these, stress-associated elicitors, representing the most extensively reported class, were further subdivided according to chemical nature and ecological context, including antibiotics, non-antibiotic clinical drugs, host-related molecules, and chemically modified molecules. This mechanism-oriented classification framework is intended to provide a conceptual guide for sourcing effective elicitors.

2.1. *Stress-associated elicitors*

Stress-associated elicitors refer to small molecules that activate silent or weakly expressed BGCs primarily by inducing physiological stress responses. In this context, elicitation arises from global or semi-global perturbations of cellular homeostasis, including disturbances to redox balance, DNA replication, transcription, translation or metabolic flux.

2.1.1. *Antibiotics*

Among stress-associated elicitors, antibiotics applied at sub-inhibitory concentrations represent one of the most extensively studied and experimentally validated classes. A series of HiTES-based studies by Seyedsayamdost group found that the majority of effective elicitors identified were antibiotics, highlighting their important role in activating silent biosynthetic pathways. For instance, the β-lactam piperacillin (**E1**) and dihydrofolate reductase inhibitor trimethoprim (**E2**) were found to activate the *mal* gene cluster in the model pathogen *Burkholderia thailandensis* E264. These compounds induced a 10–145-fold overproduction of malleilactone A (**C1**) and facilitated the discovery of a new analog, malleilactone B (**C2**). In addition to the *mal* gene cluster, trimethoprim, as a global activator, also activated at least four biosynthetic pathways. For example, it activated the *tha* gene cluster of *B. thailandensis* E264, leading to the production of thailandamide A (**C3**). Additionally, it induced the *hmq* gene cluster, leading to a sevenfold increase in the yield of the quinolone antibiotic and quorum-sensing (QS) modulator 4-hydroxy-3-methyl-2-(2-nonenyl)-quinoline (HMNQ, **C4**). Trimethoprim, as well as the *Streptomycete*-derived antitumor antibiotic drug mitomycin (**E3**) and the antimicrobial molecules flumequine (**E4**), were found to activate the *bhc* gene cluster in *B. thailandensis* E264, leading to the production of burkholdac A (**C5**), a histone deacetylase inhibitor (**Fig. 2**) (Seyedsayamdost, 2014).

A follow-up study found that trimethoprim triggered the production of over 100 compounds in *B. thailandensis* E264 that are not observed under standard growth conditions. Among these, 40 compounds were successfully identified using MS/MS and NMR, including a group of new molecules named acybolins (**C6**-**C14**), which originate from the *bta* gene cluster (**Fig. 2**) (Okada et al., 2016). Acybolins are bivalent metabolites composed of a bactobolin antibiotic scaffold covalently linked to an acyl chain derived from QS acyl-homoserine lactones (AHLs), a connection that requires a ligase not encoded within the *bta* cluster. Bactobolin itself is produced by the *bta* cluster and has been shown to be strictly regulated by AHL-mediated QS (Seyedsayamdost et al., 2010). By contrast, the combined presence of AHLs and trimethoprim elicits the formation of acybolins. This represents a distinct case in which antibiotic-induced stress reshapes the metabolic output of a known BGC, enabling the emergence of cryptic metabolites.

Beyond *B. thailandensis* E264, subinhibitory exposure to antibiotics can also activate silent or lowly expressed BGCs in diverse microbial strains. Notably, several effective elicitors identified to date are antibiotics derived from *Streptomyces*. For instance, subinhibitory streptomycin (**E5**), an aminoglycoside antibiotic, induced the *tam* gene cluster in *Microbispora* sp. BCCAGE54, promoting a 33-fold enhancement in tetarimycin B (**C15**) production alongside several putative new tetarimycin analogs (Covington et al., 2018). Although tetarimycin B was detectable at low levels in unstimulated cultures, the corresponding gene cluster is therefore considered to be lowly expressed here. Similarly, actinomycin D (**E6**), a chemotherapeutic agent, was found through

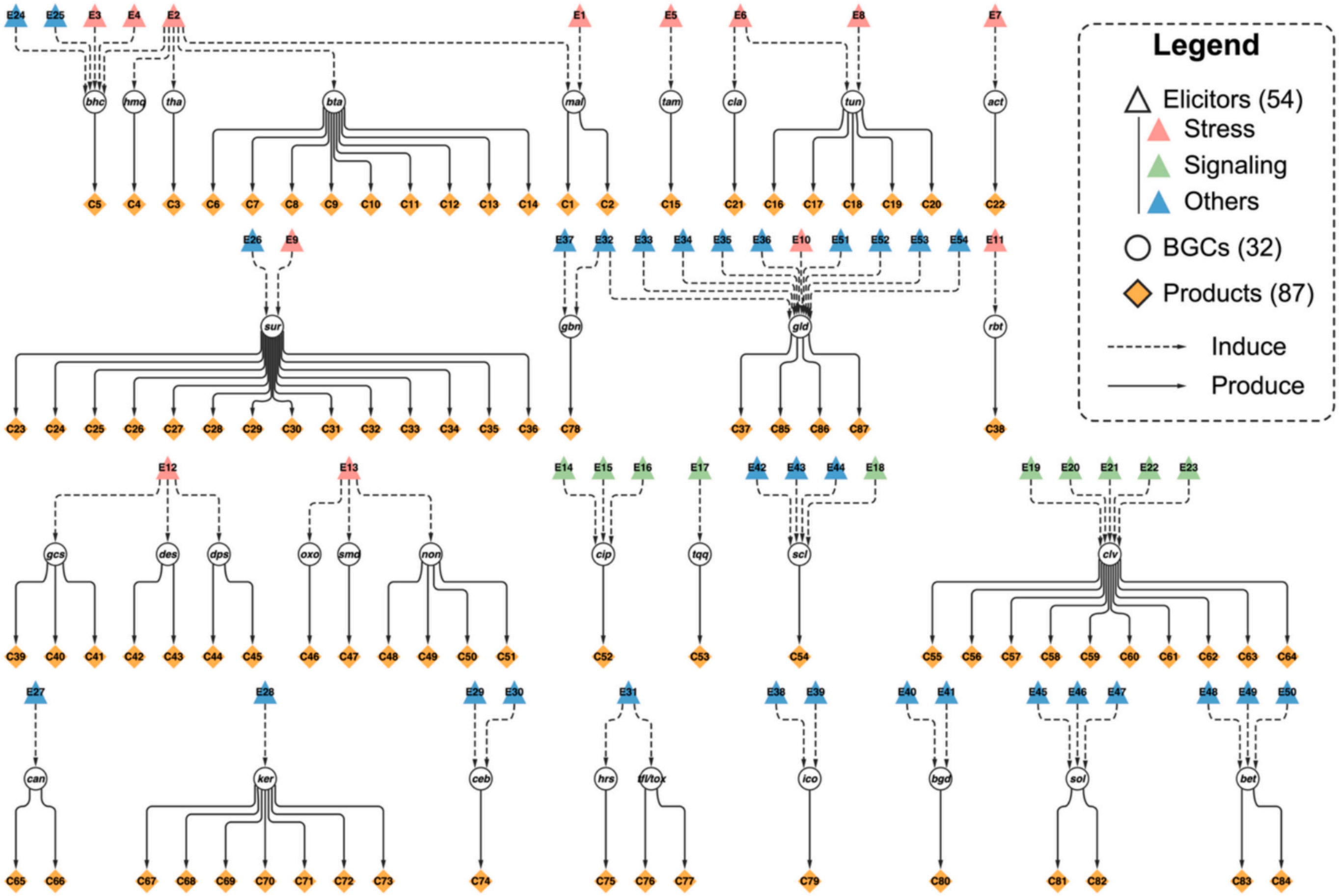


Fig. 1. A knowledge graph integrating reported chemical elicitors (triangles, $n = 54$, labeled as E1–E54), their induced BGCs (circles, $n = 32$), and metabolic products (diamonds, $n = 87$, labeled as C1–C87), corresponding to entries in Table S1. Elicitors are color-coded by category (stress-associated, signaling-associated, and others). Dashed arrows indicate induction relationships, whereas solid arrows denote biosynthetic relationships.

HiTES combined with cytotoxicity assays to activate the *tun* gene cluster in *Streptomyces clavuligerus* ATCC 27064, thereby enhancing the production of streptovirudin A2 (**C16**) and tunicamycins IIA (**C17**), IIB (**C18**), G (**C19**), and V (**C20**). Actinomycin D also activated the *cla* gene cluster in the same strain, leading to the production of clavorubin A (**C21**) (Han et al., 2022). In addition, subinhibitory lincomycin (**E7**) potentiated the *act* gene cluster in *Streptomyces coelicolor* A3(2) and *Streptomyces lividans* 1326, resulting in pronounced overproduction of the blue-pigmented antibiotic actinorhodin (**C22**), which is produced at low levels under standard conditions. Additionally, *S. lividans* 1326 produced several potential novel congeners of calcium-dependent antibiotics (CDAs) only under lincomycin exposure (Imai et al., 2015).

2.1.2. Non-antibiotic clinical drugs

In addition to antibiotics, a broad range of non-antibiotic small molecules can also impose physiological stress on microbial cells. Notably, bioactive compounds that are already used clinically are particularly enriched in effective stress-associated elicitors, likely owing to their well-defined molecular targets and pronounced cellular effects. For example, pyronaridine (**E8**), an antimalarial drug, primarily targets nucleic acids by intercalating into DNA, thereby inducing DNA damage and triggering the SOS and/or oxidative stress. Such stress responses were shown to activate the *tun* gene cluster in *S. clavuligerus* ATCC 27064, as described previously. Similarly, etoposide (**E9**), a chemotherapeutic agent with broad-spectrum antiparasitic activity, has been shown to inhibit bacterial, and particularly actinobacterial, DNA gyrase, thereby inducing the SOS response. Through this stress pathway, etoposide acts as a potent elicitor in *Streptomyces albus* J1074, strongly activating the *sur* gene cluster and resulting in the discovery of seven surugamides and seven albuquinones (**C23–C36**) (**Fig. 3 A**) (Xu et al., 2017).

Spermidine (**E10**) was found to activate the *gld* gene cluster in *Burkholderia gladioli* ATCC 10248, resulting in enhanced production of the siderophore gladiobactin (**C37**). Spermidine is a key component of the intracellular polyamine pool, and its concentration balance with other polyamines, such as putrescine, is vital for microbial physiological state and metabolic regulation. At elevated concentrations, spermidine inhibited the growth of *B. gladioli*, indicative of a stress response that may underpin enhanced gladiobactin biosynthesis. Notably, siderophore production to sequester toxic metals is often a hallmark of oxidative stress response, and high spermidine level has been reported to trigger oxidative stress in *E. coli* (Yoshimura et al., 2020).

2.1.3. Host-related molecules

From an ecological perspective, microorganisms are constantly exposed to a wide range of host-derived metabolites that shape their physiological states and metabolic behaviors. These compounds, produced by plants or animals in shared ecological niches, can act as chemical cues or stress signals that influence microbial secondary metabolism. Among such host-related molecules, *p*-coumaric acid (**E11**), a ubiquitous plant-derived phenolic compound, has emerged as a representative elicitor capable of activating cryptic metabolites. Algal *p*-coumaric acid was shown to activate the *rbt* gene cluster in its symbiotic bacterium *Phaeobacter inhibens* 2.10, leading to the discovery of an unreported siderophore, roseobactin (**C38**) (**Fig. 3B**). *p*-coumaric acid is a senescence-associated molecule produced by the microalgal *Emiliania*

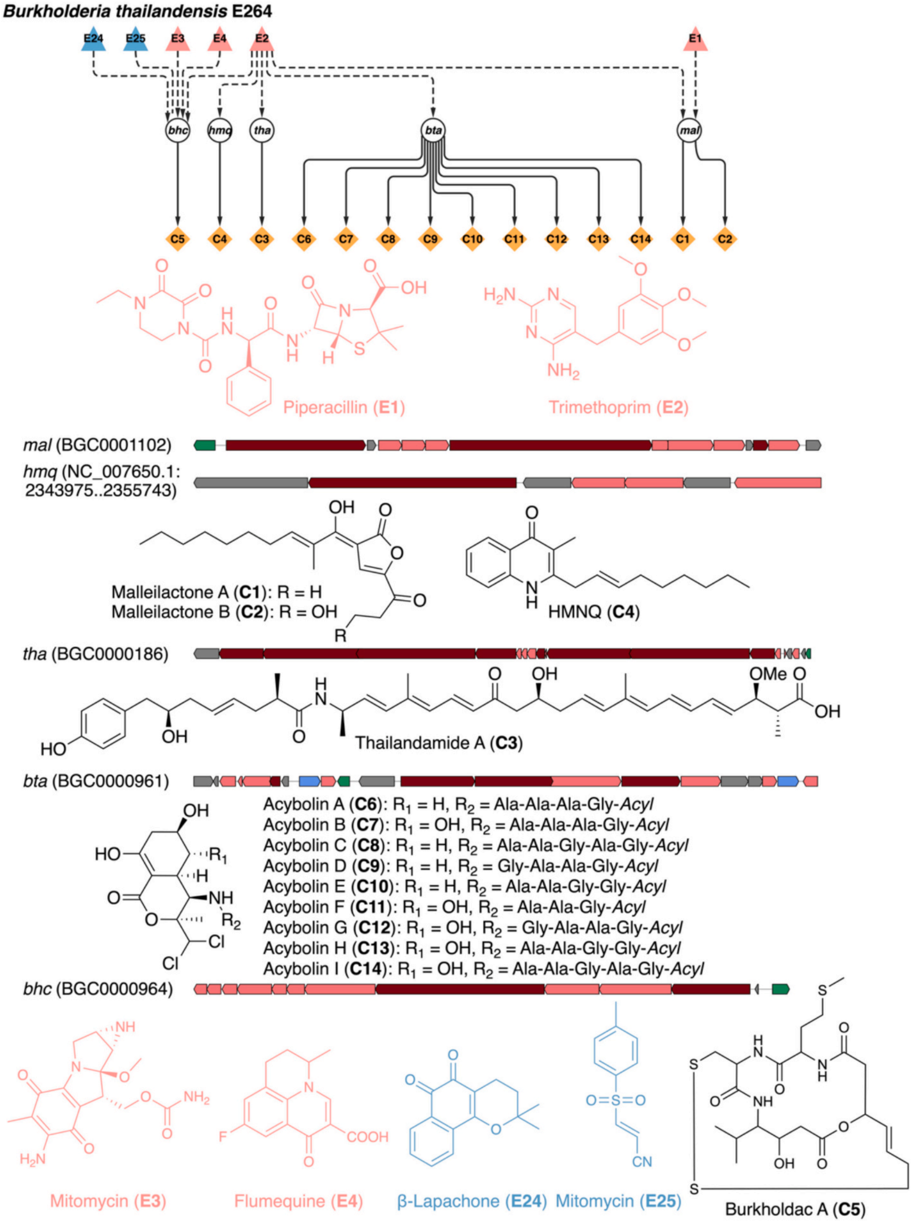


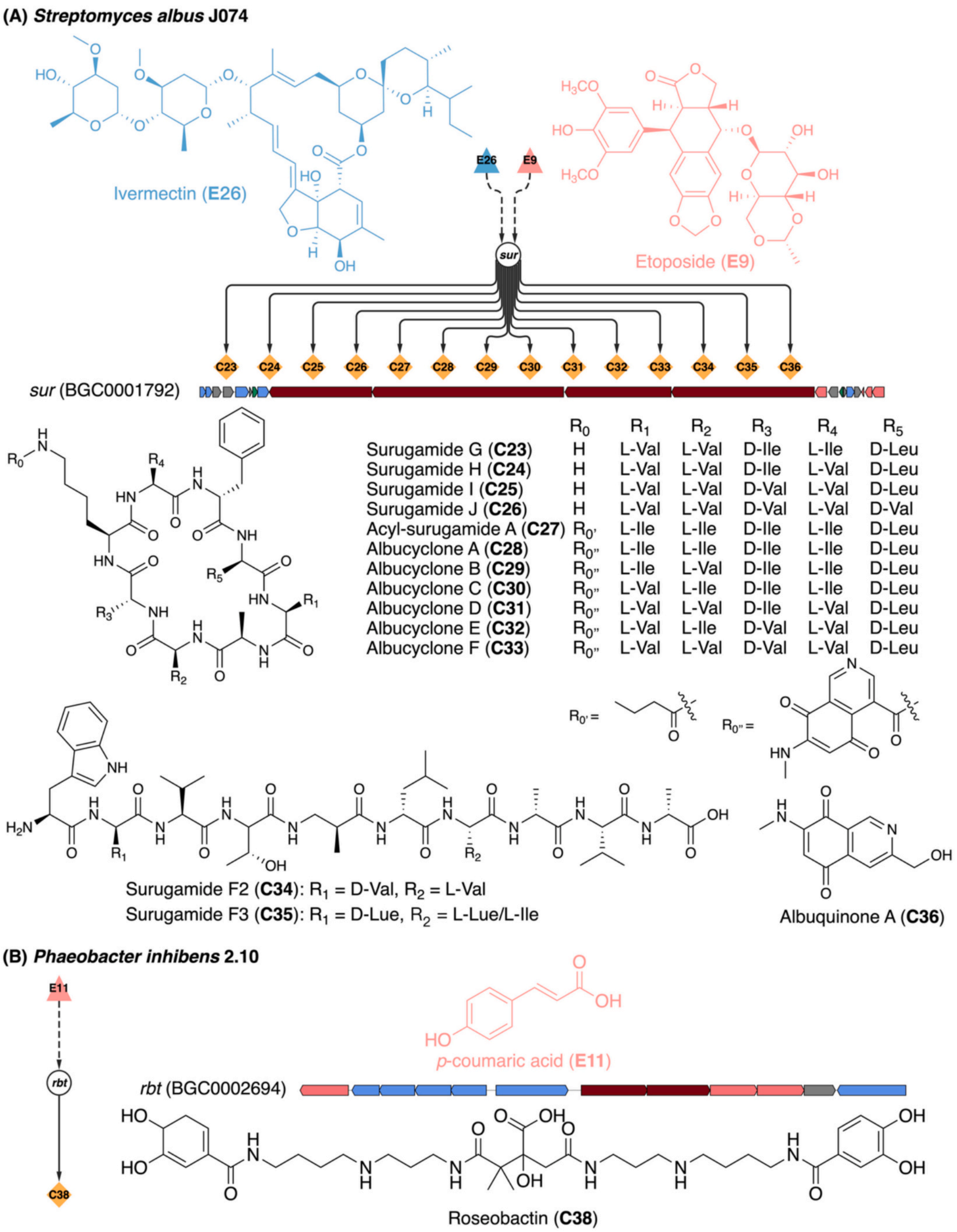
Fig. 2. A representative knowledge graph of stress-associated antibiotic elicitors with their induced BGCs and products. Piperacillin (**E1**), trimethoprim (**E2**), mitomycin (**E3**) and flumequine (**E4**) partially and collectively activate five BGCs in *B. thailandensis* E264 and lead to the production or overproduction of 14 secondary metabolites (**C1**–**C14**).

huxleyi, and its accumulation marks a shift in microalgal-bacterial symbioses from mutualistic to parasitic phases. Meanwhile, *p*-coumaric acid is toxic to the bacteria at high concentrations but induces oxidative stress response by formation of reactive oxygen species (ROS) and secondary metabolism at sub-lethal doses. These findings indicate that algal-derived phenylpropanoids, including *p*-coumaric acid as well as other compounds such as sinapic acid and ferulic acid, can induce cryptic secondary metabolism in symbiotic bacteria, underscoring the potential of ecological interactions for elicitor discovery (Wang et al., 2022a).

2.1.4. Chemically modified molecules

Chemically modified elicitors represent a class of molecules that are generated through chemical modification of known bioactive compounds, often in the form of structural analogues. Rather than originating from natural or clinical contexts, these molecules are typically derived from pre-existing scaffolds and tailored to modulate microbial physiology. Importantly, this class of elicitors can be explored through rational design strategies, such as systematic structural optimization, to identify analogues with enhanced elicitor potency or selectivity.

A representative example is the synthetic elicitor ARC2 (**E12**), an analogue of triclosan with a related mode of action. ARC2 activates the production of multiple specialized metabolites in *Streptomyces*. For instance, it activates the *gcs* gene cluster in *Streptomyces coelicolor* M145, resulting in a 3-fold increase in germicidins A–C (**C39–C41**) while decreasing the yields of prodiginines and the daptomycin-like CDA (**Fig. 4**). ARC2 also activates the *des* and *dps* gene clusters in *Streptomyces pristinaespiralis* ATCC 25486 and *Streptomyces peucetius* 27,952, enhancing the production of desferrioxamine B (**C42**) and E (**C43**) as well as doxorubicin (**C44**) and baumycin (**C45**) (Craney et al., 2012). Although ARC2 exhibits dose-dependent antibacterial toxicity at high concentrations, it primarily inhibits the enoyl reductase FabI, thereby blocking fatty acid biosynthesis. Inhibition of FabI induces a cellular stress response that depends on the global regulators AfsR and AfsS,



	R_0	R_1	R_2	R_3	R_4	R_5
Surugamide G (**C23**)	H	L-Val	L-Val	D-Ile	L-Ile	D-Leu
Surugamide H (**C24**)	H	L-Val	L-Val	D-Ile	L-Val	D-Leu
Surugamide I (**C25**)	H	L-Val	L-Val	D-Val	L-Val	D-Leu
Surugamide J (**C26**)	H	L-Val	L-Val	D-Val	L-Val	D-Val
Acyl-surugamide A (**C27**)	$R_{0'}$	L-Ile	L-Ile	D-Ile	L-Ile	D-Leu
Albucyclone A (**C28**)	$R_{0''}$	L-Ile	L-Ile	D-Ile	L-Ile	D-Leu
Albucyclone B (**C29**)	$R_{0''}$	L-Ile	L-Val	D-Ile	L-Ile	D-Leu
Albucyclone C (**C30**)	$R_{0''}$	L-Val	L-Ile	D-Ile	L-Ile	D-Leu
Albucyclone D (**C31**)	$R_{0''}$	L-Val	L-Val	D-Ile	L-Val	D-Leu
Albucyclone E (**C32**)	$R_{0''}$	L-Val	L-Ile	D-Val	L-Val	D-Leu
Albucyclone F (**C33**)	$R_{0''}$	L-Val	L-Val	D-Val	L-Val	D-Leu

Fig. 3. A representative knowledge graph of stress-associated non-antibiotic clinical drugs (**A**) and host-related molecules (**B**) with their induced BGCs and products. Etoposide (**E9**) and *p*-coumaric acid (**E11**) activate the *sur* and *rbt* gene clusters, leading to the production of surugamides (**C23**–**C36**) and roseobactin (**C38**), respectively.

linking ARC2-mediated stress to the activation of secondary metabolism (Calvelo et al., 2021a).

Building on this scaffold, Cl-ARC (E13), a chemically modified derivative of ARC2, was shown to selectively enhance yields of secondary metabolites but has relatively little effect on the rest of the metabolomic profile. Because its molecular target, FabI, is highly conserved across Actinobacteria, Cl-ARC functions as a broadly effective elicitor in most *Streptomyces* species. Specifically, Cl-ARC activates the *oxo*, *smd*, and *non* gene clusters, increasing the yields of oxohygrolidin (C46) from *S. ghanaensis* ATCC 14672, 9-methylstreptimidone (C47) from *S. hygroscopicus* ATCC 53653, and nonactin (C48), monactin (C49), dinactin (C50), and trinactin (C51) from strain WAC0256 (Pimentel-Elardo et al., 2015).

2.2. Signaling-associated elicitors

Signaling-associated elicitors represent a distinct class of elicitors that induce silent or weakly expressed BGCs by mimicking or transmitting ecologically relevant signals rather than imposing physiological stress. These elicitors are often derived from host organisms or neighboring species and are sensed through dedicated regulatory systems, enabling microorganisms to coordinate secondary metabolism with specific ecological contexts.

Zhang et al. utilized HiTES combined with matrix-assisted laser

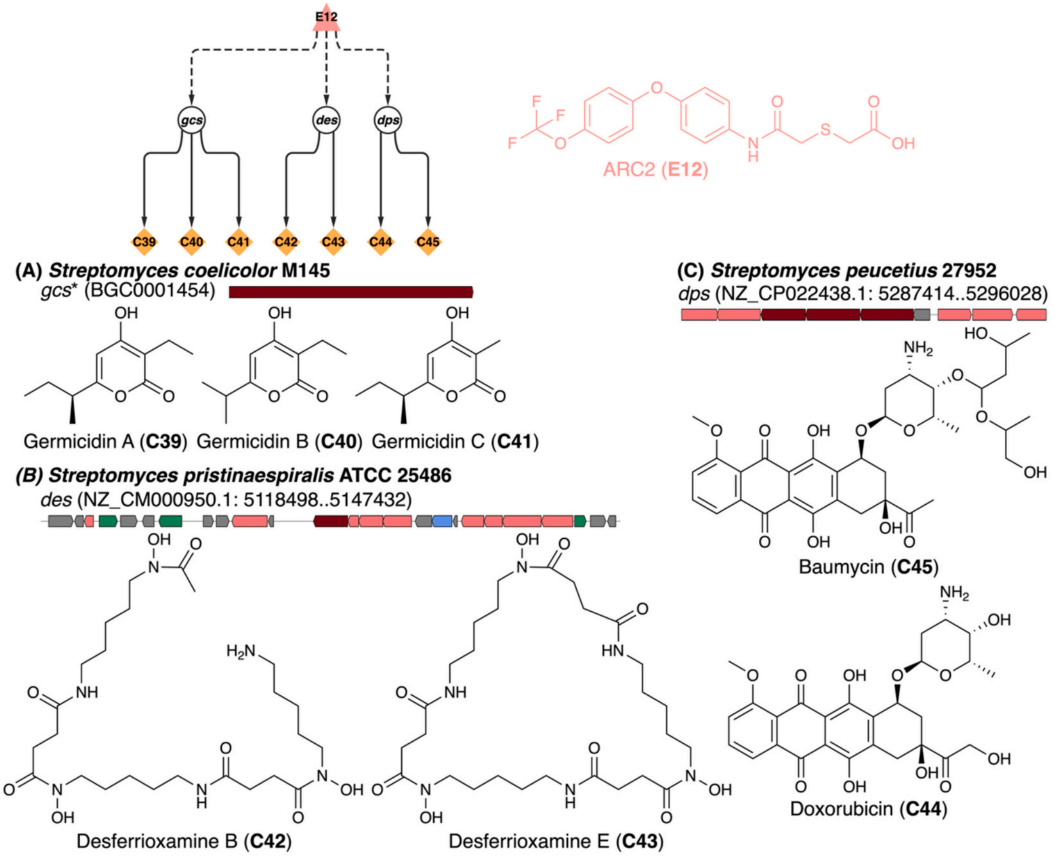


Fig. 4. A representative knowledge graph of stress-associated chemically modified molecules with their induced BGCs and products. ARC2 (**E12**) activates three BGCs and lead to the production of seven secondary metabolites (**C39–C45**).

desorption/ionization mass spectrometry (MALDI-HiTES) and revealed that plant-derived amygdalin (**E14**), sarsasapogenin (**E15**), and homobutein (**E16**) in flask cultures of *Streptomyces ghanaensis* ATCC 14672 activated the *cip* gene cluster, resulting in a 4–10-fold increase in production of a novel non-ribosomal peptide, cinnapeptin (**C52**) (Zhang and Seyedsayamdost, 2020). In a related study, Li et al. demonstrated that two adjacent LuxR-type regulators in the *gla* cluster from *Streptomyces globisporus* C-1027, which is homologous to the *cip* cluster and also directs the biosynthesis of cinnamoyl moiety-containing compounds, function as cluster-situated positive regulators (Li et al., 2022). Notably, homologous LuxR-type regulators are also present in *cip* cluster. Based on these observations, we speculated that, as a plant-associated bacterium, *S. ghanaensis* may sense these plant-derived metabolites via these two LuxR-type regulators, thereby relieving repression of the silent BGC and initiating cinnapeptin biosynthesis as an ecologically grounded signaling response. However, this hypothesis requires further experimental validation.

Vitamin B3 niacin (**E17**) was identified as a highly specific elicitor of the *tqq* gene cluster in *Streptococcus suis* ATCC 43765, whose activation led to the discovery of threoglucin A (**C53**), characterized by an aliphatic ether bond forming a substituted 1,3-oxazinane heterocycle within the peptide backbone (Covington and Seyedsayamdost, 2022). Previous evidence has indicated that *Streptococcus* species are capable of responding to extracellular niacin. In particular, the presence of NiaR has been identified in *S. suis*. NiaR is a niacin-responsive transcriptional repressor that regulates genes involved in nicotinamide adenine dinucleotide (NAD) metabolism (Rodionov et al., 2008). NiaR-mediated regulation links extracellular niacin availability to the coordinated expression of multiple genes involved in cellular metabolism (Afzal et al., 2017). From an ecological perspective, *S. suis* is a taxing agricultural pathogen and zoonotic agent, whereas niacin is an endogenous human metabolite. Therefore, niacin is likely an environmental signal that *S. suis* readily encounters and may use to modulate metabolic processes. Additionally, in the genomic context, the *tqq* gene cluster is located adjacent to an shp/rgg QS operon, suggesting that QS–related regulatory mechanisms may also be involved (Covington and Seyedsayamdost, 2022).

Using Agar-HiTES, Lee et al. identified several steroids as the most effective elicitors in the plant fungal pathogen *Sclerotinia sclerotiorum* Ss-1. Among these, the glucocorticoid prednisone (**E18**) specifically activated the *scl* gene cluster, leading to the production of a previously uncharacterized metabolite, sclerocyclane (**C54**). Further studies revealed that the plant-derived steroids β-sitosterol and brassinolide, which are structurally similar to prednisone, also function as effective elicitors. Concurrently, the induced metabolite sclerocyclane exhibits antibiotic activity against *Burkholderia plantarii*, an ecological competitor of *S. sclerotiorum*. These findings suggest that secondary metabolites may be induced in regions of active plant growth where steroids, acting as host- or environment-associated signaling molecules, are released, thereby shaping ecological interactions (Lee and Seyedsayamdost, 2022).

Similarly, Han et al. applied FRET-HiTES to *Streptomyces clavuligerus* ATCC 27064 and discovered that several steroids, including testosterone (**E19**), estrone (**E20**), cortisone (**E21**), danazol (**E22**), and 11α-hydroxyprogesterone (**E23**), activated the *clv* gene cluster, leading to the production of cryptic enediyne natural products, termed clavulynes A – J (**C55–C64**) (Fig. 5). This study suggests that steroids can play important roles in bacteria, functioning as signaling agents or carbon sources. Elicitation in *S. clavuligerus* may involve regulation of signal transduction pathways and/or an additional nutrient supply (Han et al., 2023).

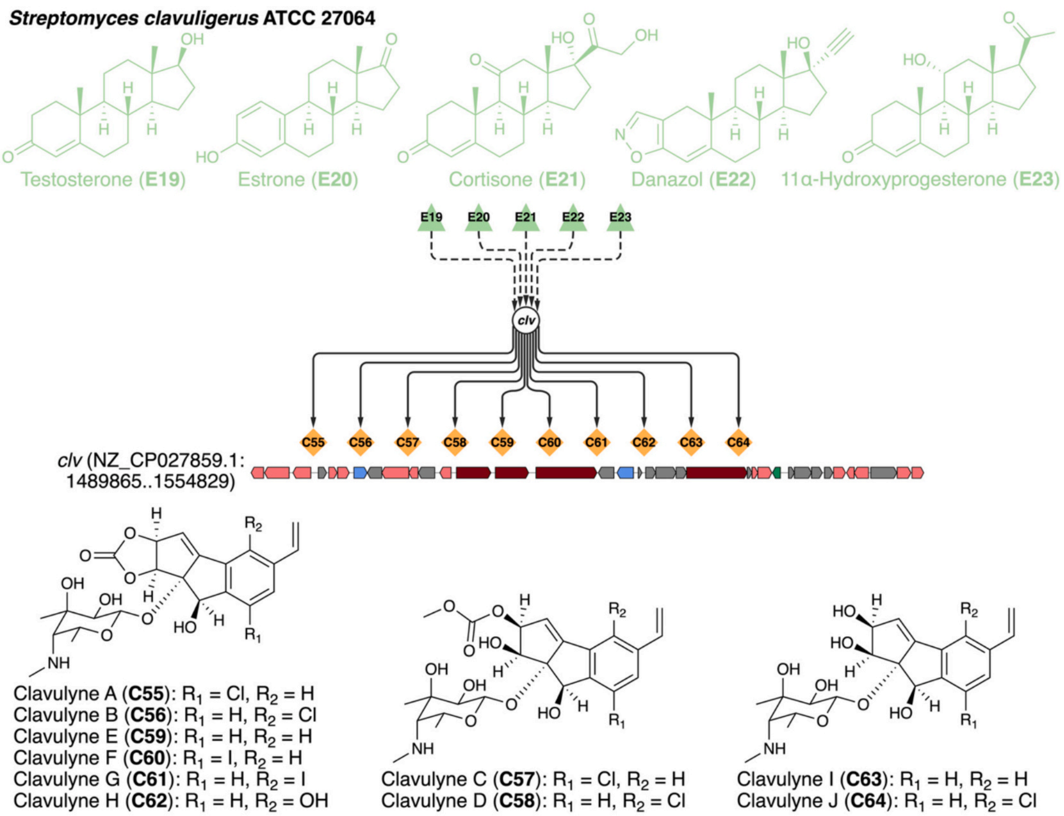


Fig. 5. A representative knowledge graph of signaling-associated elicitors with their induced BGCs and products. Five steroids (**E19–E23**) activate the *clv* gene cluster and lead to the production of clavulynes (**C55–C64**).

2.3. Others

Beyond the elicitors discussed above, many additional bioactive compounds have been reported to activate cryptic BGCs, although their mechanisms of action remain largely unexplored. A growing number of studies have demonstrated that diverse bioactive small molecules, many of which are clinically used drugs, can function as effective elicitors for cryptic BGCs when interrogated using HiTES-based strategies.

Using HiTES, Seyedsayamdost first showed that in *Burkholderia thailandensis E264*, the *bhc* gene cluster could be activated by the DNA topoisomerase I inhibitor β-lapachone (**E24**), and the nuclear factor-kappa B (NF-κB) inhibitor BAY 11–7082 (**E25**), leading to efficient synthesis of burkholdac A (**C5**) (Seyedsayamdost, 2014).

Subsequently, Xu et al. employed a genetics-dependent reporter-based HiTES to demonstrate that the broad-spectrum antiparasitic ivermectin (**E26**) strongly activated the *sur* gene cluster in *S. albus* J1074, leading to the production of surugamides and albuquinones (**C23–C36**) (Xu et al., 2017). They later developed a genetics-free strategy, HiTES in conjunction with imaging mass spectrometry (IMS-HiTES), to identify additional elicitors. Using this approach, kenpaullone (**E27**), a GSK3β inhibitor used for the treatment of chronic immune (idiopathic) thrombocytopenia, was identified as the most effective elicitor of the *can* gene cluster in *Streptomyces canus* NRRL B3980, inducing two novel cryptic lassopeptides, canucins A (**C65**) and B (**C66**). In contrast, dihydroergocristine (**E28**), a γ-secretase inhibitor clinically used to delay progressive mental decline in conditions such as Alzheimer's disease, activated the ker gene cluster in *Amycolatopsis keratiniphila* NRRL B24117, resulting in the production of seven glycopeptides, including four keratinimicins (**C67–C70**) and three keratinicyclins (**C71–C73**) (Xu et al., 2019).

Using bioactivity-coupled HiTES, Moon et al. identified the clinical diuretic furosemide (**E29**) and the cholesterol-lowering agent fenofibrate (**E30**) as the most effective elicitors for *Saccharopolyspora cebuensis* SPE 10–1. Both compounds activated the *ceb* gene cluster, leading to the production of a novel lanthipeptide, cebulantin (**C74**), which exhibited selective antibacterial activity against Gram-negative bacteria (Moon et al., 2019a). Using the same approach, Moon et al. identified the β-blocker atenolol (**E31**) as an effective global elicitor for *Streptomyces hiroshimensis* A18. Atenolol activated the *hrs* and *tfl/tox* gene clusters, thereby inducing the production of a novel naphthoquinone epoxide hiroshidine (**C75**), a new toxoflavin-type analog taylorflavin A, (**C76**), and several known metabolites, including taylorflavin B (**C77**). Notably, both taylorflavins exhibited selective growth-inhibitory activity against Gram-negative bacteria, particularly *Escherichia coli* and *Acinetobacter baumannii* (Moon et al., 2019b).

To streamline the analysis of HiTES-derived metabolomics data, Covington et al. developed MetEx, a metabolomics explorer for LC–MS-based HiTES workflows. Application of MetEx to *Burkholderia gladioli* ATCC 10248 enabled identification of multiple bioactive elicitors. Specifically, the β-lactam antibiotic oxacillin (**E32**), the calcium-channel blocker verapamil (**E33**), the antihypertension agent methyldopa (**E34**), the imidazoline decongestant naphazoline (**E35**), and the anti-inflammatory drug nabumetone (**E36**) were found to activate the *gld* gene cluster, leading to the production of gladiobactin (**C37**). The oxacillin (**E32**) and the chemotherapeutic agent azacitidine (**E37**) activated the *gbn* gene cluster, inducing gladiolin (**C78**) biosynthesis. Meanwhile, anticancer agents bleomycin (**E38**) and carboplatin (**E39**) activated the *ico* gene cluster, strongly enhancing icosalide (**C79**) production. Finally, the synthetic corticosteroid desoximetasone (**E40**) and the HIV protease inhibitor ritonavir (**E41**) activated the *bgd* gene cluster, serving as potent elicitors for burriogladin A (**C80**) (Covington and Seyedsayamdost, 2021).

Lee et al. extended the HiTES to solid media by developing Agar-HiTES, enabling systematic elicitor screening in fungi and bacteria. Using this approach, they identified the antidepressant isocarboxazid (**E42**), and the antibiotics paromomycin (**E43**) and sulfanilamide (**E44**) as the most effective elicitors of the plant fungal pathogen *Sclerotinia sclerotiorum* Ss-1, where these compounds activated the *scl* gene cluster and induced the production of a novel metabolite sclerocyclane (**C54**). In *Rhizoctonia solani* AG-3, the calcium-channel blockers nisoldipine (**E45**) and nifedipine (**E46**), together with the antifungal clotrimazole (**E47**), potently activated the *sol* gene cluster, with nisoldipine yielding 12-fold overproduction of two new siderophores, solanibactins A and B (**C81–C82**) (Lee and Seyedsayamdost, 2022). Applying Agar-HiTES to *Burkholderia* species, Lee et al. further showed that in *B. plantarii* ATCC 43733, plant-derived tropane alkaloids, including ipratropium bromide (**E48**) and atropine (**E50**), as well as the antimigraine drug zolmitriptan (**E49**), robustly induced the *bet* cluster, leading to 12–15-fold enhanced production of burkethyl A (**C83**) and B (**C84**) at 90 μM ipratropium bromide. In *B. gladioli* ATCC 10248, chemically diverse elicitors, including the synthetic steroid fluocinolone (**E51**), the antibiotics imipenem (**E52**) and rifapentine (**E53**), and the HIV protease inhibitor saquinavir (**E54**), markedly altered secondary metabolism and triggered the production of modified gladiobactins [hydroxylated B/C (**C85-C86**) and glycosylated D (**C87**)] (Lee et al., 2025).

3. Mechanisms of elicitor-mediated BGC activation

A comprehensive understanding of the regulatory mechanisms underlying BGCs is essential for the targeted activation of silent clusters using chemical elicitors. Because BGC expression is typically orchestrated by complex, multilayered regulatory networks, network-based models provide a systematic framework for deciphering these processes. Such models can illuminate the interactions among diverse regulators and thereby offer theoretical guidance for designing effective elicitor-driven strategies to activate silent BGCs. In this section, we outline the principles of regulatory networks and discuss their utility in elucidating elicitor-mediated BGC activation.

3.1. *Regulatory network models of BGC expression*

BGC expression is governed by intricate regulatory networks, which encompass interactions among DNA, RNA, proteins, and small molecules. The collective set of these interactions is often referred to as the interactome (Fig. 6A) (Luck et al., 2016; Sanchez et al., 1999). Among them, the typical gene regulatory network (GRN) models, transcription factor–target gene (TF–TG) interactions, which are often visualized as directed graphs where nodes represent genes or proteins and edges represent regulatory relationships (Fig. 6B) (Babu et al., 2004; Seshasayee et al., 2006). However, TF-based models alone cannot fully capture the complexity of BGC regulation, since gene expression is also influenced by epigenetic modifications (EMs), post-transcriptional regulation (PTR) [such as RNA-RNA interactions (RRIs)] (Silverman and Melamed, 2025), post-translational modifications (PTMs) [such as protein-protein interactions (PPIs)] (Wang et al., 2022b), and other regulatory pathways. Extended GRNs therefore integrate additional regulatory components, including independently acting molecules such as cofactors, sigma factors, small RNAs (sRNAs) (Sesto et al., 2013; Waters and Storz, 2009), as well as the *cis*-regulatory elements (CREs) and riboswitches embedded within TGs or mRNA. In addition, extended GRNs encompass regulatory components associated with modification-based mechanisms, which commonly involve EMs, PTR, and PTMs (Fig. 6B). Together, these regulatory components provide a more complete view of BGC control (Augustijn et al., 2024; Badia-i-Mompel et al., 2023).

GRNs typically exhibit a modular structure, consisting of recurring regulatory motifs (e.g., feed-forward loops [FFLs], single-input modules [SIMs], multi-input modules [MIMs]) as well as higher-order functional modules (e.g., stress response, metabolism). Global regulators (GRs) govern broad cellular functions, whereas cluster-situated regulators (CSRs) fine-tune the expression of individual BGCs. Additional layers of regulation, including various post-transcriptional and post-translational mechanisms, further contribute to the dynamic and multilayered nature of GRNs (Fig. 6B). Recent advances have facilitated the integration of GRN concepts into BGC studies. For instance, the antiSMASH7 now includes a transcription factor binding site (TFBS) prediction module, enabling the identification of key regulatory elements (Blin et al., 2023). Likewise, a recent review by Wezel and Medema emphasized the potential of GRN-based approaches to accelerate natural product discovery in Actinobacteria (Augustijn et al., 2024). Collectively, applying GRN models is expected to provide a rational framework for systematically analyzing BGC regulation and for guiding the selection of effective elicitors.

3.2. *Deciphering elicitor-mediated BGC activation through regulatory networks*

As outlined in Section 3.1, activation of BGC expression can be

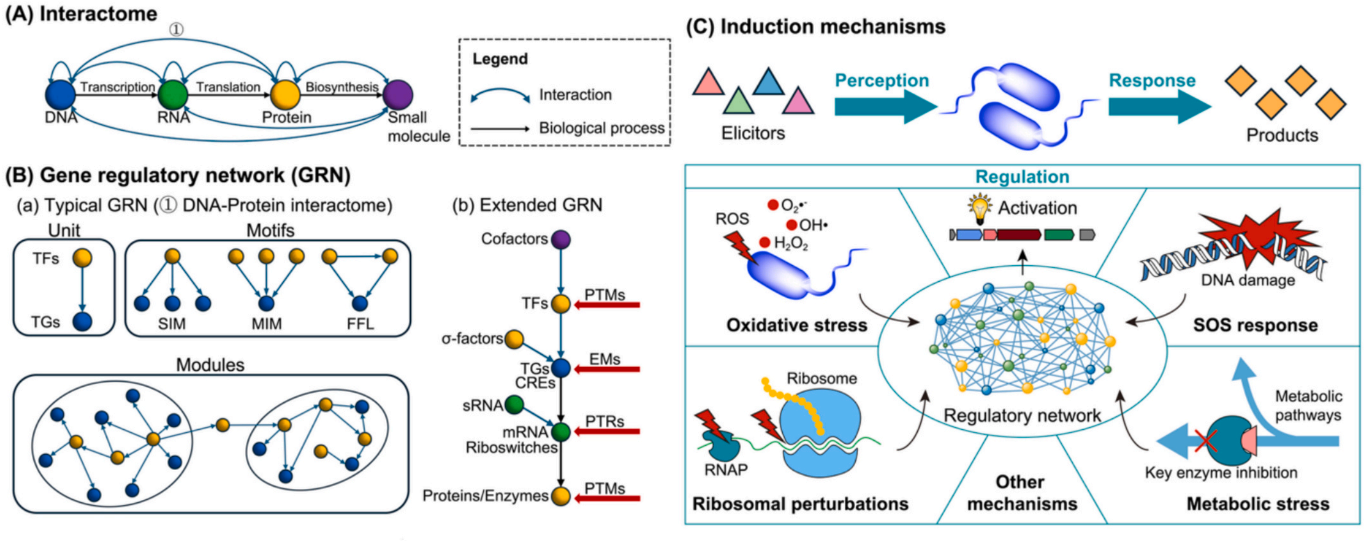


Fig. 6. Conceptual frameworks for regulatory and induction mechanisms governing BGC expression. (**A**) Key cellular components and their interactions. (**B**) A typical TF-centered GRN and an extended GRN incorporating multi-level regulatory mechanisms. (**C**) Major classes of elicitor-induced regulatory mechanisms underlying BGC activation.

systematically interpreted within the framework of regulatory networks. Based on existing studies, we summarize four major classes of elicitor-associated regulatory mechanisms, including oxidative stress–driven regulation, SOS response–mediated regulation, ribosomal perturbation–driven regulation, and metabolic stress-driven regulation (Fig. 6C). Although the molecular targets of these elicitors vary widely, their effects are ultimately perceived and processed through specific regulatory pathways, which converge on gene regulatory networks to activate the corresponding BGCs. It should be noted that the depth of mechanistic understanding differs substantially among these categories, and additional regulatory mechanisms may exist beyond those currently recognized. Further investigation will be required to achieve a more comprehensive understanding of elicitor-mediated BGC activation.

3.2.1. *Oxidative stress-driven regulation*

In the induction cases discussed in the previous section, a significant activation is associated with bacterial stress states, such as oxidative stress. Here, we first clarify the mechanisms related to stress, and then illustrate the mechanism by which BGC is induced using specific examples. Stress states induced by exogenous small molecules are mainly manifested as oxidative stress or DNA damage–triggered SOS responses, and these two processes are partially interconnected. Under normal conditions, bacterial cells maintain redox homeostasis, in which metabolically generated ROS are balanced by antioxidant systems to sustain a stable intracellular redox state. However, interference by exogenous molecules can disrupt this balance, driving cells into a stress state that may result in outcomes such as apoptosis-like cell death, autophagy, lipid peroxidation, and DNA damage (Hong et al., 2024). Meanwhile, multiple peroxide-sensor TFs, including OxyR, PerR, and SoxRS, are directly activated by ROS, thereby initiating complex downstream transcriptional programs that rewire cellular metabolic networks (Imlay, 2015). Accumulating evidence indicates that these stress conditions activate stress-responsive transcription factors and lead to extensive changes in gene expression, highlighting the systems-level impact of stress-responsive regulatory networks (Guo et al., 2024; Seo et al., 2015).

It has been proposed that many bactericidal antibiotics promote bacterial cell death not only through their specific drug–target interactions but also by inducing intracellular ROS, a hypothesis originally put forward by Kohanski et al. and widely discussed thereafter (Kohanski et al., 2007). Although this antibiotic–ROS–cell death model has remained controversial, accumulating evidence suggests that ROS can contribute to antibiotic-mediated cytotoxicity in a strain- and context-dependent manner. Based on this logic, low or sublethal doses of antibiotics may induce detectable ROS levels and engage stress-responsive pathways, such as redox-sensing TFs or the SOS response, thereby reprogramming global transcriptional and metabolic networks and potentially activating silent or weakly expressed BGCs. This notion is consistent with the pattern reported by the Seyedsayamdost group (Davies et al., 2006; Okada and Seyedsayamdost, 2017), and aligns with the concept of hormesis (Calabrese and Baldwin, 2003). For instance, Seyedsayamdost et al. demonstrated that the antifolate antibiotic trimethoprim and the β-lactam piperacillin upregulated five target genes in the malleicyprol (*mal*) gene cluster of *Burkholderia thailandensis* E264 (Seyedsayamdost, 2014). Li et al. further showed that piperacillin activates the *mal* gene cluster by inducing oxidative stress involving redox-sensing GRs. At low doses, piperacillin generates ROS, which trigger redox-sensitive TFs OxyR and SoxR. These TFs subsequently activate MalR, the CSR of the *mal* cluster (Fig. 7A) (Li et al., 2021). Meanwhile, as a LuxR-type transcriptional regulator, MalR activates the *mal* cluster in

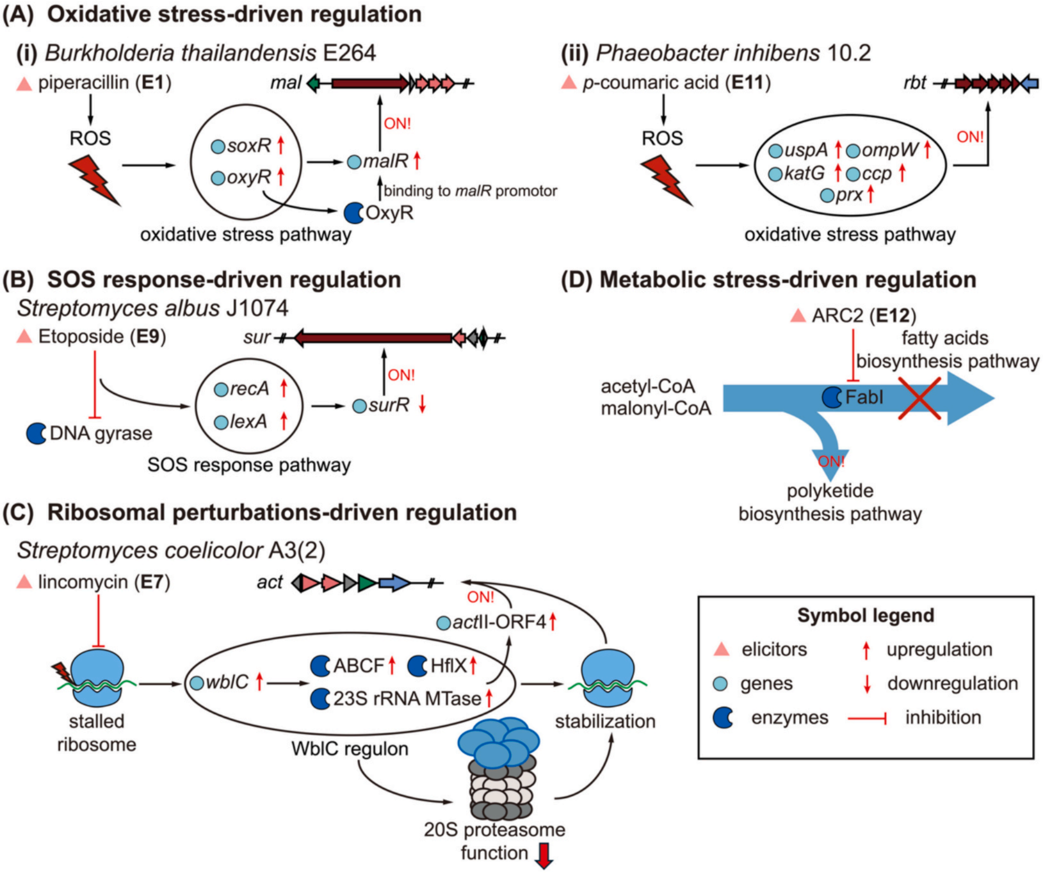


Fig. 7. Representative regulatory mechanisms of chemical elicitors in activating silent BGCs, including (**A**) oxidative stress–driven regulation, (**B**) SOS response–-driven regulation, (**C**) ribosomal perturbation–driven regulation, and (**D**) metabolic stress–driven regulation.

an AHL-independent manner by binding to a lux box-like element in the *mal* promoter, differently from the canonical AHL-based QS mechanism (Truong et al., 2015). Similarly, trimethoprim, a global regulatory elicitor, activates multiple metabolic pathways in *B. thailandensis* E264 through global regulatory mechanisms. It upregulates malR and thereby enhances *mal* expression (Okada et al., 2016). In parallel, it significantly boosts thailandamide production (8- to 36-fold), accompanied by strong induction of biosynthetic genes *thaH* (polyketide synthase, +26-fold) and *thaR* (oxidoreductase, +17-fold) (Seyedsayamdost, 2014). While the precise regulatory intermediates remain unknown, these findings illustrate how elicitors exploit global stress-response modules within GRNs to activate BGCs.

Natural metabolites can function as elicitors by activating stress-related GRN modules. For example, the algae-derived compound *p*-coumaric acid induces the production of the siderophore roseobactin in *Phaeobacter inhibens*, increasing the expression of roseobactin biosynthetic genes by 3.3- to 6.7-fold. Concurrently, it upregulates multiple antioxidant and stress-response enzymes by 4.3- to 13.7-fold and enhances the expression of genes associated with cellular repair systems by 3.3- to 14.9-fold. These effects support a model in which *p*-coumaric acid triggers ROS formation, activating oxidative stress-response modules within the GRN. The induced stress response facilitates repair of damaged biopolymers and promotes secondary metabolite biosynthesis, while cellular energy may be conserved by downregulating non-essential processes, such as motility and flagellar synthesis, thereby redirecting resources toward roseobactin production (Fig. 7A) (Wang et al., 2022a).

3.2.2. *SOS response-driven regulation*

In addition to ROS, some exogenous small molecules act as direct DNA-damaging agents, a phenomenon commonly encountered in natural environments, and can induce DNA lesions, thereby triggering the SOS response (Maslowska et al., 2019). The SOS response is regarded as one of the most fundamental and evolutionarily conserved global transcriptional regulatory systems in prokaryotes, specifically dedicated to coping with severe DNA damage. At the core of this system lies the coordinated action of two key regulators, LexA and RecA, which function as a transcriptional repressor and a damage sensor, respectively (Lima-Noronha et al., 2022). Under normal growth conditions, LexA binds as a dimer to specific DNA sequences known as SOS boxes located in the promoter regions of genes within the SOS regulon. This binding effectively prevents the recruitment of RNA polymerase, thereby maintaining SOS genes at low basal expression levels. However, when cells are exposed to stressors that lead to the accumulation of single-stranded DNA (ssDNA), RecA is rapidly recruited to ssDNA and forms an activated ATP-dependent nucleoprotein filament, referred to as RecA*. Beyond its role in recombinational repair, RecA* acts as a co-protease for LexA, inducing LexA autoproteolysis. The cleavage of LexA results in loss of its promoter-binding affinity, thereby derepressing the transcription of numerous genes involved in DNA damage tolerance and repair, as well as global transcription reprogramming (Podlesek and Žgur Bertok, 2020).

In such contexts, elicitors that induce DNA damage and activate the SOS response have the potential to trigger the expression of silent BGCs. A representative example is the activation of the *sur* gene cluster by the clinical DNA-damaging agent etoposide. Etoposide has been shown to inhibit actinobacterial DNA gyrase. At low concentrations, etoposide strongly induces the expression of *recA* and *lexA* in the SOS response pathway (Fig. 7B). Subsequent RT-PCR and gene knockout analyses revealed that etoposide downregulates SurR, a pathway-specific transcriptional repressor, thereby derepressing the cluster and enhancing the production of cryptic metabolites. Collectively, this case illustrates that elicitor-mediated activation of the SOS response can establish a global regulatory context in which relief of pathway-specific transcriptional repression enables BGC activation, linking DNA damage responses with secondary metabolism (Xu et al., 2017). In addition, mitomycin and bleomycin, as mentioned in the preceding examples, have also been reported to induce the SOS response in *E. coli* (Wei et al., 2001; Xu et al., 2012). However, their mechanisms of action as elicitors have not yet been experimentally examined.

3.2.3. *Ribosomal perturbations–driven regulation*

Some elicitors, particularly translation-inhibiting antibiotics, can rewire cellular metabolic networks by perturbing ribosomal homeostasis. Ochi and co-workers discovered that mutations in ribosomal proteins can profoundly reshape cellular metabolic phenotypes in *Streptomyces lividans* (Shima et al., 1996), an observation that later gave rise to the concept of ribosome engineering. In this strategy, mutations in ribosomal components or RNA polymerase (RNAP) are introduced to reprogram transcriptional and translational states (Zhu et al., 2019). These findings established the ribosome and RNAP as regulatory targets for inducing cryptic metabolites. Perturbation of ribosome or RNAP often disrupts translational dynamics, leading to ribosomal pausing or stalling. Such disturbances are rapidly sensed by stress-responsive regulatory networks, such as WhiB7/WlbC TFs, which coordinate ribosome rescue, translational reprogramming, and broader metabolic adaptation, ultimately leading to the activation of silent BGCs (Hurst-Hess et al., 2023; Lee et al., 2020).

A representative example of this ribosome-centered elicitation mechanism is provided by lincomycin. The Hosaka group identified lincomycin at subinhibitory concentrations as an effective elicitor for actinorhodin biosynthesis in *S. coelicolor* A3(2) and further elucidated its regulatory mechanisms (Imai et al., 2015). Lincomycin, a ribosome-targeting antibiotic, temporarily inhibits ribosome function by binding to the peptidyl transferase loop of 23S rRNA in 70S ribosomes. This perturbation elicits a rapid response mediated by the WblC regulatory network (Mukai et al., 2023). As a master regulator, the TF WblC coordinates lincomycin resistance and ribosome reconstruction by upregulating genes that encode ABCF proteins (facilitating lincomycin efflux), HflX proteins (ribosome-splitting factors that resolve stalled ribosomes), and 23S rRNA methyltransferase (structurally protecting ribosomes against inhibition) (Ishizuka et al., 2018). In the context of WblC regulation, reduced 20S proteasome activity stabilizes ribosome-associated proteins and enhances translational efficiency. This multilayered regulation promotes the downstream expression of the CSR actII-ORF4, ultimately increasing actinorhodin production (Fig. 7C). Moreover, streptomycin, described in the above cases as an elicitor, can be classified as a ribosome-targeting antibiotic, and its elicitor mechanism remains unreported (Lin et al., 2018).

3.2.4. *Metabolic stress-driven regulation*

Primary metabolism comprises the core biochemical pathways that sustain cellular growth, energy production, and structural integrity. Because these pathways operate at high flux and are deeply interconnected, their activities are highly sensitive to perturbation. Certain small molecules can directly target and inhibit key enzymes within primary metabolic pathways, disrupting metabolic flux balance and homeostasis, giving rise to the metabolic stress. Cells respond to this stress by engaging global TFs, leading to the reprogram of gene expression and metabolic flux.

The ARC2 compound series provides an example of a metabolic stress-driven regulation, in which elicitors redirect metabolic flux by inhibiting core primary metabolic pathways. Due to its structural similarity to triclosan, a known FabI inhibitor, validation experiments and gene complementation studies confirmed that ARC2 functions as an effective inhibitor of the FabI enoyl reductase in fatty acid biosynthesis (Craney et al., 2012). Subsequent studies demonstrated that the ARC2-induced metabolic response depends on the global TFs AfsR and AfsS (Calvelo et al., 2021b). Notably, there is currently no evidence that AfsR or AfsS act as direct sensors of metabolic stress; instead, they are generally regarded as global regulators of antibiotic production in *Streptomyces* (Yan and Xia, 2024). As a regulatory outcome, inhibition of fatty acid biosynthesis diverts shared precursors such as acetyl-CoA and malonyl-CoA toward polyketide and siderophore production, including actinorhodin, germicidin, doxorubicin, baumycin, and the acetyl-CoA-dependent desferrioxamine, while reducing the yields of fatty acid-containing metabolites such as prodiginines and CDAs (Fig. 7D). Additionally, as mentioned above, elicitor trimethoprim inhibits folate metabolism, thereby blocking one-carbon metabolism and nucleotide biosynthesis, and can therefore be regarded as a form of metabolic stress (Quinlivan et al., 2000).

4. Targeted activation of BGCs: A guide to elicitor selection

Based on the integrated knowledge graph and the mechanistic insights summarized above, we propose a set of strategies to guide elicitor selection toward the targeted activation of silent or lowly expressed BGCs. These strategies are not intended as deterministic rules, but rather as knowledge-driven heuristics supported by bioinformatic and cheminformatic reasoning. Given the diversity of regulatory architectures across microorganisms, truly targeted activation of BGCs is not always achievable for arbitrary strains. Therefore, we introduce the following strategies in an order that reflects their relative reliability, from approaches grounded in shared regulatory mechanisms to more exploratory strategies based on homology-based extrapolation. Together, this framework aims to provide practical guidance and facilitate the identification of additional elicitor-induced BGC activation cases.

4.1. Regulatory mechanism-based elicitor selection

Effective elicitors can be extendedly discovered based on the mechanism or observable effects. For instance, Li et al. observed a clear correlation between ROS levels and the expression of the *mal* BGC in *B. thailandensis* E264. Consequently, they successfully induced the *mal* BGC using a series of well-known ROS elicitors, including plumbagin, paraquat, and menadione (Li et al., 2021). Based on the mechanistic insights summarized above, our understanding of elicitor–microbe interactions provides valuable clues for the selection of elicitors. Different classes of elicitors act through distinct mechanisms that can reshape regulatory networks and trigger metabolic reprogramming.

Specifically, small molecules capable of inducing detectable ROS production provide a rational basis for elicitor selection. For example, β-lactam antibiotics, such as ampicillin, meropenem, and ceftriaxone, can be prioritized as candidate elicitors. Classical models propose that these compounds act by binding to transpeptidases and interfering with peptidoglycan cross-linking in the bacterial cell wall, while subsequent studies have shown that this perturbation leads to enhanced cellular respiration and elevated intracellular levels of ROS (Dwyer et al., 2014; Van Acker et al., 2016). ROS induction can also be achieved through non–small-molecule approaches (Memar et al., 2018); however, these strategies are not discussed further here.

Similarly, DNA-damaging antibiotics, such as quinolones including nalidixic acid, ciprofloxacin, norfloxacin, and moxifloxacin, exert their effects by inhibiting DNA gyrase and topoisomerase IV (Bush et al., 2020; Dörr et al., 2010; Newmark et al., 2005). This interference with DNA replication induces DNA damage and activates the bacterial SOS response system. Accordingly, quinolones emerge as candidate elicitors operating through the SOS response.

Based on ribosome perturbation, translation-targeting antibiotics represent a particularly attractive class of elicitors, as they directly interfere with ribosomal function and have been extensively reported to be effective in actinomycetes, especially *Streptomyces* and *Mycobacterium*, including aminoglycoside antibiotics (streptomycin, kanamycin, gentamicin) and other antibiotics such as tetracycline, erythromycin, and chloramphenicol (Krause et al., 2016; Lin et al., 2018; Morris et al., 2005). Beyond translation-targeting antibiotics, small molecules capable of triggering ribosome rescue pathways through alternative mechanisms, such as common alkylating agents that cause mRNA damage (Thomas et al., 2020) or puromycin that induces premature transcription termination by blocking nascent chain elongation (Aviner, 2020), may also serve as potential elicitors (Keiler, 2015).

From the metabolic stress perspective, small molecules that perturb core primary metabolic processes can be considered candidate elicitors. For example, linoleic acid has been reported to perturb central metabolism and redox balance, leading to a pronounced metabolic stress state (Senizza et al., 2020). Inhibition of fatty acid biosynthesis by cerulenin and thiolactomycin disrupts lipid metabolic flux and imposes metabolic constraints on cellular growth (Price et al., 2001). Glycolytic stress can be induced by iodoacetate and iodoacetamide through inhibition of glyceraldehyde-3-phosphate dehydrogenase, resulting in impaired energy metabolism (Schmidt, 2009). Fosmidomycin targets the methyl-D-erythritol phosphate (MEP) pathway by inhibiting 1-deoxy-D-xylulose 5-phosphate reductoisomerase, thereby constraining isoprenoid precursor biosynthesis and inducing metabolic stress (Zhang et al., 2011). Collectively, these examples illustrate how mechanism-guided interrogation of existing literature can facilitate the identification of previously unexplored elicitors.

4.2. Structure and activity-based elicitor selection

It is well established that similar compounds often share the same mechanism of action. For example, third- and fourth-generation cephalosporins, which share high structural similarity, almost showed the effective induction of the *mal* BGC (Li et al., 2021). Inspired by this, based on the chemical structures of reported elicitors, we can quickly identify potential chemical elicitors from a specific compound dataset by calculating the Tanimoto coefficient to quantify structural similarity among compounds. These analogs are likely to have the potential to activate corresponding BGCs. For example, among the reported 54 elicitor structures in our knowledge graph, we quickly identified 118 analogs of 38 known elicitors from a DrugBank database containing over 11,000 clinical drugs by applying a Tanimoto coefficient threshold of 0.6 (Fig. 8A**,** Table S2). The identified analogs were mainly categorized into 37 lipids and lipid-like molecules, 14 phenylpropanoids and polyketides, 12 benzenoids, 10 organic acids and derivatives and others, which were discretely distributed in multiple molecular families.

It is worth noting that once the structure-activity relationship between elicitor chemical structures and their inducing activity is better understood, the clustering criteria for compounds can be more accurately defined, further improving the accuracy of discovering novel elicitors. For example, trimethoprim (**E2**) has been shown to activate the *bhc*, *mal*, *bta*, *hmq*, and *tha* gene clusters. It is likely that its structural analogs, brodimoprim (**DB13795**), tetroxoprim (**DB15976**), and 2,4-diamino-5-(3,4,5-Trimethoxy-Benzyl)-pyrimidin-1-Ium (**DB03125**), could also activate these gene clusters; however, their specific effects on the production of secondary metabolites encoded by these gene clusters still require experimental validation. Similarly, the analogs of piperacillin (**E1**), mezlocillin (**DB00948**), and azlocillin (**DB01061**) are likely candidates to activate the *mal* gene cluster, and the *N*-methyl derivative of mitomycin (**E3**), porfiromycin (**DB06478**), is probably an effective chemical elicitor for the *bhc* gene cluster (Fig. 8B).

4.3. BGC homology-driven elicitor selection

Given the vast number of unidentified BGCs encoded by microorganisms, the 32 activated BGCs exhibit significant diversity, as they were identified as 32 singletons by BIG-SCAPE at the default threshold of 0.3. Moreover, it is likely that homologous BGCs can be activated by the same chemical elicitors. By leveraging these known BGCs, numerous homologous gene clusters can be identified in microbial gene cluster databases, which may also be activated by the corresponding chemical elicitors. For example, using the cblaster (v1.3.19) (Gilchrist et al., 2021), we identified 1274 gene clusters highly similar with these activated BGCs from an in-house database containing over 1.3 million

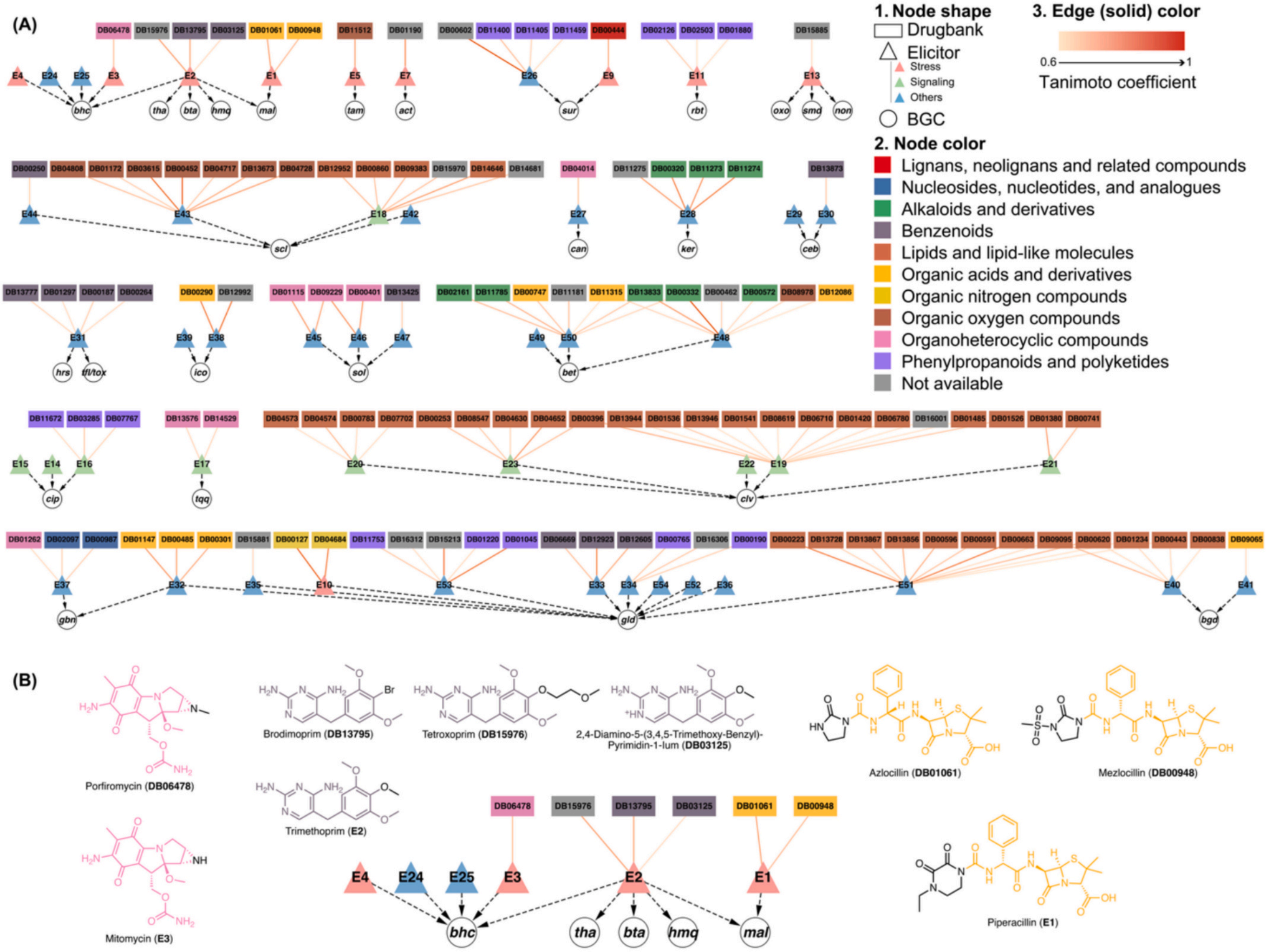


Fig. 8. (**A**) The chemical similarity network of 38 known elicitors and 118 analogs identified from the DrugBank database. In the network, connections between compounds indicate a Tanimoto coefficient greater than 0.6. The 27 gene clusters that can be activated by the elicitors (represented by triangles) are also added to the network with dashed arrows. The color of the rectangles represents the structural class of the drugs. (**B**) Chemical structures of six analogs identified from the database for elicitors **E1–E3**, with the color of the core structure matching their structural class.

microbial BGCs (Fig. 9A, Table S3) (Zhou et al., 2026). Out of the 32 activated BGCs, 23 were found to have similar BGCs, while 9 did not. These similar BGCs come from eight genera, with the majority derived from *Burkholderia*, *Streptomyces*, and *Streptococcus*. By utilizing the elicitors of these activated BGCs, it is possible to conduct the targeted discovery of novel analogs of their encoded secondary metabolites, which would significantly enhance the efficiency of natural product discovery.

Additionally, we conducted a preliminary validation using the *sur* gene cluster as an example, demonstrating that ivermectin has the ability to induce high expression of its homologous gene clusters identified from *Streptomyces albidoflavus* LHW61002 (Fig. 9B). After processing the LC-MS^2 data using a feature finding tool MZmine3 (Schmid et al., 2023), the outputs were uploaded to global natural products social molecular networking (GNPS) for feature-based molecular networking (FBMN). Two features within one molecular family were annotated as known compounds surugamide A and D, while another feature remained unannotated, which may represent a novel analog (Fig. 9C). Based on literature and the mass spectrum mirror plot, we speculate that the unannotated compound may be acyl-surugamide I (Xu et al., 2017). This case study highlights that homologous gene clusters can indeed be activated by the same elicitor, offering a promising strategy for the targeted discovery of novel natural products.

4.4. *Regulatory gene-based elicitor selection*

Even non-homologous BGCs derived from different bacteria may be governed by similar regulatory mechanisms. For example, a class of BGCs regulated by LuxR-type QS has been reported to produce metabolites such as clinically used antibiotics carbapenem and mupirocin, as well as other antibiotics bactobolin and enacyloxin. The regulatory mechanism of LuxR-type QS involves the activation of LuxR, which acts as a receptor for signal molecules, mostly AHLs or others unknown, and subsequently controls the expression of the associated BGCs. In one case, the exogenous addition of *Vibrio fischeri* AHL (3-oxohexanoyl-L-homoserine lactone) resulted in the early onset and significantly elevated levels of QS-regulated bioluminescence (Septer and Stabb, 2012). This finding suggests that identifying a set of effective inducers targeting LuxR-type regulators could potentially activate this class of LuxR-type QS-regulated BGCs. Greenberg et al. investigated *luxR* homolog-linked BGCs in *Proteobacteria* and revealed that associations between *luxR* homolog genes and BGCs have evolved independently multiple times (Brotherton et al., 2018). These associations were observed in functionally diverse gene clusters, highlighting the convergent evolution of regulatory linkages across distinct BGCs despite their non-homologous nature. They identified a large number of LuxR-type QS-regulated BGCs using bioinformatics methods, which could serve as potential

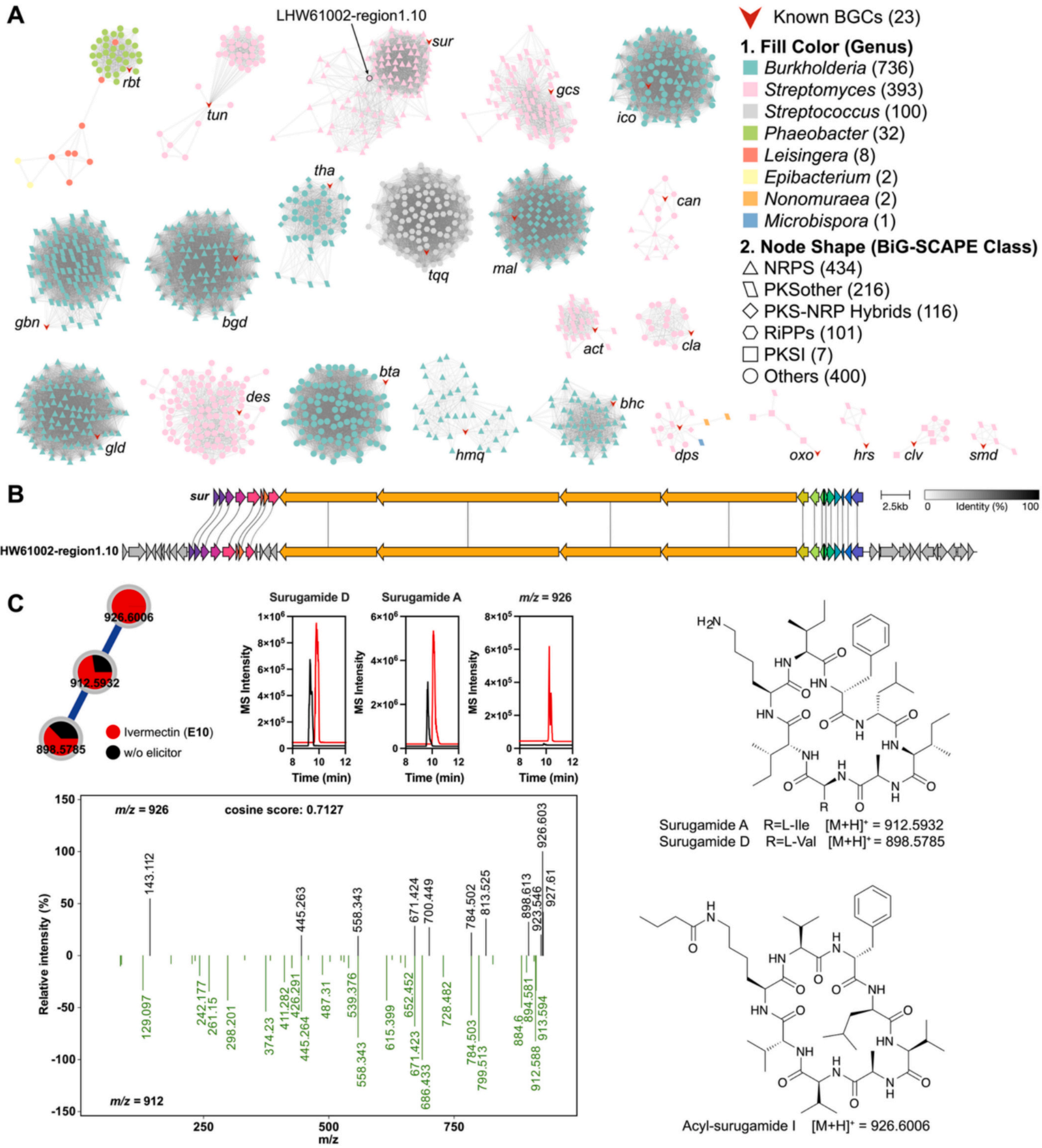


Fig. 9. (**A**) The sequence similarity network is composed of BGCs, identified from an in-house bacterial gene cluster database, that show high similarity to activated BGCs. Each node represents a BGC, with the fill color indicating the genus source of the BGC. The node shape indicates the BGC category as classified by BIG-SCAPE. (**B**) Comparative analysis of a homologous gene cluster of *sur* identified from an in-house strain *Streptomyces albidoflavus* LHW61002. (**C**) Ivermectin can induce *S. albidoflavus* LHW61002 to produce a series of surugamides, including a potential novel analog.

research targets for activation using inducers.

Mechanistic studies have shown that chemical elicitors can induce the substantial biosynthesis of secondary metabolites by altering the expression or activity of regulatory genes within the biosynthetic pathway. This suggests that these elicitors might also be able to activate BGCs containing homologous regulatory genes. Consequently, it may be possible to achieve targeted activation of a wide range of silent BGCs based on these specific genes. Based on the identified genes that can be regulated by specific chemical elicitors, including *malR*, *thaR*, *act*II-ORF4, *surR*, *recA*, and *lexA*, local BLAST analysis was conducted against our in-house bacterial gene cluster database. This analysis revealed 608 BGCs containing highly homologous protein-coding genes of these identified genes (Table S4). Using BiG-SCAPE (v1.1.9) with a default threshold of 0.3, 334 gene clusters were grouped into 62 gene cluster

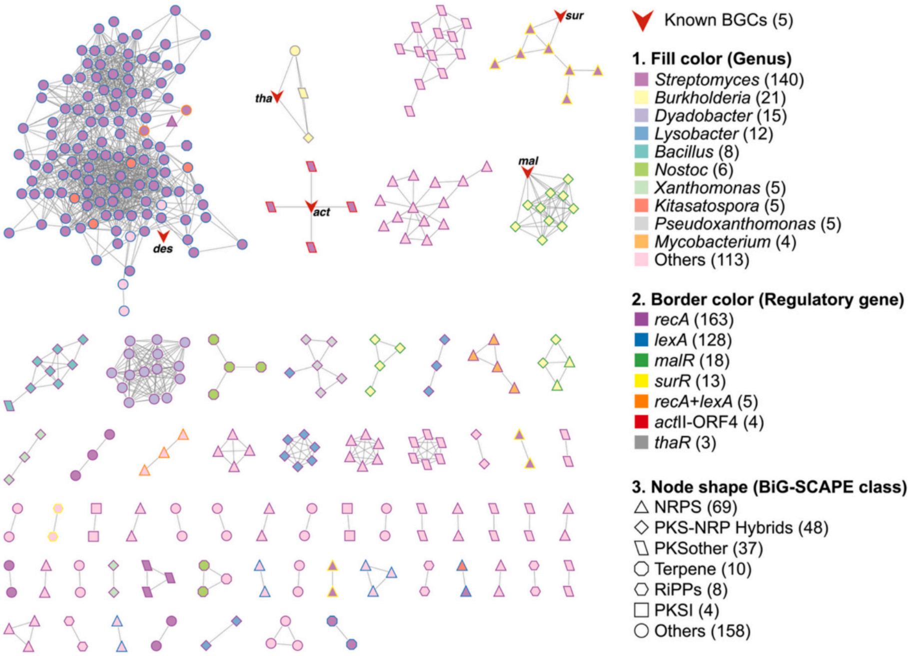


Fig. 10. Sequence similarity network of BGCs identified from an in-house bacterial gene cluster database that contain genes homologous to known regulatory genes. Each node represents a BGC, with the fill color indicating the genus source of the BGC. The border color denotes the type of regulatory genes present within the BGC, while the node shape indicates the BGC category as classified by BIG-SCAPE.

families (GCFs) (Fig. 10), while 274 remained as singletons. Notably, five of these GCFs contain 144 BGCs, each featuring a gene cluster (*des*, *tha*, *sur*, *act*, and *mal*) that can be activated by small molecules, as described earlier. This suggests that these gene clusters not only exhibit high similarity to the five previously mentioned BGCs but also share homologous regulatory genes, indicating a higher likelihood of activation by their corresponding elicitors. These BGCs display considerable diversity, originating from 182 different genera, with *Streptomyces* being the most prevalent. It is worth noting that *rec*A and *lex*A are distributed across various BGC types and genera, playing crucial roles in DNA repair and the regulation of the SOS response. Future research can investigate the effects of the corresponding chemical elicitors on the secondary metabolite profiles of strains encoding these homologous genes, which offers valuable insights for the targeted discovery of natural products based on these reported regulatory mechanisms.

5. Conclusion and future perspectives

In conclusion, this review provides a focused overview of the current use of chemical elicitors to activate silent BGCs and their targeted application in the discovery of new natural products. Given the vast number of uncharacterized BGCs revealed through genome mining, the strategic use of chemical elicitors is crucial for unlocking the full secondary metabolic potential of microorganisms. We constructed an integrated knowledge graph of elicitor–BGC–metabolite relationships and classified elicitors into three categories based on their functional modes: stress-associated elicitors, signaling-associated elicitors and other compounds. However, many elicitor-activated gene clusters and their underlying regulatory mechanisms remain insufficiently characterized, presenting an important direction for further research.

Additionally, we summarized the regulatory mechanisms of elicitors activating silent BGCs. From a regulatory network perspective, despite their chemical diversity, elicitors often converge on a limited set of common upstream regulatory routes or stress-responsive pathways, which can be broadly categorized into four major types: oxidative stress-associated regulation, DNA damage-induced SOS responses, ribosomal perturbation-mediated regulation, and metabolic stress-driven regulation. Although the primary molecular targets of elicitors differ, signals generated from these perturbations are integrated through gene regulatory networks, ultimately leading to derepression and activation of BGCs. However, mechanistic dissection of elicitor-mediated BGC activation remains incomplete. With the rapid development of multi-omics technologies, future studies integrating time-resolved bulk transcriptomic data with proteomic, metabolomic, and epigenomic data will enable construction of GRNs or extended regulatory networks. In this context, machine learning- or deep learning-based network inference frameworks provide powerful tools to identify common upstream regulators and regulatory modules underlying BGC activation (Hegde et al., 2025). In addition, single-cell transcriptomic approaches may further complement bulk analyses by resolving population heterogeneity in elicitor responses that is masked in averaged measurements. Together, these advances are expected to deepen mechanistic understanding of elicitor–regulatory network interactions and guide rational strategies for BGC activation and natural product discovery.

Furthermore, based on the knowledge graph and mechanistic insights, we propose four key strategies for the targeted activation of silent BGCs: (1) regulatory mechanism-based elicitor selection, in which elicitors are chosen according to shared stress or signaling pathways; (2) structure and activity-based elicitor selection, which explores analogs of existing elicitors to activate related gene clusters; (3) BGC homology-driven elicitor selection, which involves using known elicitors to activate gene clusters with similar characteristics; and (4) regulatory gene-based elicitor selection, focusing on targeting gene clusters that share similar regulatory genes with those already activated by known elicitors. In practice, for a genome-sequenced strain, elicitor selection can be guided by prior knowledge from related taxa. If small molecule perturbation has been reported in the same genus or closely related lineages, these compounds and their structural analogs can be prioritized. Subsequently, genome annotation, including BGC annotation, can be used to assess whether the genome harbors gene clusters or regulatory genes homologous to those represented in our knowledge graph, thereby prioritizing the corresponding elicitors. It should be acknowledged that these strategies have inherent limitations, as they are primarily applicable to strains or elicitors that exhibit relevant associations within the existing knowledge graph, and therefore cannot provide guidance for elicitor selection in an arbitrary microbial strain. Compared with blind screening, this knowledge-guided framework increases the likelihood of identifying positive elicitors, although it does not guarantee successful activation outcomes. Overall, these approaches aim to facilitate the expansion of elicitor-induced BGC activation cases, and ultimately support both mechanistic investigations and practical applications.

Nonetheless, significant challenges remain in the targeted activation of specific BGCs using elicitors, requiring careful consideration of several key factors. One critical aspect is the concentration of the elicitor, particularly antibiotic-based elicitors, which tend to stimulate metabolism at subinhibitory levels. Another crucial factor is the choice of growth medium, as its components can significantly influence the expression of silent BGCs. Variations in media composition, such as different carbon, nitrogen, or phosphate sources, can markedly affect the yield and types of secondary metabolites. For instance, high concentrations of glucose, phosphate, or ammonia typically inhibit secondary metabolism, while lower levels of these components often enhance secondary metabolite production (Bode et al., 2002). Previous studies have also shown that the same elicitor can induce different outcomes when applied in different growth media, reflecting the dependence of secondary metabolism on cultivation conditions (Pimentel-Elardo et al., 2015). The type of culture medium, whether solid or liquid, can lead to distinct metabolite profiles even when the same elicitors are added (Lee et al., 2025). Environmental factors like temperature and pH also impact BGC expression and should be considered. Therefore, beyond selecting suitable elicitors, researchers should explore the potential of different media combinations and physical conditions. Advanced techniques such as LC-MS (Yu et al., 2026), bioactivity assays (Gaudêncio et al., 2023), and reporter gene systems (Ji et al., 2024) can be employed to detect new metabolites or activated BGCs under these varied conditions.

Elicitors can be viewed as valuable “medicines” for microbes, effectively “curing” their silence by activating dormant BGCs and unveiling cryptic metabolic capabilities. Similar to the rational design of drugs that has led to the discovery of numerous effective pharmaceuticals, an approach based on known elicitors could similarly yield more potent and specific elicitors. As more cases of elicitor-induced activation are reported and mechanistic insights accumulate, our understanding of how elicitors interact with microbial regulatory networks will deepen. This growing knowledge will enable rational elicitor design and deeper mechanistic understanding, thereby advancing strategies to boost the production of specific metabolites and benefiting fields such as pharmaceuticals, biotechnology, and beyond.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data involved in this review are publicly available on Zenodo at https://doi.org/10.5281/zenodo.1781079.

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