

Review

Signal Molecules Regulate the Synthesis of Secondary Metabolites in the Interaction between Endophytes and Medicinal Plants

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Abstract: Signaling molecules act as the links and bridges between endophytes and host plants. The recognition of endophytes and host plants, the regulation of host plant growth and development, and the synthesis of secondary metabolites are not separated by the participation of signaling molecules. In this review, we summarized the types and characteristics of signaling molecules in medicinal plants and the recent processes in intracellular conduction and multi-molecular crosstalk of signaling molecules during interactions between endophytic bacteria and medicinal plants. In addition, we overviewed the molecular mechanism of signals in medical metabolite accumulation and regulation. This work provides a reference for using endophytic bacteria and medicinal plants to synthesize pharmaceutical active ingredients in a bioreactor.

Keywords: endophyte; medicinal plants; interaction relationship; signaling molecules; secondary metabolite



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1. Introduction

Secondary metabolites of higher plants are the main source of many natural medicines, and the plant origins of which are gradually occupying a dominant position in the field of medicine and healthcare on a global scale. Among these chemicals are ginsenosides [1], tanshinones [2], vinblastine [3], camptothecin [4], paclitaxel [5], and others with anti-fatigue, anti-blood pressure, anti-thrombotic, and anti-tumor properties, which serve as a foundation for novel medications development. However, the majority of these natural active components have distinct chemical structures, which means artificial synthesis to directly replace natural plant resources is difficult. The existing single extraction and separation method is far from satisfying the complex and diversified extraction of natural active compounds in medicinal plants, resulting in resource waste. In addition, the wave of economic development has also brought about the destruction of wild medicinal resources and unplanned and unregulated exploitation. This has accelerated the formation of the global shortage of herbal resources.

However, after medicinal plants are invaded by specific environmental microorganisms, they establish symbiotic relationships including partial symbiosis and mutualistic symbiosis, which was been accompanied by in-depth understanding and exploration of medicinal plant microbiota in recent years. Symbiosis includes offset symbiosis and mutualism. Endophytic bacteria and host plants have evolved a comparatively robust equilibrium maintenance symbiosis mechanism through ongoing synergistic evolution and balanced confrontation [6]. In the symbiotic system involving endophytic bacteria, the convenient generation of secondary metabolites of medicinal plants fully reflects the characteristics

of high yield, rapid generation, high plasticity, convenient operation, and mild reaction. Therefore, endophytic bacteria act as a special "inducer" signal to regulate plant growth and metabolism [7] and biotic and abiotic resistance [8] and to induce specific secondary metabolites [9] in a mutualistic system with plants. The signaling molecules such as organic molecules and signaling hormones in the symbiotic system are also key to the processes of endophytic bacteria recruitment, infestation, colonization [10], signal integration [11], and regulation of plant secondary metabolite synthesis. In this paper, we focus on the types and characteristics of signaling molecules involved in the symbiotic system induced by endophytic bacteria, as well as the role and regulation mechanism of signaling molecules in signal transduction and secondary metabolite synthesis in medicinal plant cells, providing ideas for the effective synthesis of medicinal ingredients.

2. Signaling Molecules Involved in the Interactions between Endophytic Bacteria and Medicinal Plants

Endophytes and host plants have formed a unique symbiotic system under long-term symbiotic synergy, becoming a functional symbiosis with diverse structures, complex composition, and dynamic change. The orderly operation of various signaling molecules and symbiotic systems provides a basis for subsequent research on the synthesis pathways of secondary metabolites in medicinal plants. Plants recognize endophytes through selective metabolic signals to restrict other microorganisms from entering the plant. After initiation of intracellular symbiotic signaling pathways, endophytes successfully colonize host plants.

2.1. Interaction of Metabolic Signaling Molecules in Endophytic Bacteria and Medicinal Plants

Symbiosis is a complex nutrient environment. In a symbiotic system, many chemicals can be used as signals to recruit and identify endophytic bacteria. These metabolites generally include (i) nutrients available only to specific microorganisms, (ii) antibacterial substances toxic to some microorganisms, and (iii) a metabolite that attracts specific microorganisms. For example, organic acids such as citric acid, malic acid, fumaric acid, and salicylic acid have been shown to play important roles as nutrients in the recruitment of endophytic bacteria [12]. Triterpenoids are another large and diverse group of plant metabolic nutrients that mediate the establishment of symbiotic systems by promoting and limiting the growth of endophytic bacteria [13]. Similarly, plants may produce a wide range of antimicrobial substances, but the regulatory mechanisms of how these molecules allow endophytic bacteria to proliferate while resisting pathogenic bacteria have not been fully described in studies. Plant-derived coumarins have antimicrobial activity against some pathogenic bacteria, but not against endophytic bacteria [14]. Similarly, rhizobacteria have evolved resistance to the toxic structural mimic of arginine (cotinine) produced by legumes, thus allowing proliferation in the inter-rhizosphere of legumes [15]. These examples show that plants can use antimicrobial products to select specific endophytes while excluding other microorganisms. Plant-secreted metabolites can also serve as signals used by hosts in symbiotic systems to attract specific endophytic bacteria. Nitrogen-fixing rhizobacteria can sense the presence of plant flavonoids through bacterial regulators that biosynthesize in conjunction with Nod factors [16]; the phytohormone strigolactone can trigger the germination of mycorrhizal (AM) fungal spores, thus signaling the presence of a potential plant host [17,18]. Symbionts can also use the presence of plant metabolites, including polyamines [19], amino acids, organic acids, or sugars [20], to indicate the presence of a plant host. Thus, the secretion of induced signals such as nutrients, antimicrobial substances, and metabolites provides the basis for plants to invoke only beneficial endophytes in a complex microbiota. The release of plant metabolic signals may be a major determinant of the formation of specific symbiotic systems between host plants and endophytes.

2.2. Receptor Signaling Molecules in the Interactions between Endophytic Bacteria and Medicinal Plants

Endophytic bacteria form a symbiosis with the host plant after successfully competing for nutrients in the host and surviving the attack of host antimicrobial metabolites. Plant receptors need to sense and integrate multiple signaling cues to successfully recognize the symbiont and determine the pathway to initiate symbiosis. Plant genomes encode hundreds of structure-specific membrane-associated pattern recognition receptors (PRRs) [21] to specifically recognize microbial-associated molecular patterns (MAMP). MAMPs that play a role in symbiotic pathways include chitosan, bacterial extracellular polysaccharides (EPS) [22], lipopolysaccharides (LPS) [23], and various protein components. In addition to this, endophytic bacteria have evolved effectors that can also act as receptor signaling molecules involved in the symbiotic pathway between endophytes and plants. The symbiosis between rhizobia and legumes begins when rhizobia secrete lipid chitoooligosaccharides (LCO) and release Nod factors [24]. Both the effector and the host plant have multiple LysM structural domains, and the LysM structural domain receptors of the host plant need to recognize the correct Nod factor separately, regulating parallel signaling pathways [25]. For example, *Tribulus Terrestris* NFP and LYK3 recognize the non-reducing and reducing ends of Nod factors, respectively [26], and initiate the signaling pathways of NFP and LYK2 [27]. In summary, the symbiont signal can be selectively, and with high affinity, delivered to downstream intracellular signaling molecules through successful recognition of multiple receptors' signaling molecules by host plants for MAMP signaling and effector signaling of endophytes, and transduction of invasion colonization signals by endophytes (schematized in Table 1).

Table 1. Signal molecules and their sources in the interaction between endophytes and medicinal plants.

Source	Action Category	Signal Molecule	Strain (Genus)	Reference
Metabolic signal molecules	Nutrients	Citric acid	<i>Rhizoctonia</i>	[28]
		Malic acid	<i>Bacillus subtilis</i> FB17	[29]
		Fumaric acid	<i>Pseudomonas fluorescens</i>	[30]
	Antibacterial substances	Succinic acid	<i>Bacillus amylolyticus</i>	[31]
		Coumarin	<i>Pseudomonas</i>	[32]
		Concanavaline	<i>Nitrogenous Rhizobium</i>	[33]
	Specific products	Triterpene	<i>Endophytic flora</i>	[34]
		Salicylic acid	<i>Endophytic flora</i>	[35]
		Flavonoid	<i>Nitrogenous Rhizobium</i>	[36]
	Metabolites	Unicornolactone	<i>Arbuscular mycorrhiza</i>	[37]
		Polyamine	<i>Pseudomonas</i>	[38]
		Amino acid	<i>Nitrogenous Rhizobium</i>	[39]
		Organic acid	<i>Nitrogenous Rhizobium</i>	[39]
		Sugar	<i>Nitrogenous Rhizobium</i>	[39]
		Extracellular polysaccharide	<i>Nitrogenous Rhizobium</i>	[40]
Receptor Signal Molecules	Conservative MAMP	Lipopolysaccharide	<i>Nitrogenous Rhizobium</i>	[41]
		Cell wall polysaccharide	<i>Verticillium dahluricum</i>	[42]
		Phospholipid protein	<i>Phytophthora camphora</i>	[43]
		Ribosomal protein	<i>Phytophthora cryptogea</i>	[44]
		Nod factor	<i>Laccaria bicolor</i>	[45]
Intracellular signal molecule	Second Messenger	LCO	<i>Laccaria bicolor</i>	[45]
		Ca ²⁺	<i>Nitrogenous Rhizobium</i>	[46]
	Hormone molecule	NO	<i>Soybean Stalk Rot Pathogen</i>	[47]
		ROS	<i>E.festuae</i>	[48]
		JA	<i>Epichloë gansuensis</i>	[49]
		SA	<i>Penicillium citri</i>	[50]

3. Endophytes Mediate the Transduction and Integration of Major Signaling Molecules for the Accumulation of Secondary Metabolites in Medicinal Plants

The signal transduction system that exists within the cell is a key bridge between endophytic bacteria and plant secondary metabolites. Endophytes can influence host metabolic pathways and gene expression processes by acting as "inducers". By recognizing the colonized signaling system, plants can gain insight into the release of endophytic bacterial molecules, which in turn activates the signaling network and biological response of the plant, influencing the related gene expression activity and mediating the synthesis and accumulation of secondary metabolites in the plant. The intracellular signals that generate the secondary metabolite pathway through signal transduction in response to endophyte elicitor stimulation in plants include JA and SA, in addition to the generally recognized second messengers. Among them, hydrogen peroxide (H_2O_2), jasmonic acid (JA), salicylic acid (SA), and other signaling molecules have special chemical structures (Figure 1) and key effects. Together with other messengers such as the calcium signaling system, they play a particularly prominent role. These signaling molecules integrate through a signaling network that conducts external stimuli to various transcription factors (TFs), and the different transcription factors will activate the relevant products to synthesize the genes.

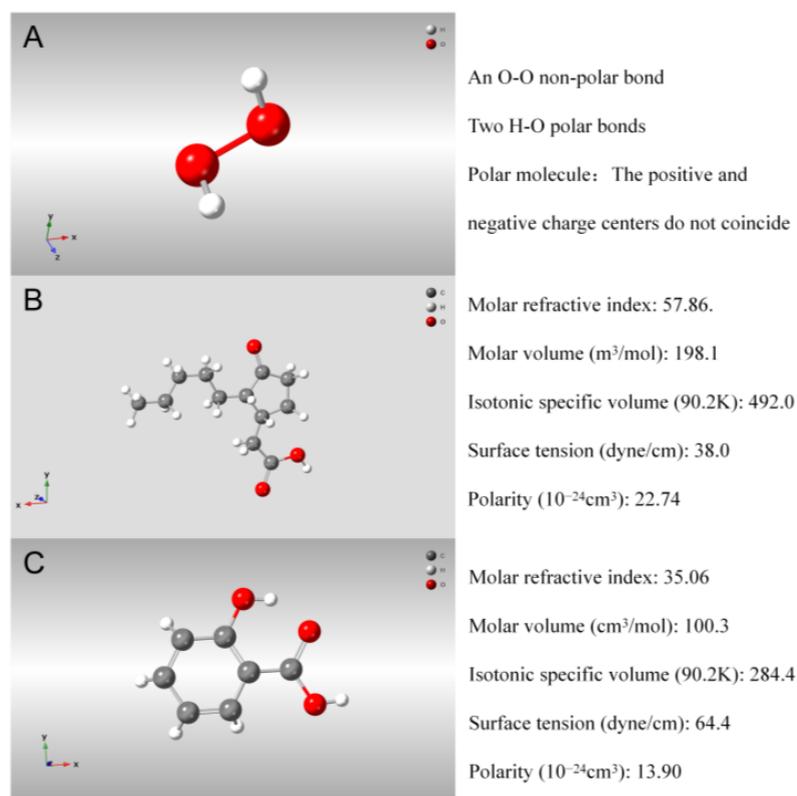


Figure 1. Molecular structure diagram of H_2O_2 , JA, SA ((A): H_2O_2 , (B): JA, (C): SA).

3.1. Intracellular Signal Transduction System of Endophytic Bacteria Mediating the Accumulation of Secondary Metabolites in Medicinal Plants

After endophytic signals are translated through the cell membrane, they are generally amplified by second messengers via the corresponding intracellular messenger system, which amplifies the signal cascade into a cellular signal. The commonly accepted second messengers are Ca^{2+} , IP3, DAG, cAMP, cGMP, and intracellular components of specific signaling such as nitric oxide [51], hydrogen peroxide, arachidonic acid, and cADPR. The calcium messenger system, the inositol phospholipid messenger system, and the cyclic nucleotide messenger system are currently widely recognized intracellular messenger

systems. In the intracellular calcium messenger system, the content of intracellular calcium ions oscillates with a certain amplitude and frequency of calcium. The cellular signaling system based on inositol phosphate metabolism generates two signaling molecules (IP₃, DAG) through the hydrolysis of phospholipase C (PLC) located on the plasma membrane after the endophytic inducer signal is recognized by the membrane receptor. The cyclic nucleotides cAMP and cGMP are produced by AIP via adenylate cyclase and GTP via guanylate cyclase, respectively. Research on the intracellular cyclic nucleotide messenger system located in the host plant has been slow, but it is clear that protein kinase (PKA) is the center of action in this signaling system, which also has synergistic effects with the calcium signaling system. Thus, it can be seen that there is crosstalk between the complex and diverse signaling systems in host plants when they are stimulated by endophytic bacteria, and this results in the formation of a signal transduction network in plant cells.

3.2. Signal Integration of Endophytic Bacteria Mediating the Accumulation of Secondary Metabolites in Medicinal Plants

Transcription factors are DNA-binding proteins with specific structures that activate gene expression in signaling pathways through specific binding with promoter elements. In the signal transduction of the interaction between host plants and endophytes, different transcription factors are responsible for regulating specific secondary metabolic pathways (schematized in Table 2). Studies have shown that transcription factors can be phosphorylated to regulate the expression of disease resistance and defense genes. For example, when *Botrytis cinerea* is inoculated with *Arabidopsis*, it can induce the activation of the MPK3/6 signaling pathway, phosphorylate ERF6 by MPK3/MPK6, improve the stability of the ERF6 protein, and regulate the expression of defense genes in *Arabidopsis* and the resistance to *Botrytis cinerea* [52]. In addition, the specific transcription factors involved in the reaction can combine with other proteins and change the functions of other transcription factors in addition to regulating the promoters of target genes, thus regulating the genes related to the synthesis of secondary metabolites.

Table 2. Categories of key transcription factors regulating plant metabolites.

Regulate the Type of Metabolite	Botany	Category	Name	Medicinal Products	Reference
Terpenoids	<i>Artemisia annua</i> Linn.	WRKY	AaWRKY17	Artemisinin	[53]
		AP2/ERF	AaERF1, AaERF2	Artemisinin	[54]
			AaORA	Artemisinin	[55]
	<i>Taxus chinensis</i>	WRKY	WRKY1	Taxol	[56]
		AP2/ERF	TcAP2	Taxol	[57]
			TcDREB	Taxol	[58]
Flavonoids	<i>Antirrhinum majus</i> L.	bHLH	TcJAMYC	Taxol	[59]
		R2R3MYB	Rosea1, Rosea2, Venosa	Anthocyanin	[60]
	<i>Gentiana scabra</i> Bunge <i>Gentiana trichotoma</i> Kusnez.	bHLH	Delila	Anthocyanin	[61]
		bHLH	GtbHLH1	Anthocyanin	[62]
		R2R3MYB	GtMybP3	Xanthone alcohol	[63]
			GtMybP4		
<i>Tartary buckwheat</i>	MYB	FtMyb1, FtMyb2, FtMyb3	Rutin	[64]	
<i>Scutellaria baicalensis</i> Georgi	MYB	SbMYB	Baicalin	[65]	

Table 2. Cont.

Regulate the Type of Metabolite	Botany	Category	Name	Medicinal Products	Reference	
Alkaloids	<i>Catharanthus roseus</i> (L.) G.Don	bHLH	CrMYC1	Vinblastine/vincristine	[66]	
			CrMYC2	Indole alkaloid	[67]	
		AP2/ERF	ORCA1, ORCA2	Vinblastine/vincristine	[68]	
			ORCA3	Vinblastine	[69]	
		bZIP	CrGBF1, CrGBF2	Indole alkaloid	[70]	
		WRKY	CrWRKY1	Vinblastine/vincristine	[71]	
		C2H2 Zinc-Finger	ZCT1, ZCT2, ZCT3	Vinblastine/vincristine	[72]	
Phenylpropanoids	<i>Coptis chinensis</i>	bHLH	CjbHLH1	Berberine	[73]	
		WRKY	CjWRKY1	Isoquinoline alkaloid	[74]	
	<i>Salvia miltiorrhiza</i> Bge.	bHLH	SmMYC	Salb	[75]	
		<i>Lonicera japonica</i> Thunb.	R2R3-MYB	LmMYB15	Chlorogenic acid	[76]
			TCP	TCP1	Brassinolide	[77]
Steroids	<i>Arabidopsis thaliana</i>	Sp/KLF	Sp1	Paris polyphylla saponin I	[78]	
		NF-κB	NF-κB	Paris polyphylla saponin II	[79]	
Quinones	<i>Hypericum</i> Linn	bcl-2	Bax	Hypericin	[80]	
Tannins	<i>Populus</i> L.	MYB	PtrMYB57	Tannin	[81]	
	<i>Onobrychis viciifolia</i> Scop.	MYB	OvMYBPA2	Condensed tannins	[82]	
Other	<i>Arabidopsis thaliana</i>	MYB-bHLH- WD40	GL1	Trichome	[83]	

4. Molecular Mechanisms of Signaling by Endophytic Bacteria Mediating the Accumulation of Secondary Metabolites in Medicinal Plants

4.1. Calcium Signal Transduction Mechanism

After elicitor treatment, plant cells demonstrate electron flow within less than 5 minutes [84], which is the early response of plant cells to the emergence of endophytic elicitors. Among them, Ca²⁺ flow is a key signal in physiological changes and is also a common second messenger in the interaction between endophytes and plants [85]. After the signaling molecules of the interacting symbiont are recognized, they activate the downstream signal transduction proteins and DMI1 [86], CNGC15, and MCA8. These channel proteins and transporters constitute calcium signal encoders. Calcium ions periodically enter and exit the cell through the calcium signal encoder, causing the calcium concentration in the cell to oscillate [87]. As one of the most conservative Ca²⁺ receptors, CaM located in the nucleus can be regarded as a decoder, which can interact with CYCLOPS to sense and decode calcium oscillation signals [88,89], activate a large number of downstream target proteins, and transcribe symbiotic signals. Transcription factors NSP1 and NSP2 induce gene expression [90] and finally produce secondary metabolites, completing the whole symbiotic interaction pathway. On the basis of the information described in the present review, it is possible to delineate the course of the essential events triggered by the elicitor as follows (schematized in Figure 2).

4.2. ROS Signal Transduction Mechanism

It has been shown that ROS can regulate the expression of plant resistance genes and plant regulatory processes that cause hypersensitivity reactions (HR) in plant cells as well as kill pathogens [91]. Endophytic bacterial inducers acidify plant cytoplasm under induction and produce two common ROS (superoxide anion radical, H₂O₂) to promote the synthesis of plant secondary metabolites. In the induction reactions of different plants, H₂O₂ and superoxide anion radicals appear with different probabilities as the major intermediates. For example, anthropogenic acidification of tobacco cytoplasm leads to increased transcript levels of phenylalanine deaminase (PAL) and 3-hydroxy-3-methylglutaryl coenzyme

A reductase (HMGR) genes, which are the basic enzymes of the phenylalanine-like synthetic pathway and isoprenoid pathway [92]. In the induced metabolism of some plants, the production of ROS is mainly dependent on NADPH oxidases and other oxidases present in mitochondria, chloroplasts, and peroxisomes [93]. Additionally, it was shown that the appearance of intracellular Ca^{2+} peaks is a prerequisite for ROS production [94]. It is pointed out that in plant cells, catalase can inhibit the activation of phenylalanine deaminase by endophytic inducers, and superoxide dismutase (SOD) can inhibit the promotion of antioxidant inducers in the synthesis of secondary metabolites [95]. Although the exact mechanism by which ROS regulate secondary metabolite production is not fully understood, they are abundantly present in pathways directly related to secondary metabolite gene expression and serve as one of the key signals for many induced metabolic reactions.

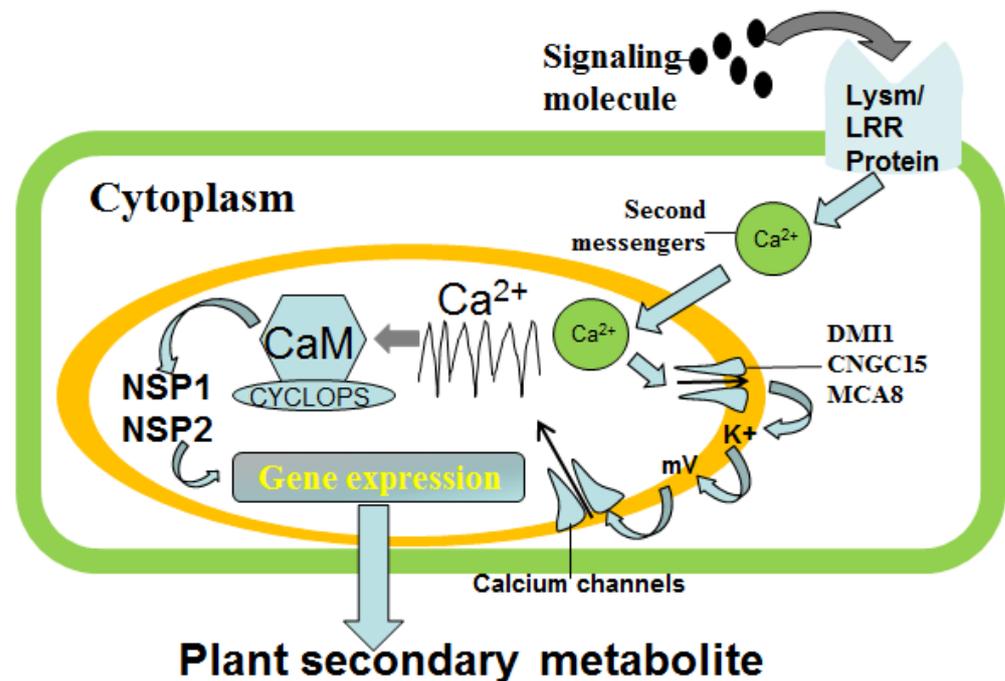


Figure 2. Ca^{2+} signal regulation in the process of endophyte-mediated accumulation of secondary metabolites of medicinal plants.

4.3. Mechanisms of Jasmonic Acid (JA) Signaling

JA, as another important signaling molecule, has a key role in signaling pathways that promote plant growth [96], enhance plant resistance [97], and promote the synthesis of secondary plant metabolites [98]. In contrast, the accumulation effect of JA content is overwhelmingly seen in the pre-response of plant cells after treatment by endophytic bacterial inducers. Endophyte inducers can induce the specific expression of relevant genes in the metabolic pathway of plant cells, while IBU and DIECA, which are inhibitors of JA synthesis, can inhibit the JA synthesis pathway. Upon induction of plants by endophytic bacteria, JA content increases and forms a complex JA-Ile complex with Ile catalyzed by JAR1. JA-Ile specifically binds to the jasmonate receptor COI1 and promotes JAZ protein ubiquitination using the E3 ubiquitin ligase SCFCOI1 complex, which in turn degrades the JAZ protein by 26S protease, releasing the regulatory MYC2, and activates transcription of JA-responsive genes [99]. On the basis of the information described in the present review, it is possible to delineate the course of the essential events triggered by the elicitor as follows (schematized in Figure 3). Many researchers have identified the presence of many transcription factors at the level of JA-mediated gene transcription, including the bHLH class, AP2 /ERF class, and some WRKY family members. This shows that JA has an important role in signaling processes in the synthesis pathways of secondary metabolites in plant cells.

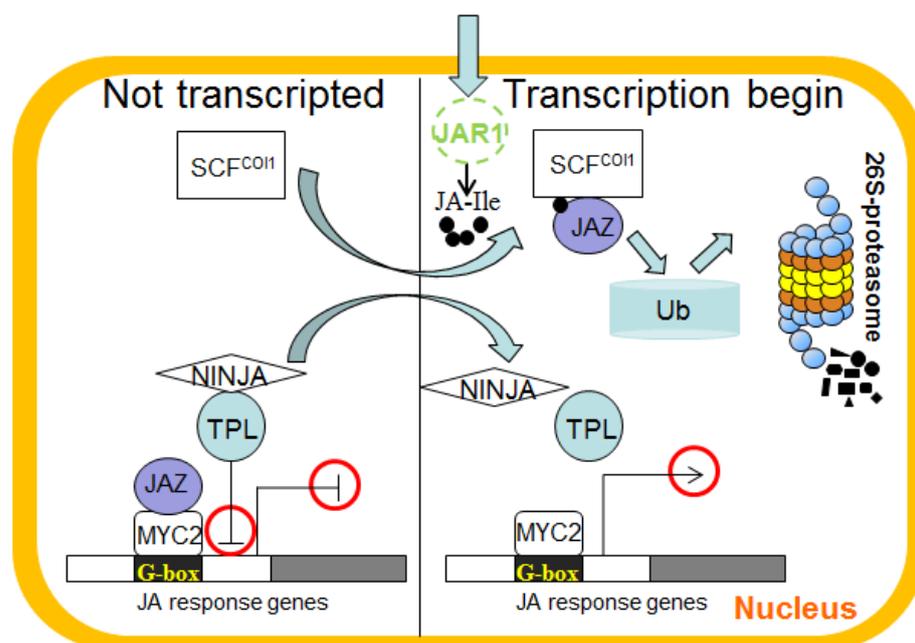


Figure 3. JA signal regulation during the accumulation of secondary metabolites of medicinal plants mediated by endophytes.

4.4. Salicylic Acid (SA) Signal Transduction Mechanism

SA, as an important endogenous hormone signaling molecule involved in the regulation of plant secondary metabolite production by endophytic bacteria, is widely present in plants and secreted by their specific cells. The basic outline of the SA signaling pathway has also been gradually developed in recent years [100]. First, SA acts as a single electron donor substrate through a complex with SA-binding proteins (SABPs) and transmits information to intracellular second messengers by inhibiting the activities of catalase, ascorbate peroxidase, etc. SA enhances the signal through its own peroxidation and self-feedback mechanism of oxidation products. Intracellular signaling triggers the activation and interaction of transcription factors such as NPR1, TGA, and WRKY [101], which ultimately induce the expression of PRs genes and the development of allergic responses, leading to the development of acquired resistance and the accumulation of secondary metabolites in the host. SA has multiple SABP-binding proteins in plants, which also reveal the possible existence of different pathways for SA-induced responses. Among them, NPR1, which is located downstream of SA accumulation and upstream of PR gene expression, is a very important positive regulator of SA-induced responses and a central regulator of plant responses [102]. This shows that the signal transduction pathway involved in SA is a key link in the synthesis of secondary metabolites induced by endophytic bacteria in plant cells.

4.5. Multi-Molecular Tandem Interaction Mechanism

A large number of studies reported above demonstrated that Ca^{2+} , ROS, JA, and SA are the signaling molecules required to participate in the induction of secondary plant metabolite synthesis. The whole process of signaling is finally completed by specific synergistic interactions among these signaling molecules. In addition, NO is also an important signaling molecule that exists in the inter-regulatory role of different signaling molecules. Related studies found that NO-specific scavengers (cPTIO) could inhibit the promotion of JA synthesis by *Aspergillus niger* cell wall inducers, and lipoxygenase inhibitors (NDGA) could inhibit the effect of NO on the synthesis of plant secondary metabolites [103]. It is thus speculated that NO may be located in the upstream position of the JA pathway. It was shown that NO could increase JA content when treated alone in *A. chrysogenum* cells [104], suggesting that NO has a facilitative effect on the JA signaling pathway in cells and that a unique autocatalytic mechanism may also exist between NO and JA. cPTIO,

an NO scavenger, can also hinder fungal inducers from inducing SA synthesis in powdered kudzu cells to some extent [105]. Therefore, it is hypothesized that NO in plant cells can not only participate in the SA signaling pathway but also mediate the inducible effect of inducers through this signaling pathway. On the other hand, it was obtained that there may be specific interactions between JA and SA. For example, JA has an antagonistic effect on the SA signaling pathway and SA-dependent gene expression [106], while SA acts as a potent inhibitor in the JA signaling pathway and JA-dependent defense gene expression. Furthermore, JA and SA can activate different defense systems and express different genes in the plant defense response [107]. When JA biosynthesis is inhibited, SA accumulation is enhanced, whereas when SA accumulation is impaired, JA production or signal transduction pathways can replace the role of SA.

The NO signaling pathway is partly dependent on ROS to some extent. The study found that the cell wall inducer of *Penicillium citrinum* can induce NO accumulation and oxidative burst. Nitric oxide scavengers (cPITO, PBITU) have a certain inhibitory effect on the induction effect of the inducer. At the same time, the single exogenous NO has a certain promotion on the production of ROS in *Taxus* cells [108], indicating that ROS synthesis and accumulation is a signal transduction event downstream of the NO pathway. Oxidative burst inhibitors (DPI) can inhibit the induction of exogenous NO on the synthesis of secondary metabolites in plant cells, indicating that NO depends on the oxidative burst to induce the synthesis of products. However, when ROS accumulation is completely inhibited, NO can still promote the synthesis of products in cells. This indicates that NO can mediate the process of elicitor-induced plant secondary metabolite synthesis through two different signal transduction pathways. On the basis of the information described in the present review, it is possible to delineate the course of the essential events triggered by the elicitor as follows (schematized in Figure 4).

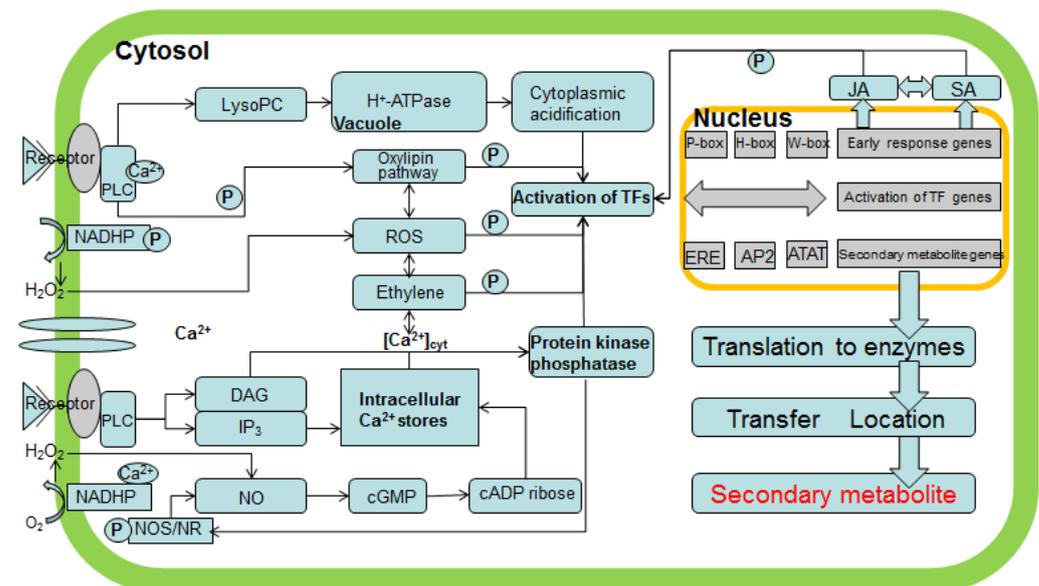


Figure 4. Multi-molecule tandem interaction mechanism in the process of endophyte-mediated accumulation of secondary metabolites of medicinal plants.

5. Conclusions

At present, a large number of studies have confirmed the role of endophytic bacteria in promoting the accumulation of secondary metabolites of medicinal plants. Compared with the primary metabolic process, the synthetic pathway of plant secondary metabolites has more complex and diverse mechanisms, which are strictly regulated by various factors inside and outside the cell. The intracellular signal transduction process is the key to connecting the external endogenous inducers and the plant secondary metabolites. In the positive response of plants to the action of endophytic bacteria, each signaling molecule

(pathway) represents a specific expression pattern in the regulatory metabolic pathway stimulated by it and ultimately translates into a cognitive physiological response to the synthesis and accumulation of secondary metabolites. The full dissection of the plant cell signaling pathway activated in the event of endophytic action in this paper may lead to strategic improvement and guide the biotechnology production of plant compounds with medical and industrial value.

6. Prospects

As our view on plant–microorganism interaction gradually expands, the exploration of a few model systems is not enough to support our full understanding of plant–microorganism interaction. In the context of the development of the microbiome, it is critical to re-examine the innate immunity or symbiosis paradigm of microorganisms and plants. In order to avoid the limitation of imagination and prediction ability, we focus on and look forward to the following research.

First of all, the biosynthesis of active substances in medicinal plants is influenced by the long-term interaction of their genotypes and environmental conditions. Only in specific ecological environments can the main enzyme genes in the secondary metabolic processes of medicaments be fully expressed. At present, most studies focus on the impact of geographical environmental factors such as climate, soil, water quality and altitude of medicinal plants. Further research is needed on the types, characteristics and distribution of the more critical medicinal plants themselves and the surrounding microbial groups. On the basis of fully recognizing the influence of external factors, the interaction between key genes, key enzymes and active components in the regulation process should be analyzed. Fully and comprehensively exploring the favorable conditions of endophytic bacteria influencing the synthesis and accumulation of secondary metabolites of medicinal herbs not only can aid in analyzing the molecular mechanism of interactions from the perspective of correlation between intrinsic factors, such as genes of medicinal plants and endophytic bacteria themselves, and signaling molecules of inter-collaboration system and biosynthesis of active ingredients of medicinal herbs, but also is a milestone to achieve a high degree of unification of internal and external factors.

Second, plant cell secondary metabolic signaling regulatory mechanisms also exhibit differences among biological taxa. Current plant endophyte species cover a wide range of microbial taxa including bacteria, archaea, fungi, and algae. In addition to the commonly used isolation and culture methods, the use of high-throughput sequencing technology based on 16S rDNA sequence analysis is gradually providing conditions for analyzing diverse endophytic bacteria. Therefore, it is necessary to conduct systematic studies on the metabolic pathways and regulatory pathways of specific endophytic bacteria within a large group of microorganisms. To further analyze the specific physiological patterns of plant responses to endophytes during the intercropping process, through the differences in the interactions between different taxa and host plants, more efficient endophytic species can be selected to improve the quality of medicinal herbs.

In recent years, some results have been achieved in the field related to the application of endophytic inducers in medicinal plants to enhance the accumulation and synthesis of their metabolites. However, the specific structure of membrane receptors, the specific recognition and binding of molecular patterns associated with endophytes, the signal transduction pathways of bilayer membrane structures, and the specific targets of action of specific endophytes are yet to be further elucidated. At the same time, the process of how plants successfully recognize beneficial endophytes while resisting pathogenic bacteria still needs to be further explored through systematic studies and deeper understanding of secondary metabolic pathways and their molecular regulatory mechanisms in medicinal plants. Based on the elucidation of the signaling molecules and the response mechanisms of endophytic bacteria affecting the biosynthesis of secondary metabolites in medicinal plants, we have established a network of signaling molecules, genes, and secondary metabolites in medicinal plants by combining high-throughput screening, combinatorial chemistry,

bioinformatics, and multidisciplinary and multi-omics to clone and deeply explore the roles of genes related to secondary metabolism regulation, analyze the roles of key enzymes and products, and construct the molecular mechanisms of signal transduction. The network of signaling molecules, genes, and secondary metabolites in medicinal plants has been established, which provides a theoretical basis for further elucidation of the role of endophytes in medicinal plants.

Relevant researchers should fully understand the laws of secondary metabolic regulation of plant cells by the interactions between endophytic bacteria and hosts, focus on the exploration of symbiotic mutualism system between endophytic bacteria and medicinal plants in a comprehensive, lasting, and specific manner with an eye on the future, actively explore and enrich the resources of endophytic plant strains, construct bioengineering reaction systems, and form a large-scale resource bank. Under the optimized culture conditions, specific and efficient endophytic bacteria will be used for intercropping with medicinal plants, which will be directly put into production and applied to the cultivation, breeding, and resource conservation of medicinal plants through biotechnological means in order to further improve their quality and yield, realize the efficient recycling of resources in the real sense, and effectively solve the current situation faced by medicinal resources.

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