Review Paper:

Chemical elicitors and their impact on secondary metabolite production in *Streptomyces*

Madhuri Mukindrao Moon and John Godwin Christopher*
School of BioSciences and Technology, Vellore Institute of Technology, VIT University, Vellore, Tamil Nadu, INDIA
*godwinj@vit.ac.in

Abstract

Streptomyces are typically known for their varieties of secondary metabolites, many of which hold significant pharmaceuticals. However, a major share of their biosynthetic capacity remains hidden because secondary metabolite-biosynthetic gene clusters are often silent under standard cultivation conditions. To switch on these cryptic genes, there is a need for such signaling molecules as physiological or environmental stress. These numerous signals, signal transduction proteins and transcription factors form an elaborate regulatory network. This triggers various responses, chief among them being the activation of secondary metabolism.

In this review, we present evidence of how chemical elicitors can induce the expression of such cryptic genes. These cryptic genes can be activated with a better understanding of the genome sequencing, leading to novel drug discovery. Elicitors increase the synthesis of secondary metabolite compounds by signal transduction mechanisms and impact the growth and other cellular activities in Streptomyces such as carbohydrate and lipid metabolism protein production and gene activity. This underlined the importance of elicitor approaches and signaling-based strategies in developing and advancing secondary metabolite research and development.

Keywords: Streptomyces sp., Cryptic genes, Secondary metabolites, Signalling pathways.

Introduction

Secondary metabolites are a broad category of naturally occurring compounds that have been found to exhibit a wide range of biological properties such as antibacterial, anticancer, anthelminthic, antidiabetics, anticholesterol and other immunomodulatory effects[18]. Since these compounds are frequently synthesized in response to various environmental stressors, such as nutrient depletion or exposure to other microorganisms or chemicals, they serve as crucial lead targets in the field of drug research[21].

These compounds are the source of around 80% of anticancer agents and about 50% of all FDA-approved pharmaceuticals[50]. In fact, over the last 25 years, more than half of all anti-infective and anti-cancer chemicals have been derived from natural products with *Actinomycetes* being major contributors[1]. *Actinomycetes* are the source of two-thirds of all known antibiotics, with *Streptomyces* being the most common[51]. *Streptomyces* sp. is a desirable target for this approach due to its well-known ability to produce a wide range of bioactive secondary metabolites[68].

The history of natural product discovery can be divided into three separate stages, the first 30 years (the 1940s–1970s) of phenotypic screening, the second 30 years (1970s–2000s) of knowledge-based approaches and the last 20 years (since the 2000s) of genomics-based approaches[37]. The discovery and characterization of secondary metabolites in microbial systems now rely heavily on genomic-based methods and the cryptic genes involved in the biosynthesis of secondary metabolites that can be identified using genomic-based methods[57].

Overall, it has the potential to create new methods for boosting yields or creating novel analogs with higher bioactivity by fusing these genomic-based approaches with established fermentation procedures and analytical methods[47]. Current investigations on antibiotic cryptic gene activation or enhancement in *Streptomyces* primarily focus on elicitors that boost gene cluster expression[51].

Elicitors are molecules or substances that encourage the synthesis of secondary metabolites in microbes, plants and other living things[15]. Elicitors function by turning on particular cryptic genes involved in pathways for the biosynthesis of secondary metabolites[10]. Generally, at a given time not all the genes are active, only a few are functional or active. Most of them are silent or inactive, but various stimuli can activate them and stimuli can be chemical molecules such as DMSO, ethanol, scandium etc. called elicitors[81]. Elicitors can be categorized as either biotic or abiotic; *Streptomyces* frequently cohabit with other microbes in a variety of habitats during biotic elicitation[23] allowing for contact-dependent activation of cryptic gene clusters that may result in the creation of novel secondary metabolites and chemical defense mechanisms[13].

Recent research has also investigated abiotic elicitation methods, including chemical, physical and molecular methods as efficient ways to activate silent gene clusters[82]. On the other hand, abiotic elicitors are non-living chemical compounds or physical factors such as metal ions, salts, changes in temperature, dark and light, or UV radiation that can also stimulate secondary metabolites production of *Streptomyces.*

In this review study, we focused on chemical elicitors. These are often preferred over other elicitors because they can be easily available, can be purified and can be standardized for large-scale fermentation processes[25]. It can also tend to have more defined mechanisms of action compared to elicitors derived from living organisms or environmental factors. Exposure to particular chemical elicitors that work at transcriptional and translational regulation and expression of previously dormant genes, can be used to activate cryptic biosynthetic gene clusters (Figure 1)[56].

The binding of these elicitors to regulatory pathways, including transcription factors, is the mechanism underpinning this occurrence which results in the creation of natural products. New natural compounds with therapeutic promise against a variety of infectious disorders brought on by pathogenic bacteria, have been found using this method with effectiveness. The emphasis of this review is on the chemical elicitor and the different techniques used for the expression of cryptic genes.

Cryptic genes and elicitors: Cryptic genes are DNA sequences that do not usually express themselves during the life cycle of an organism and remain phenotypically silent, but via genetic processes such as recombination, insertion, or mutation, they might be made active in a tiny number of creatures[28].

Even so, such sequences might still act as regulators of gene expression or be transcribed into RNA molecules. Despite their importance, cryptic genes frequently lack recognizable features like promoter elements and open reading frames, so it might be challenging to identify them[72]. Therefore, discovering cryptic genes necessitates complex genomic methods like transcriptomics and bioinformatics analysis. To harness the potential of cryptic genes, researchers often employ chemical elicitors to trigger the expression of cryptic gene clusters by testing several compounds to induce secondary metabolite production.

Secondary metabolites that arise from cryptic genes usually have pharmacological properties that make them useful for pharmaceuticals and biotechnology. Scientists discovered the best conditions for activating cryptic metabolic pathways and increasing metabolite production by testing different chemical triggers. Chemical-based strategies have proven useful for activating cryptic biosynthetic genes in *Streptomyces* under controlled laboratory conditions, with promising results observed during research studies. Chemical elicitors are thought to be widely useful for increasing secondary metabolite production and potentiating the outputs of cryptic secondary metabolites in *Streptomyces.*

Target and expression of cryptic genes by chemical elicitor: Several techniques have been developed by reawakening cryptic gene clusters including co-cultivation, epigenetic modification, metabolic stimulation and CRISPR/Cas9 genome editing[17]. These techniques allow for the induction of novel compounds under varied laboratory settings by using a variety of chemical elicitors including DMSO, ethanol, scandium (Sc3+), scandium and lanthanum, sodium butyrate, dimethyl sulphone, antibiotic remodelin' complex (ARC) etc.

In our review, we provide an in-depth analysis of elicitor DMSO, ethanol and scandium, as recent studies have demonstrated their efficacy in enhancing the production of secondary metabolites.

Elicitors specifically encourage a gene's overexpression and this activation may occur for a gene that is silent or has low expression. The active defensive mechanisms influence the elicitation response including which are activated and how powerfully they are stimulated. A complete list of chemical elicitors that have been demonstrated to activate cryptic genes and stimulate secondary metabolism in Streptomyces is presented in table 1.

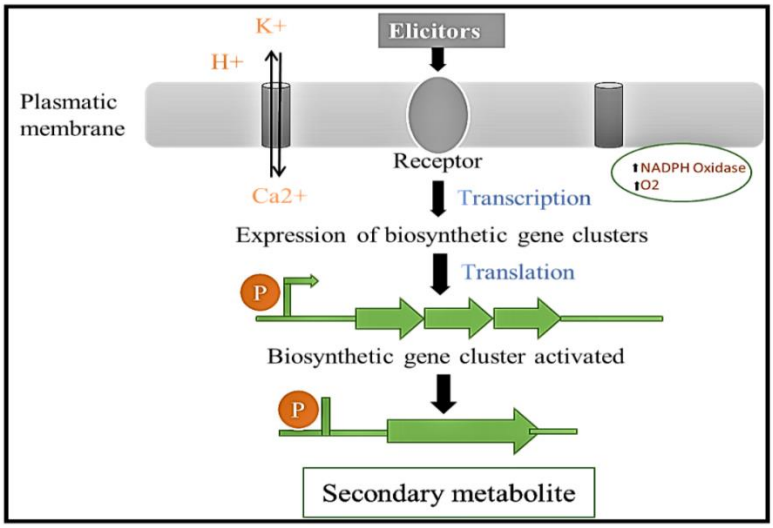

Fig. 1: Elicitor signal transduction leading to secondary metabolite synthesis (P represents Promoter)

Table 1
List of chemical elicitors used in different species of *streptomyces* and the product

S.N.	Elicitor	*Streptomyces*	Cryptic gene	Product
1	DMSO[33,58,73]	*S. venezuelae ATCC 10712*	*cml*	Chloramphenicol
		S. glaucescens	*tcmA* and *tcmR*	Tetracenomycin C
		S. azureus ATCC 14921	*TsrA*	Thiostrepton
		S. lividans	*tipA*	Thiostrepton
		S. lividans	*afsB*	Prodigiosin
		S. hygroscopicus	*aroA, fkbN and luxR*	Ascomycin
2	Ethanol[22,58,79]	*S. glaucescens*	*tcmKLM*	Tetracenomycin C
		S. venezuelae ISP5230	*Jad, JadR1, JadR2*	Jadomycin B
		S. hygroscopicus	*Val-A, AfsR, GlnR*	Validamycin
3	Scandium (Sc^{3+})[20,41,66,67]	*S. coelicolor*	*act*	Actinorhodin
		S. diastatochromogenes SD3145	*toyF* and *toyG*	Toyocamycin
		S. antibioticus and S. parvulus	*acmA* or *acmB*	Actinomycin D
		S. griseus	*strB1, strD* and *strF*	Streptomycin
		S. lividans	*actII-ORF4*	Actinorhodin
4	Scandium and Lanthanum[53,54]	*S. coelicolor A3*	*actII-ORF4*	Actinorhodin
5	Sodium butyrate[46,80]	*S. coelicolor*	*act*II-ORF4	Actinorhodin
6	Dimethyl sulphone[58]	*S. venezuelae ATCC 10712*	*sven0929*	Chloramphenicol
7	Antibiotic remodeling complex (ARC)[6,19,54,64]	*S. coelicolor*	*AfsK AfsR AfsS*	Actinorhodin
		S. coelicolor	*RpsL And rpoB*	Germicidin
		S. griseorubiginosus	*rpoB*	Promomycin, Salinomycin, Monensin, Nigericin
		S. avermitilis	*Frr*	Avermectin

Activation mechanism of DMSO: Dimethyl sulfoxide (DMSO) is a ubiquitous organic solvent that is extensively utilized as an elicitor in various biochemical and cellular investigations, particularly within drug development programs. It possesses several advantageous characteristics including its small molecular size, polar nature and ability to dissolve in polar and nonpolar solvents. These properties contribute to the exceptional cell permeability exhibited by DMSO. The remarkable ability of DMSO to readily diffuse across cell membranes enables efficient penetration into cells[4].

The "DMSO effect" refers to the observed enhancement in the production of various antibiotics including chloramphenicol in *S. venezuelae* ATCC 10712, tetracenomycin C in *S. glaucescens*, prodigiosin in *S. lividans*, ascomycin in *S. hygroscopicus* and thiostrepton in *S. aureus* ATCC 14921 when DMSO is present at varying concentrations[82]. The underlying mechanisms involve the modulation of specific metabolic pathways such as the pentose phosphate pathway (PPP), glycolysis and amino acid metabolism. In particular, DMSO has been found to elevate PPP activity in certain cellular contexts, leading to higher NADPH production and enhanced ribose-5-phosphate generation for nucleotide biosynthesis[78]. This upregulation of PPP serves a protective role by facilitating increased NADPH availability which helps to counteract oxidative stress within cells.

These metabolic pathways have been identified as major contributors to antibiotic production processes. Notably, DMSO influences carbon flow by diverting it away from pyruvate within the tricarboxylic acid (TCA) cycle and pyruvate metabolism. The transcriptional response triggered by DMSO appears to have pleiotropic effects or a widespread impact across multiple cellular processes. Furthermore, DMSO modifies metabolic fluxes towards primary metabolism pathways such as the pentose phosphate pathway, glycolysis and TCA cycle, as shown in figure 2. This redirection provides an increased supply of precursors necessary for optimal antibiotic synthesis.

Upon the addition of the DMSO solution to fermentation media, *Streptomyces* sp. strains exhibited enhanced production of various metabolites belonging to different biosynthetic families. For instance, *S. venezuelae* ATCC 10712 demonstrated a three-fold increase in chloramphenicol synthesis by activating the cryptic *cml* gene[62]. It has been proposed that *JadR1* acts as a repressor for the chloramphenicol biosynthetic pathway by binding to

promoter regions of structural genes associated with its production. Through comparative genome analysis, seven previously unidentified structural genes (*sven0909 to sven0915*) were discovered adjacent to the known chloramphenicol biosynthetic gene cluster in *S. venezuelae* strain ATCC 10712, which was absent in closely related *Streptomyces* strains unable to produce chloramphenicol (*sven0916-sven0928*)[11].

Notably, no cluster-situated regulator specific to the chloramphenicol biosynthetic gene (*cml)* cluster was found. To investigate the particular interactions between *JadR1* and the *cmlJ* promoter region, researchers identified the transcription start point of *cmlJ* located at 439 nucleotides upstream from its putative start codon. Subsequent analyses revealed that downstream regions ranging from nucleotides 186-335 became protected by *JadR1* upon binding. This prompted further investigations into whether *JadR2* also plays a role in regulating chloramphenicol biosynthesis. To ascertain if *JadR1* directly inhibits chloramphenicol production, band shift tests were conducted using each possible promoter region within the chloramphenicol biosynthetic gene cluster as targets for *JadR1* binding assays. Interestingly, it was found that *JadR1* binds specifically to the intergenic region between *cmlI* and *cmlJ*[63], an area previously established as essential for chloramphenicol biosynthesis. These findings confirm that *JadR1* directly governs the translation of *cml* genes involved in chloramphenicol biosynthesis represented in figure 3.

Activation mechanism of ethanol: Ethanol, a potent elicitor of secondary metabolite, exerts significant effects on various cellular processes in *Streptomyces* species. Its molecular properties, including size, polarity and chemical composition, enable it to readily permeate plasma membranes via passive diffusion. This high permeability allows ethanol to exert a "pull" effect on antibiotic production by influencing steady-state growth, cryptic gene expression, carbon metabolism and membrane structure. In the context of antibiotic biosynthesis induction, ethanol demonstrates preferential promotion of pathway-specific transcriptional regulation and activation of oxidative stress mechanisms. These selective modulations contribute to the enhancement of secondary metabolite synthesis upon exposure to ethanol stimulation. For instance, ethanol significantly enhances the synthesis of tetracenomycin C by *S. glaucescens* through activation of the cryptic *tcmKLM* gene[58,73].

Similarly, ethanol supplementation in D-galactose-L-isoleucine fermentation media increases jadomycin B production by *S. venezuelae* ISP5230, a pigmented benzoxazole phenanthridine antibiotic. The mechanism behind this enhancement involves potentially triggering a heat shock response or modification in membrane permeability caused by ethanol exposure in *S. venezuelae* ISP5230. These effects are believed to influence the activity of cryptic genes such as *JadJ*, *JadR1* and *JadR2*[22]. Studies have shown that *JadR2* acts as a repressor within the jadomycin biosynthetic gene cluster[30], while *JadR1* is an essential activator for jadomycin B biosynthesis located nearby but divergent from *jadR2*.

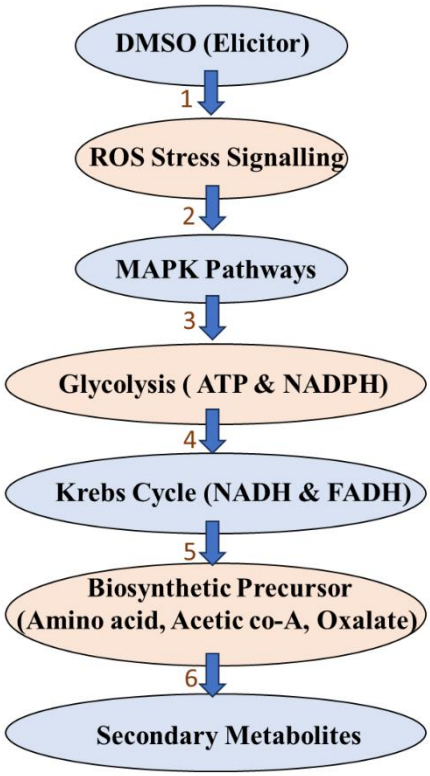

Fig. 2: Overview steps followed by DMSO

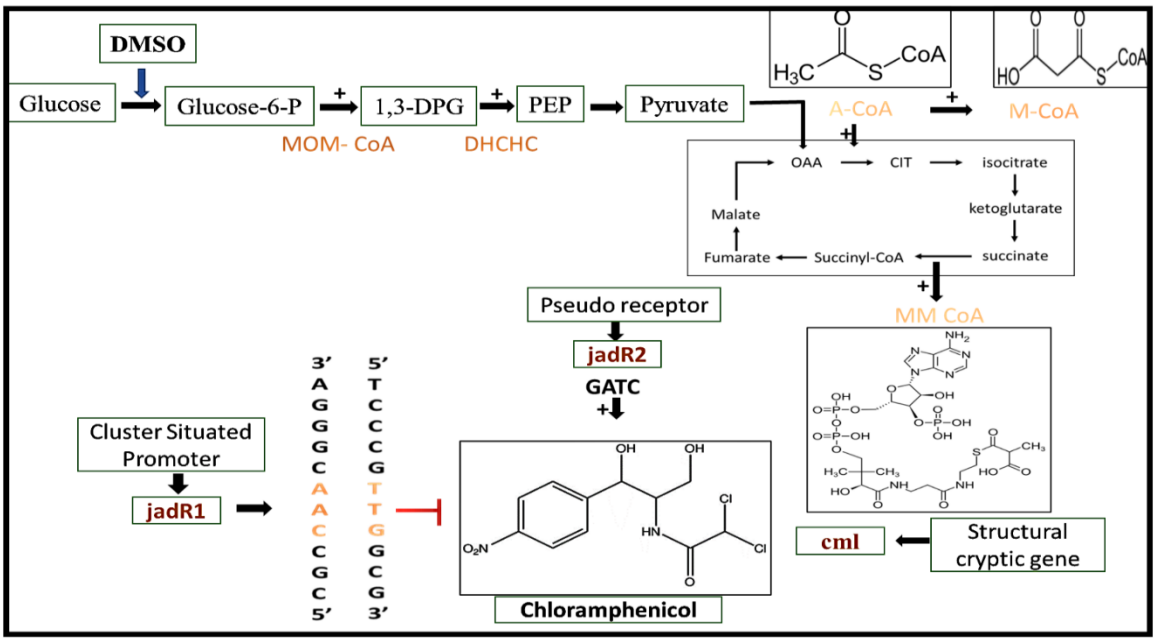

Fig. 3: DMSO role in the molecular expression of cryptic gene. A-CoA: acetyl-CoA; m-CoA: malonyl-CoA; CIT: citrate; FK506: tacrolimus; DMSO: dimethyl sulfoxide; Glu-6-P: glucose 6-phosphate; M-CoA: malonyl-CoA; MM-CoA: methyl malonyl-CoA; MOM-CoA: methoxymalonyl-CoA; OAA: oxaloacetate; TCA: tricarboxylic acid; 1,3-DPG: 1,3-bisphosphoglyceric acid.

When Jodamycin B production occurs under ethanol exposure, synthesis becomes independent of ethanol stress when *JadR2* is disrupted[75]. Band shift tests using *JadR2* were conducted with all possible promoter sites within the Jodamycin biosynthetic gene cluster. The results suggest that repetitive sequences in promoter regions inhibit RNA polymerase binding at the *jadR1* promoter site by *JadR2*. This inhibition prevents the activation of Jodamycin biosynthesis mediated by *JadR1* through a dependent mechanism involving interaction between these two proteins.

Furthermore, it has been observed that Jodamycin B causes dissociation of *JadR1* from its target protein by binding to the N-terminal receiver domain of *JadR1*. Additionally, a genuine butyrolactone receptor called *JadR3* is modulated by butyrolactone SVB1, influencing DNA binding and leading to stimulation of *JadR1* transcription while suppressing the transcription of *JadR2*[76]. These findings highlight autoregulatory mechanisms involved in controlling Jodamycin B production, where high expression levels of biosynthetic enzyme genes result in product accumulation, reaching concentrations that inhibit initial activation shown in figure 4. Therefore, ethanol exposure influences the secondary metabolite synthesis of jadomycin B by activating or suppressing specific cryptic genes. Understanding these regulatory mechanisms and autoregulatory processes provides valuable insights into optimizing antibiotic production strategies using microbial sources.

Activation mechanism of scandium: Scandium (Sc^{3+}) has been demonstrated to act as an elicitor, stimulating the production of diverse secondary metabolites in *Streptomyces* including antibiotics, anticancer drugs and immunosuppressants. Despite the lack of understanding regarding the molecular mechanism behind scandium's action, it is believed that Sc^{3+} ions interact with regulatory proteins or DNA, leading to changes in gene expression profiles[41]. The effectiveness of scandium as an elicitor depends on factors such as the metal ion's concentration, the culture's growth phase and the specific strain of *Streptomyces* being studied. Overcoming these challenges is crucial for maximizing secondary metabolite production using scandium[82]. In the case of actinorhodin production by *S coelicolor A3* (*Act*), it was found that adding scandium significantly increased antibiotic synthesis compared to control sets.

The presence of scandium led to elevated levels of transcripts associated with *actII-ORF4*, a key regulator involved in antibiotic biosynthesis as shown in figure 5. This transcriptional activation is essential for successfully expressing Actinorhodin biosynthetic structural genes. The *act* cluster encompasses the *ActII-ORF4* determinant, which acts as a major controlling factor for actinorhodin synthesis[11,27]. Multiple known regulatory proteins play both positive and negative roles, directly targeting the promoter region containing *actII-ORF4*. These include *AdpA* (a pleiotropic regulator)[55], *LexA* (a global DNA damage response regulator)[35], *AbsA2* (a global repressor)[65,70], *DasR* (mediator responding to N-acetylglucosamine)[59], *DraR* and *AfsQ1* (activators responding to nitrogen excess)[75,77], *AtrA* (transcriptional activator linked with acetyl-CoA metabolism) and *ROK7B7* (SCO6008) binding targets related to xylose operon repression[52].

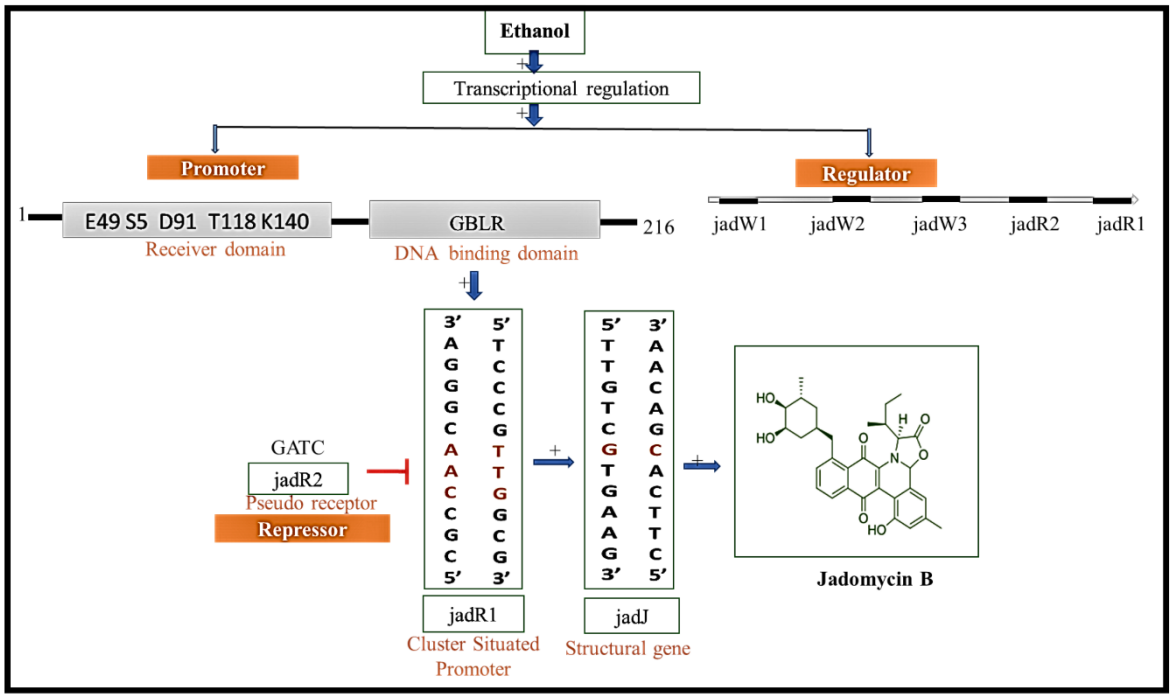

Fig. 4: Ethanol role in the expression of the cryptic gene (jadw1, jadw2, jadw3, jadJ, jadR1, jadR2: Jadomycin biosynthesis gene clusters)

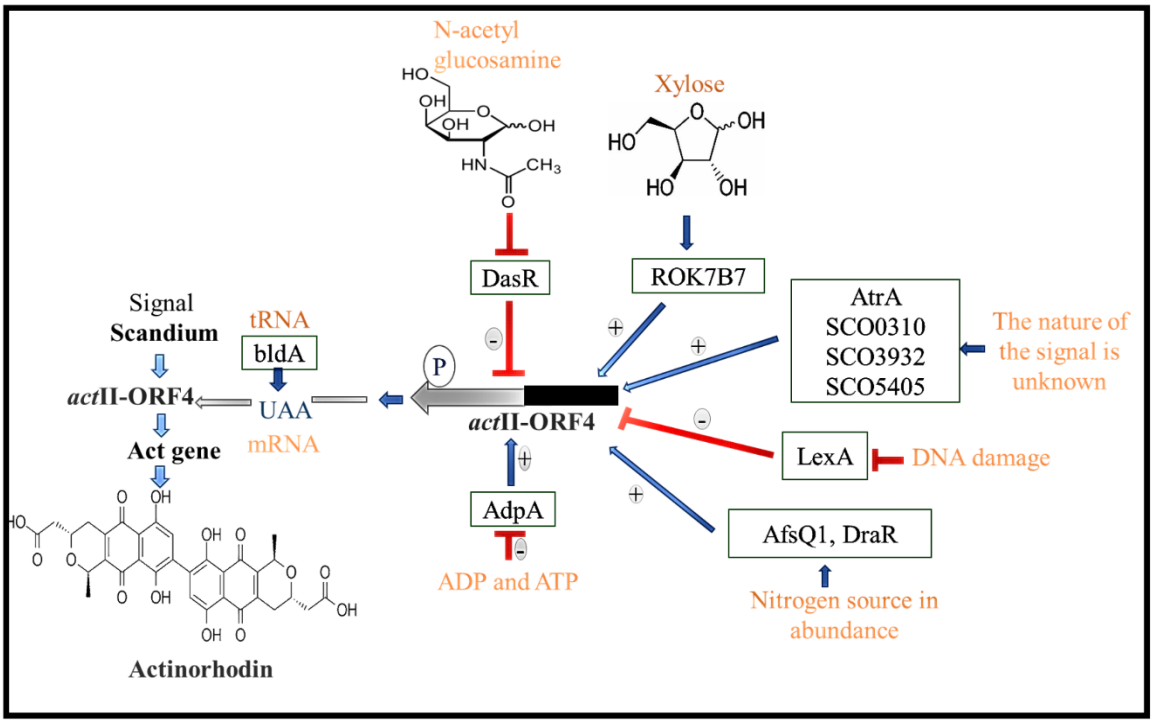

Fig. 5: Molecular action of scandium. (DasR: GntR-family transcriptional regulator, ROK7B7: ROK family regulator, AtrA: transcriptional activator linked with acetyl-CoA metabolism, LexA: Global DNA damage response regulator, AFsQ1 and DraR: activators responding to nitrogen excess, AdpA: Pleiotropic regulator, bldA: Global regulator protein, Act: Actinorhodin)

Unraveling the complex regulatory network involving these regulators binding at different or overlapping locations is an ongoing challenge. Additionally, *actII-ORF4* mRNA contains a rare codon (UUA) and its translation relies on a developmentally relevant tRNA encoded by the *bldA* gene. Further exploration of scandium's effects and elucidation of the underlying molecular interactions will provide valuable insights for optimizing secondary metabolite production in *Streptomyces* species

Application of chemical elicitors in *Streptomyces* for enhancing secondary metabolite production: The use of elicitors can greatly benefit industries such as industrial biotechnology, pharmaceuticals and agriculture[40]. Chemical elicitors are small exogenous molecules that can trigger the membrane-specific receptors of the metabolic pathway, induce or can enhance the production of secondary metabolites, can modify the microbes, or can influence the interaction of the microbes with their environment[29]. Chemical elicitors have been used in microbiology since the 1980s to increase antibiotic synthesis and to find new secondary metabolites. For example, in the 1980s, investigations found that adding sodium butyrate or valproic acid to *Streptomyces* cultures boosted antibiotic synthesis by modifying gene expression or metabolic pathways.

In the 2000s, elicitors such as DMSO were employed to increase the production of cryptic compounds such as coelimycin P1, revealing previously unknown biosynthetic potential. This technique remains a cornerstone in drug discovery and microbial biotechnology[26]. Elicitor triggers act via various mechanisms including the activation of signal transaction pathways, the triggering of stress response and the modulation of metabolic processes[45]. For example, elicitors such as jasmonic acid and salicylic acid, which are originally plant hormones, can mimic signals in microorganisms, leading to the activation of biosynthetic pathways for the production of secondary metabolite[37] and some elicitors like lincomycin at a sub-inhibitory concentration resulted in an elevated expression of the CSR activator gene actII-ORF4 and therefore increased (ACT) actinorhodin metabolite overproduction in *S. coelicolor*[34].

Elicitors promote the formation of secondary metabolites through complex interaction at microbes' molecular, cellular and physiological levels. This interaction often involves signaling pathways, transcriptional regulation and metabolic changes ultimately leading to increased production of desired metabolites[36].

Signal transduction pathways activation in *Streptomyces* - Mechanism and Implications: Streptomyces, a Gram-positive bacterial species known for its complex life cycle and subsequent generations of secondary metabolites, relies heavily on receptor-mediated signaling and calcium signaling to respond to external stimuli. Elicitors are chemical compounds that activate these signaling pathways, leading to various physiological responses including the production of secondary metabolites. Understanding how elicitors act in receptor-mediated signaling in *Streptomyces* is critical for application in biotechnology, particularly for increasing the production of drugs and other useful chemicals.

In receptor-mediated signal transactions, specialized receptors recognize the triggers which are often membrane-bound proteins which are outer domains that interact with signaling molecules[24]. *Streptomyces* contains numerous types of receptors including histidine kinases[16], G protein-coupled receptors (GPCRs)[42] and receptor-like kinases[8]. These receptors recognize various triggers and activate downstream signaling pathways. When the receptor binds to a trigger, it undergoes a structural change that enables signal transduction across the membrane. This process usually requires phosphorylation of the receptor or related proteins[61]. Two-component systems are common in *Streptomyces*[60]. The phosphorylated receptor (histidine kinase) transfers the phosphate group to a reaction regulator. The reaction regulator then controls the target genes' expression in secondary metabolite and other cellular processes.

Signal transmission often leads to the formation of secondary messengers such as cyclic AMP (cAMP), cyclic di-GMP and other smaller molecules. This messenger spreads the signal throughout the cell and amplifies the cellular responses. For example, cyclic AMP can activate cAMP-dependant protein kinases which subsequently phosphorylate other proteins involved in gene expression such as transcription factors controlling secondary metabolite formation receptor-mediated signaling resulting in gene expression regulation[32]. *Streptomyces* frequently activates genes responsible for manufacturing secondary metabolites such as antibiotics, antifungal and anticancer drugs[3].

In calcium-mediated signaling, binding of the elicitor can trigger cell surface receptors that can cause an influx of calcium ions (Ca^{+2}) into the cells. Increased intracellular Ca^{+2} levels are secondary messengers that transmit the signal throughout the cell. This often leads to the activation of calcium-dependent protein kinases which then stimulate transcription factors that stimulate genes involved in producing secondary metabolites[9].

Transcriptional Regulation: Elicitors can stimulate the expression of genes encoding molecules involved in producing secondary metabolites. In *Streptomyces* species, elicitors can increase the expression of polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) gene clusters[71]. Elicitors can modulate the activity of these global regulators, leading to an increased expression of PKS and NRPS genes such as AdpA, AtrA, or DasR which control the expression of numerous genes[80]. Elicitor-induced signals lead to the activation of two-component systems (TCS) or other regulatory proteins that can directly or indirectly enhance the expression of PKS and NRPS gene clusters including those involved in secondary metabolism[28]. Elicitors can modulate the activity of these global regulators, leading to an increased expression of PKS and NRPS genes.

Induction of Stress Responses: Many chemical elicitors including heavy metal ions like copper (Cu^{2+}) or cobalt (Co^{2+}), induce oxidative stress within microbial cells[49]. This stress leads to generating reactive oxygen species (ROS), serving as signaling molecules that can activate secondary

metabolic pathways[40]. Whenever oxidative stress occurs, the accumulation of ROS can lead to a physiological response involving the activation of various transcription factors and signaling pathways[7]. These pathways often promote the upregulation of genes involved in secondary metabolism[48]. In *Streptomyces coelicolor*, for example, ROS produced under oxidative stress can stimulate gene expression in producing pigmented antibiotics like actinorhodin[82]. Activating this metabolic pathway is a defense strategy that enables the organism to produce secondary metabolites that can hinder competitors or are neutralize the effects of oxidative stress.

ROS activates secondary metabolite formation in *Streptomyces* through various regulatory proteins and pathways. *SoxR*, a redox-sensitive transcription factor, responds to intracellular redox changes. *SoxR* undergoes a conformational change when oxidized by ROS. This allows it to bind DNA and to stimulate the expression of genes involved in secondary metabolites like ɣ-actinorhodin[43].

In *S. clavuligerus*, ROS can also influence the function of the alternative sigma factor such as *RpoE* and two-component regulators control the transcription of genes *cutS* and *cutR*, encoding biosynthetic enzymes for holomycin secondary metabolites[44]. Oxidative stress can also affect global regulatory proteins such as *BldA* (a leucyl-tRNA synthetase that controls the expression of several secondary metabolite pathways) and increases the production of actinorhodin and prodiginine of secondary metabolites[5].

Epigenetic Modifications: Certain elicitor triggers lead to epigenetic changes including DNA methylation or histone modification which alter the accessibility of DNA to the transcription machinery. Epigenetic modifications can activate previously silent or cryptic expressed secondary metabolite gene clusters, leading to the synthesis of new or higher levels of secondary metabolites[2]. In a recent study, epigenetic modification is a key tool for secondary metabolite production in microorganisms[12].

Conclusion

Advances in genome sequencing have transformed the discovery of natural products by revealing the hidden metabolic potential of microorganisms, with genome mining playing a crucial role in linking biosynthetic gene clusters to their metabolites. This has enabled the prioritization of microbial strains with novel synthetic capabilities while minimizing the rediscovery of natural products. Despite these advances, the activation of silent gene clusters remains a major problem, mainly due to the lack of knowledge of the regulatory mechanisms controlling secondary metabolic pathways. This study focuses on elicitor-based techniques as promising tools for enhancing secondary metabolite production in *Streptomyces* and demonstrates their diverse applicability in uncovering obscure biosynthetic pathways.

Combining these tactics with cutting-edge technologies such as omics, synthetic biology and machine intelligence holds great promise for overcoming these obstacles. By deciphering the intricate regulatory networks of secondary metabolism and building high-throughput systems to optimize triggers, the field can tap the full metabolic capacities of microbes and pave the way for breakthroughs in medicine, agriculture and sustainable bioproduction.

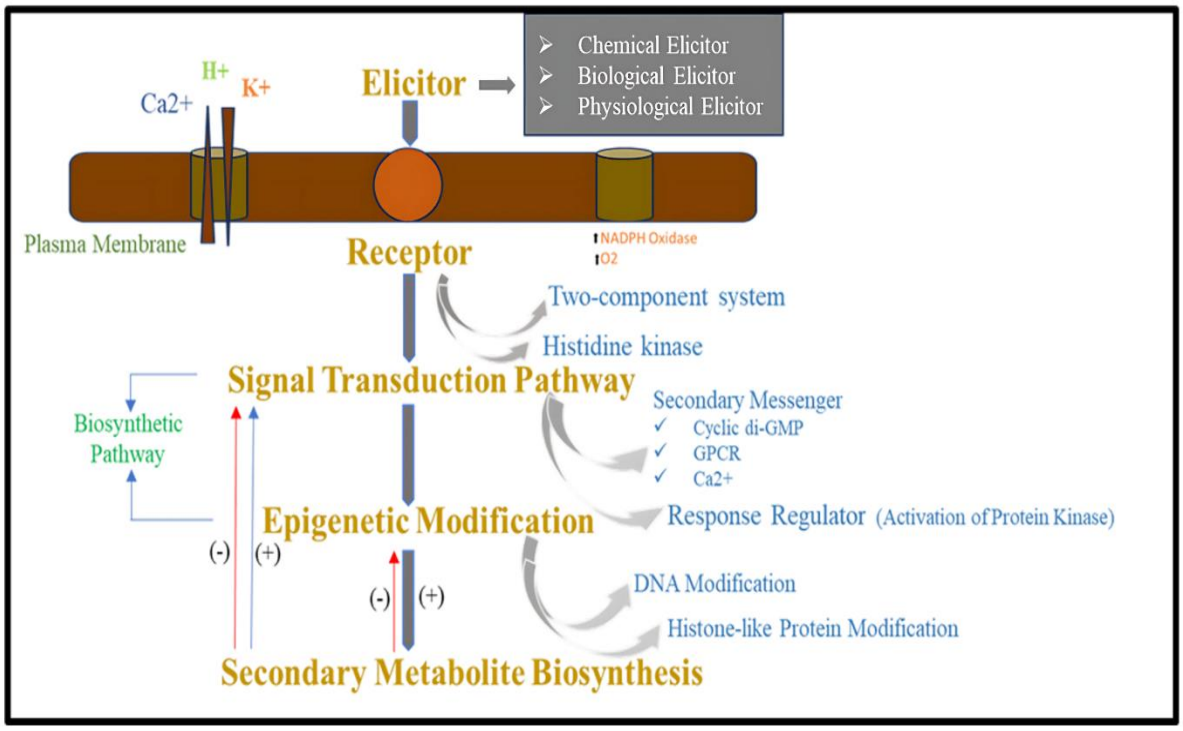

Fig. 6: Pathways followed by secondary metabolite production

References

1. Abdellah Ali Salah F., Aljabori Tamam M.S. and El- Rafey E., The Functional and Biodegradation Characteristics of High-Density Poly-ethylene, Poly-caprolactone and Poly-ethylene Maleic Anhydride Composites, *Res. J. Chem. Environ*., **28(1)**, 115-120 **(2019)**

2. Akone S.H. et al, Epigenetic modification, co-culture and genomic methods for natural product discovery, *Physical Sciences Reviews*, **4**, 20180118 **(2019)**

3. Alam K. et al, Streptomyces: The biofactory of secondary metabolites, *Frontiers in Microbiology*, **13**, 968053 **(2022)**

4. Albayati N., Langley N., Mohd H., Michniak-Kohn B. and Osborne D.W., Dimethyl sulfoxide in topical pharmaceutical drug development: A fresh perspective, *Int. J. Pharm. Excip*, **1**, 10-24 **(2024)**

5. Allenby N.E.E., Laing E., Bucca G., Kierzek A.M. and Smith C.P., Diverse control of metabolism and other cellular processes in Streptomyces coelicolor by the PhoP transcription factor: Genome-wide identification of in vivo targets, *Nucleic Acids Res*, **40**, 9543–9556 **(2012)**

6. Amano S.I. et al, A cryptic antibiotic triggered by monensin, *Journal of Antibiotics*, **64**, 703 **(2011)**

7. Apel K. and Hirt H., Reactive oxygen species: Metabolism, oxidative stress and signal transduction, *Annual Review of Plant Biology*, **55**, 373–399 **(2004)**

8. Baier A. and Szyszka R., Compounds from natural sources as protein kinase inhibitors, *Biomolecules*, **10**, 1–30 **(2020)**

9. Baz M. et al, Calcium- and ROS-mediated defence responses in BY2 tobacco cells by nonpathogenic Streptomyces sp., *J Appl Microbiol*, **112**, 782–792 **(2012)**

10. Bharatiya P., Rathod P., Hiray A. and Kate A.S., Multifarious Elicitors: Invoking Biosynthesis of Various Bioactive Secondary Metabolite in Fungi, *Appl Biochem Biotechnol*, **193**, 668–686 **(2021)**

11. Bibb M.J. et al, New insights into chloramphenicol biosynthesis in Streptomyces venezuelae ATCC 10712, *Antimicrob Agents Chemother*, **58**, 7441–7450 **(2014)**

12. Bind S., Bind S., Sharma A.K. and Chaturvedi P., Epigenetic Modification: A Key Tool for Secondary Metabolite Production in Microorganisms, *Frontiers in Microbiology*, **13**, 784109 **(2022)**

13. Brencic A. and Winans S.C., Detection of and Response to Signals Involved in Host-Microbe Interactions by Plant-Associated Bacteria, *Microbiology and Molecular Biology Reviews*, **69**, 155–194 **(2005)**

14. Caicedo-López L.H. et al, Elicitors: Bioethical implications for agriculture and human health, *Revista Bioetica*, **29**, 76–86 **(2021)**

15. Chamkhi I. et al, Plant-microbial interaction: The mechanism and the application of microbial elicitor induced secondary metabolites biosynthesis in medicinal plants, *Plant Physiology and Biochemistry*, **167**, 269–295 **(2021)**

16. Chater K.F., Recent advances in understanding Streptomyces, *F1000 Res*, **5**, 2795 **(2016)**

17. Collemare J. and Seidl M.F., Chromatin-dependent regulation of secondary metabolite biosynthesis in fungi: Is the picture complete?, *FEMS Microbiology Reviews*, **43**, 591–607 **(2019)**

18. Cot M. et al, Lipoteichoic acid in streptomyces hygroscopicus: Structural model and immunomodulatory activities, *PLoS One*, **6**, e26316 **(2011)**

19. Craney A., Ozimok C., Pimentel-Elardo S.M., Capretta A. and Nodwell J.R., Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism, *Chem Biol*, **19**, 1020–7 **(2012)**

20. Crnovčić I., Lang M., Ortel I., Süssmuth R.D. and Keller U., Comparison of actinomycin peptide synthetase formation in Streptomyces chrysomallus and Streptomyces antibioticus, *J Basic Microbiol*, **59**, 148–157 **(2019)**

21. Dhakal D., Sohng J.K. and Pandey R.P., Engineering actinomycetes for biosynthesis of macrolactone polyketides, *Microbial Cell Factories*, **18**, 1-22 **(2019)**

22. Doull J.L., Singh A.K., Hoare M. and Ayer S.W., Conditions for the production of jadomycin B byStreptomyces venezuelae ISP5230: Effects of heat shock, ethanol treatment and phage infection, *J Ind Microbiol*, **13**, 120–125 **(1994)**

23. Elsherbiny G., Moghannem S., Kalaba M.H., El-Sherbiny G.M. and Moghannem S.A., Enhancement of Streptomyces Baarnensis Mh-133 Activity Against Some Antibiotic Resistant Bacteria Using Biotic Elicitation Enhancement of Streptomyces Sp. Mh-133 Activity Against Some Antibiotic Resistant Bacteria Using Biotic Elicitation, *Al Azhar Bulletin of Science*, **9**, 275-288 **(2017)**

24. Falke J.J. et al, The Two-Component Signaling Pathway of Bacterial Chemotaxis: A Molecular View of Signal Transduction by Receptors, Kinases and Adaptation Enzymes, *Annu Rev Cell Dev Biol*, **13**, 457–512 **(1997)**

25. Gakuubi M.M. et al, Enhancing the Discovery of Bioactive Secondary Metabolites from Fungal Endophytes Using Chemical Elicitation and Variation of Fermentation Media, *Front Microbiol*, **13**, 898976 **(2022)**

26. Genilloud O., The re-emerging role of microbial natural products in antibiotic discovery, *Antonie Van Leeuwenhoek*, **106**, 173–88 **(2014)**

27. Gramajo H.C., Takano E. and Bibb M.J., Stationary-phase production of the antibiotic actinorhodin in Streptomyces coelicolor A3(2) is transcriptionally regulated, *Mol Microbiol*, **7**, 837–845 **(1993)**

28. Gupta Aanchal, Sharma Chandra Kant and Sharma Monika, Cultivation, Phytochemical screening and Antimicrobial analysis of caterpillar mushroom Cordyceps militaris and fruiting body, *Res. J. Biotech*., **19(1)**, 22-32 **(2024)**

29. Halder M., Sarkar S. and Jha S., Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures, *Engineering in Life Sciences*, **19**, 880–895 **(2019)**

30. Han L. et al, An Acyl-Coenzyme A Carboxylase Encoding Gene Associated with Jadomycin Biosynthesis in Streptomyces Venezuelae ISP5230, *Microbiology*, **146**, 903-910 **(2000)**

31. Hayward R.D., Leong J.M., Koronakis V. and Campellone K.G., Exploiting pathogenic Escherichia coli to model transmembrane receptor signalling, *Nature Reviews Microbiology*, **4**, 358–370 **(2006)**

32. Hengge R., Gründling A., Jenal U., Ryan R. and Yildiz F., Bacterial signal transduction by cyclic di-GMP and other nucleotide second messengers, *Journal of Bacteriology*, **198**, 15–26 **(2016)**

33. Horinouchi S. and Beppu T., Production in large quantities of actinorhodin and undecyl- prodigiosin induced by afsb in streptomyces lividans, *Agricultural and Biological Chemistry*, **48**, 2131–2133 **(1984)**

34. Imai Y., Sato S., Tanaka Y., Ochi K. and Hosaka T., Lincomycin at Subinhibitory Concentrations Potentiates Secondary Metabolite Production by Streptomyces spp, *Appl Environ Microbiol*, **81**, 3869–3879 **(2015)**

35. Iqbal M. et al, Extracting regulator activity profiles by integration of de novo motifs and expression data: Characterizing key regulators of nutrient depletion responses in Streptomyces coelicolor, *Nucleic Acids Res*, **40**, 5227–5239 **(2012)**

36. Jain D. et al, Effective Biotic Elicitors for Augmentation of Secondary Metabolite Production in Medicinal Plants, *Agriculture (Switzerland)*, **14**, 796 **(2024)**

37. Jeyasri R. et al, Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secondary metabolites in medicinal plants: an updated review, *Plant Cell, Tissue and Organ Culture*, **153**, 447–458 **(2023)**

38. Joshi J.B., Maupin-Furlow J.A. and Uthandi S., Microbial elicitors: Positive and negative modulators of plant defense, In Mitigation of Plant Abiotic Stress by Microorganisms, Elsevier, 77–102 **(2022)**

39. Katz L. and Baltz R.H., Natural product discovery: past, present and future, *Journal of Industrial Microbiology and Biotechnology*, **43**, 155–176 **(2016)**

40. Kaur S. et al, Beneficial elements for agricultural crops and their functional relevance in defence against stresses, *Archives of Agronomy and Soil Science*, **62**, 905–920 **(2016)**

41. Kawai K., Wang G., Okamoto S. and Ochi K., The rare earth, scandium, causes antibiotic overproduction in Streptomyces spp., *FEMS Microbiol Lett*, **274**, 311–315 **(2007)**

42. Lahiani A., Yavin E. and Lazarovici P., The molecular basis of toxins' interactions with intracellular signaling via discrete portals, *Toxins*, **9**, 107e **(2017)**

43. Lee K.L., Singh A.K., Heo L., Seok C. and Roe J.H., Factors affecting redox potential and differential sensitivity of SoxR to redox-active compounds, *Mol Microbiol*, **97**, 808–821 **(2015)**

44. Martínez-Burgo Y., Álvarez-Álvarez R., Rodríguez-García A. and Liras P., The pathway-specific regulator ClaR of Streptomyces clavuligerus has a global effect on the expression of genes for secondary metabolism and differentiation, *Appl Environ Microbiol*, **81**, 6637–6648 **(2015)**

45. Meena M. et al, Role of elicitors to initiate the induction of systemic resistance in plants to biotic stress, *Plant Stress*, **5**, 100103 **(2022)**

46. Moore J.M., Bradshaw E., Seipke R.F., Hutchings M.I. and McArthur M., Use and Discovery of Chemical Elicitors That Stimulate Biosynthetic Gene Clusters in Streptomyces Bacteria, *In Methods Enzymol.*, **517**, 367–385 **(2012)**

47. Mushtaq S., Abbasi B.H., Uzair B. and Abbasi R., Natural products as reservoirs of novel therapeutic agents, *EXCLI Journal*, **17**, 420–451 **(2018)**

48. Nascimento N.C. Do. and Fett-Neto A.G., Plant secondary metabolism and challenges in modifying its operation: An overview, *Methods in Molecular Biology*, **643**, 1–13 **(2010)**

49. Tripathi B.N. and Singh V., Role of metal ions as potential abiotic elicitors of secondary metabolites in plant cell, tissue and organ culture techniques **(2012)**

50. Newman D.J. and Cragg G.M., Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010, *J Nat Prod*, **75**, 311–335 **(2012)**

51. Nguyen C.T., Dhakal D., Pham V.T.T., Nguyen H.T. and Sohng J.K., Recent advances in strategies for activation and discovery/characterization of cryptic biosynthetic gene clusters in streptomyces, *Microorganisms*, **8**, 616 **(2020)**

52. Nothaft H. et al, The permease gene nagE2 is the key to N-acetylglucosamine sensing and utilization in Streptomyces coelicolor and is subject to multi-level control, *Mol Microbiol*, **75**, 1133–1144 **(2010)**

53. Ochi K. and Hosaka T., New strategies for drug discovery: Activation of silent or weakly expressed microbial gene clusters, *Applied Microbiology and Biotechnology*, **97**, 87–98 **(2013)**

54. Ochi K., Tanaka Y. and Tojo S., Activating the expression of bacterial cryptic genes by rpoB mutations in RNA polymerase or by rare earth elements, *J Ind Microbiol Biotechnol*, **41**, 403–414 **(2014)**

55. Ohnishi Y. and Horinouchi S., The A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces*, *Biofilms*, **1(4)**, 319-328 **(2004)**

56. Okada B.K. and Seyedsayamdost M.R., Antibiotic dialogues: Induction of silent biosynthetic gene clusters by exogenous small molecules, *FEMS Microbiology Reviews*, **41**, 19–33 **(2017)**

57. Ongley S.E., Bian X., Neilan B.A. and Müller R., Recent advances in the heterologous expression of microbial natural product biosynthetic pathways, *Natural Product Reports*, **30**, 1121–1138 **(2013)**

58. Pettit R.K., Small-molecule elicitation of microbial secondary metabolites, *Microbial Biotechnology*, **4**, 471–478 **(2011)**

59. Rigali S. et al, Feast or famine: The global regulator DasR links nutrient stress to antibiotic production by Streptomyces, *EMBO Rep*, **9**, 670–675 **(2008)**

60. Rodríguez H., Rico S., Díaz M. and Santamaría R.I., Two-Component Systems in Streptomyces: Key Regulators of Antibiotic Complex Pathways, *Microb. Cell Fact*., **12**, 1-10 **(2013)**

61. Rozengurt E., Mitogenic signaling pathways induced by G protein-coupled receptors, *Journal of Cellular Physiology*, **213**, 589–602 **(2007)**

62. Sandbakken I.S. Synthetic biology for drug discovery: construction of biosensors for detection of endogenously produced bacterial secondary metabolites, M.S. thesis, Institutt for bioteknologi **(2013)**

63. Sekurova O.N., Zhang J., Kristiansen K.A. and Zotchev S.B., Activation of chloramphenicol biosynthesis in Streptomyces venezuelae ATCC 10712 by ethanol shock: Insights from the promoter fusion studies, *Microb Cell Fact*, **15**, 1-10 **(2016)**

64. Schulman M.D., Valentino D. and Hensens' O., Biosynthesis of the avermectins by *Streptomyces avermitilis*: incorporation of labeled precursors, *J. Antibiot*., **39(4)**, 541-549 **(1986)**

65. Sheeler N.L., MacMillan S.V. and Nodwell J.R., Biochemical activities of the absA two-component system of Streptomyces coelicolor, *J Bacteriol*, **187**, 687–696 **(2005)**

66. Shentu X.P. et al, Substantial improvement of toyocamycin production in Streptomyces diastatochromogenes by cumulative drug-resistance mutations, *PLoS One*, **13**, e0203006 **(2018)**

67. Tanaka Y., Tokuyama S. and Ochi K., Activation of secondary metabolite-biosynthetic gene clusters by generating rsmG mutations in streptomyces griseus, *Journal of Antibiotics*, **62**, 669–673 **(2009)**

68. Tangerina M.M.P. et al, Metabolomic study of marine Streptomyces sp.: Secondary metabolites and the production of potential anticancer compounds, *PLoS One*, **15**, e0244385 **(2021)**

69. Thakur M., Bhattacharya S., Khosla P.K. and Puri S., Improving production of plant secondary metabolites through biotic and abiotic elicitation, *J Appl Res Med Aromat Plants*, **12**, 1–12 **(2019)**

70. Uguru G.C. et al, Transcriptional activation of the pathway-specific regulator of the actinorhodin biosynthetic genes in Streptomyces coelicolor, *Mol Microbiol*, **58**, 131–150 **(2005)**

71. van Bergeijk D.A., Terlouw B.R., Medema M.H. and van Wezel G.P., Ecology and genomics of Actinobacteria: new concepts for natural product discovery, *Nature Reviews Microbiology*, **18**, 546–558 **(2020)**

72. Vandecraen J., Chandler M., Aertsen A. and Van Houdt R., The impact of insertion sequences on bacterial genome plasticity and adaptability, *Critical Reviews in Microbiology*, **43**, 709–730 **(2017)**

73. Wang C. et al, Generation of Streptomyces hygroscopicus cell factories with enhanced ascomycin production by combined elicitation and pathway-engineering strategies, *Biotechnol Bioeng*, **116**, 3382–3395 **(2019)**

74. Wang L., Tian X., Wang J., Yang H., Fan K., Xu G., Yang K. and Tan H., Autoregulation of antibiotic biosynthesis by binding of the end product to an atypical response regulator, Proc. Natl. Acad. Sci. U.S.A., **106(21)**, 8617-8622 **(2009)**

75. Wang R. et al, Identification of two-component system AfsQ1/Q2 regulon and its cross-regulation with GlnR in Streptomyces coelicolor, *Mol Microbiol*, **87**, 30–48 **(2013)**

76. Xu G. et al, "Pseudo" γ-Butyrolactone Receptors Respond to Antibiotic Signals to Coordinate Antibiotic Biosynthesis, *Journal of Biological Chemistry*, **285**, 27440–27448 **(2010)**

77. Yu Z. et al, Differential regulation of antibiotic biosynthesis by DraR-K, a novel two-component system in Streptomyces coelicolor, *Mol Microbiol*, **85**, 535–556 **(2012)**

78. Zhang J., Hassan H.A., Abdelmohsen U.R. and Zahran E.M., A glossary for chemical approaches towards unlocking the trove of metabolic treasures in actinomycetes, *Molecules*, **27**, 142 **(2022)**

79. Zhou W.W., Ma B., Tang Y.J., Zhong J.J. and Zheng X., Enhancement of validamycin A production by addition of ethanol in fermentation of Streptomyces hygroscopicus 5008, *Bioresour Technol*, **114**, 616–621 **(2012)**

80. Zhu H., Sandiford S.K. and Van Wezel G.P., Triggers and cues that activate antibiotic production by actinomycetes, *Journal of Industrial Microbiology and Biotechnology*, **41**, 371–386 **(2014)**

81. Zhu S., Duan Y. and Huang Y., The application of ribosome engineering to natural product discovery and yield improvement in streptomyces, *Antibiotics*, **8**, 133 **(2019)**

82. Zong G. et al, Use of elicitors to enhance or activate the antibiotic production in streptomyces, *Critical Reviews in Biotechnology*, **42**, 1260–1283 **(2022)**.

(Received 13th December 2024, accepted 13th February 2025)

*****