

Review

Emerging Technologies Empowering the Biosynthesis of Paclitaxel

Xiaonan Liu ^{1,*}, Yuanyuan Zhang ^{1,†}, Jing Li ^{2,†} and Huifeng Jiang ^{3,4,*}

¹ Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province), Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei Key Laboratory of Industrial Microbiology, National “111” Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Wuhan 430068, China; 502510059@hbut.edu.cn (Y.Z.)

² College of Life Sciences, Nankai University, Tianjin 300071, China; lijing@tib.cas.cn (J.L.)

³ Key Laboratory of Engineering Biology for Low-Carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

⁴ College of Bioengineering, Tianjin University of Science and Technology, Tianjin 300457, China

* Corresponding author. E-mail: liuxiaonan@hbut.edu.cn (X.L.); jiang_hf@tib.cas.cn (H.J.)

† These authors contributed equally to this work.

Received: 26 January 2026; Revised: 24 February 2026; Accepted: 30 April 2026; Available online: 18 May 2026

ABSTRACT: Paclitaxel (Taxol) is a clinically important diterpenoid anticancer drug whose industrial production remains constrained by limited *Taxus* resources and semi-synthetic routes. Driven by the rapid advancement of genome mining and synthetic biology technologies, the past two years have witnessed substantial breakthroughs in elucidating the biosynthetic pathway of paclitaxel. The pathway constitutes an exceptionally complex biosynthetic network comprising approximately 20 enzymatic steps, predominantly catalyzed by cytochrome P450 monooxygenases, 2-oxoglutarate-dependent dioxygenases (ODDs), and acyltransferases. Nevertheless, microbial production of paclitaxel remains highly obstructed, largely due to inefficient catalytic abilities, enzyme promiscuities, and complex metabolic fluxes. This review summarizes recent progress in elucidating the evolutionary origins and catalytic mechanistic basis of the paclitaxel biosynthetic pathway, with particular emphasis on the emerging technologies and catalytic mechanism studies. Furthermore, current challenges and perspectives for constructing efficient artificial biosynthetic pathways are discussed, providing insights into the future biotechnological production of paclitaxel.

Keywords: Paclitaxel; Pathway analysis; P450 enzyme; Enzyme modification; Synthetic biology

1. Introduction

Paclitaxel (Taxol) is a tetracyclic diterpene compound isolated from the rare *Taxus* species, standing out as one of the most potent natural anticancer agents ever discovered. Its remarkable therapeutic efficacy stems from a unique mechanism of action: it stabilizes microtubules by preventing their depolymerization, thereby arresting mitosis and inhibiting cancer cell proliferation [1]. This compound has attracted widespread scientific interest not only for its clinical importance but also for its limited natural availability, complex molecular structure, and the intricate biosynthetic pathway required for its production. Recent research on paclitaxel biosynthesis has achieved great progress, and almost all the enzymes involved in its



pathway have been identified. However, substrate and functional promiscuity among key enzymes transform paclitaxel biosynthesis into a complex metabolic network, making it highly challenging to fully decipher the reaction process [2]. Moreover, although the synthesis of taxa-4(5),11(12)-diene in microorganisms can reach gram-scale yields, subsequent oxidation steps remain difficult to advance. Several recent review articles have summarized progress in elucidating the biosynthetic mechanism of paclitaxel, as well as the key challenges hindering the sustainable production of taxane compounds, and also proposed the state-of-the-art synthetic biology and artificial intelligence strategies to overcome these challenges [3–5]. This work focuses mainly on advances in the evolutionary origin, pathway identification, and artificial biosynthesis of paclitaxel formation. The emerging technologies, such as complex genomic sequencing, the mpXsn method, MALDI-IMS, dropout experiments, and the oxidases toolbox, will also be discussed, as well as the catalytic mechanism studies of key catalytic components.

The yew tree is a rare species listed as a national key protected wild plant, often praised as “Green Gold” for its unique medicinal, timber, and ornamental value. The sequencing of the *Taxus* genome, especially the releases of high-quality genomes for *Taxus chinensis* [6], *Taxus wallichiana* [7], and *Taxus yunnanensis* [8], has substantially accelerated research on paclitaxel biosynthesis [9]. These genomic resources, integrated with comprehensive transcriptomic datasets and advanced spatial metabolomics techniques like MALDI-IMS, have enabled unprecedented insights into the evolutionary origins and metabolic intricacies of taxane biosynthesis. For instance, MALDI-IMS analysis of *Pseudotaxus chienii*, combined with its genome sequencing, has provided crucial clues about the origin and evolution of this pathway [10]. Furthermore, innovative methodologies such as multiplexed perturbation \times single nuclei sequencing (mpXsn), which couples single-nucleus RNA-seq with high-throughput phenotypic perturbation, offer a powerful platform for deconstructing complex plant metabolic networks and identifying regulatory factors, such as the nuclear transport factor 2 (NTF2)-like protein FoTO1 [11].

Significant progress has been made in elucidating the paclitaxel biosynthetic pathway, with nearly all involved enzymes now identified. Recent research has yielded pivotal discoveries that overcome long-standing barriers. Key breakthroughs include the elucidation of the oxetane ring-forming mechanism at C4, C5, and C20 positions [12–15], the identification of the nuclear transport factor 2 (NTF2)-like protein FoTO1 (which complexes with TXS and T5 α OH to enhance early pathway yield by 10 to 17-fold) [11], T9 α OHs for different pathways, T7AT and different T1 β OHs, deacetylases and taxane C-9-oxidase (T9ox), β -phenylalanine-CoA ligase (PCL), and the clarification of final steps such as C2' α hydroxylation and 3'-N benzoylation [16]. Concurrently, enzyme engineering has produced innovative tools, such as artificial oxidase toolboxes derived from TteUPO mutants, enabling site-directed oxidation of taxanes at multiple positions and offering unconventional strategies for creating paclitaxel analogs [17]. In addition, versatile 2-oxoglutarate-dependent dioxygenases, such as Tm576, TcOGD1, and TcOGD2, could catalyze skeleton reconstructions and oxidation modifications of toxoids, which expanded the chemical space of taxane diterpenoids [2,18].

While advances in synthetic biology have made green and sustainable paclitaxel intermediates (particularly taxadiene), production via engineered cell factories is increasingly viable. Reconstruction of the downstream pathway after taxa-4(5),11(12)-diene still faces critical bottlenecks, including intrinsically low P450 catalytic efficiency, poor compatibility with heterologous hosts, and metabolic flux diversion due to enzyme promiscuity [4]. Consequently, a central research focus is the reconstruction and enhancement of the complete pathway in amenable heterologous hosts such as *Escherichia coli*, yeast, *Nicotiana benthamiana*, and cyanobacteria. Current strategies to overcome these hurdles include: (1) developing novel chassis systems (such as non-conventional yeasts, fungi, algae) for better compatibility with plant P450s; (2) employing hybrid biosynthetic-chemical approaches; (3) deploying regulatory scaffolds like FoTO1 to minimize off-target oxidation; (4) utilizing machine learning and synthetic microbial consortia for pathway optimization [5]. This review summarizes recent progress across evolutionary origin studies,

pathway identification, and artificial biosynthesis strategies (Figure 1). By offering a comprehensive analysis of key enzymatic discoveries and cutting-edge synthetic biology approaches, it aims to enhance the understanding of paclitaxel biosynthesis and catalysis, thereby laying a foundation for the systematic development of sustainable production platforms.

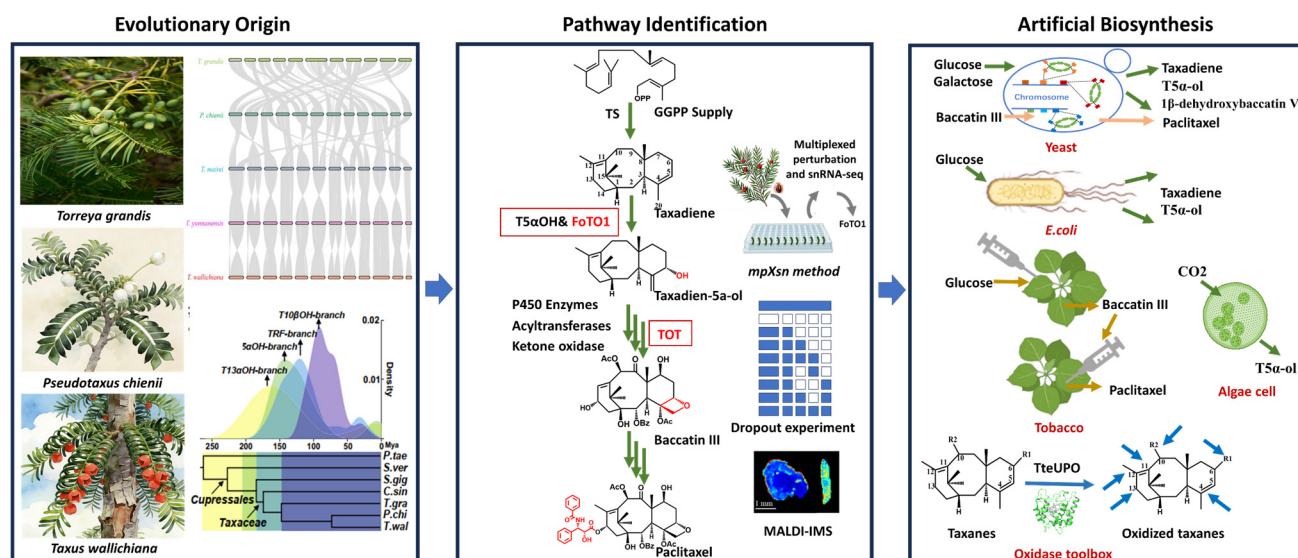


Figure 1. The evolution and synthetic biology research on paclitaxel biosynthesis. Single arrow represents a one-step reaction, three arrows represent multi-step reactions. Green arrows indicate reactions catalyzed by enzymes naturally involved in the taxol biosynthesis pathway, blue arrows indicate catalysis by oxidases not derived from the *Taxus* species. Red boxes highlight key catalytic steps.

2. Advances in the Paclitaxel Biosynthetic Pathway Identification

As a starting point for understanding the paclitaxel synthesis, a systematic review of its biosynthetic pathway is provided below. The biosynthetic pathway of paclitaxel is generally divided into three stages. The first stage involves the synthesis of the key taxane core skeleton, which leads to the formation of taxa-4(5),11(12)-diene. The second stage involves the formation of baccatin III from taxadiene via several hydroxylation and acetylation modifications. The third stage comprises the synthesis of the β -phenylisoserinoyl-CoA side chain and attaching this side chain to baccatin III followed by a series of additional modifications that ultimately result in the formation of paclitaxel (Figure 2).

2.1. Biosynthesis of the Taxa-4(5),11(12)-Diene

The biosynthesis of the taxane core begins with geranylgeranyl pyrophosphate (GGPP), which is a universal precursor to diterpenes. GGPP is formed through the condensation of one molecule of dimethylallyl pyrophosphate (DMAPP) with three molecules of isopentenyl pyrophosphate (IPP), catalyzed by geranylgeranyl pyrophosphate synthase (GGPPS). IPP and DMAPP are primarily synthesized through the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway and the mevalonate (MVA) pathway [19]. Additionally, an isomerization reaction between DMAPP and IPP can be catalyzed by isopentenyl diphosphate isomerase (IDI). Subsequently, taxa-4(5),11(12)-diene synthase (TS) catalyzes the cyclization of GGPP to generate the taxane diterpene backbone [19,20], which comprises approximately 95% taxa-4(5),11(12)-diene, and taxa-4(20),11(12)-dienetaxadiene with other minor diterpene scaffolds. In plant cells, although the cytosolic MVA pathway can also generate IPP, both GGPPS and TS harbor intrinsic plastid-targeting peptides and thus preferentially utilize IPP synthesized via the MEP pathway in plastids [20]. Following the taxa-4(5),11(12)-diene backbone, a cascade of tailoring modifications ultimately leads to the formation of baccatin III.

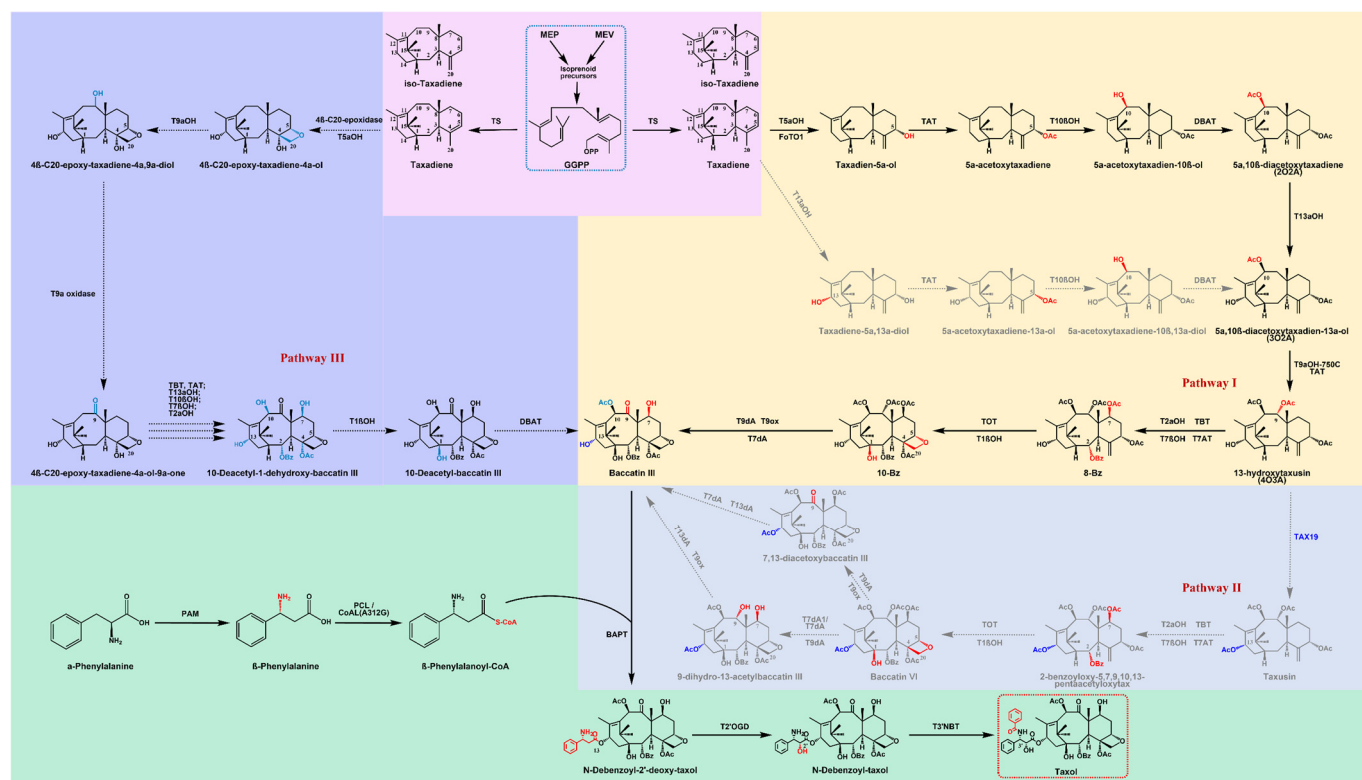


Figure 2. The recently discovered biosynthetic pathway of paclitaxel. TS, taxa-4(5),11(12)-diene synthase; T5 α OH, taxane 5 α -hydroxylase; TAT, taxa-4(5),11(12)-diene-5 α -ol-O-acetyl transferase; T10 β OH, taxane 10 β -hydroxylase; T13 α OH, taxane 13 α -hydroxylase; T2 α OH, taxane 2 α -hydroxylase; T9 α OH, taxane 9 α -hydroxylase; T7 β OH, taxane 7 β -hydroxylase; T1 β OH, taxane 1 β -hydroxylase; TBT, taxane 2 α -O-benzoyltransferase; DBAT, 10-deacetyl baccatin III-10-O-acetyltransferase; BAPT, baccatin III-3-amino, 13-phenylpropanoyltransferase; TOT, taxane oxetanase; TAX19, taxane C13-O-acetyltransferase; PAM, phenylalanine aminomutase; PCL, β -phenylalanine coenzyme A ligase; CoA(A312G), a variant of a CoA ligase from *Penicillium chrysogenum*; T7AT, taxane C-7 β -O-acyltransferase; T7dA/T7dA1, taxane 7 β -O-deacetylase; T9dA, taxane 9 α -O-deacetylase; T9ox, taxane C-9-oxidase; T13dA, taxane C13-O-deacetylase; T2/OGD, taxane 2' α -hydroxylase; T3'NBT, 3'-N-debenzoyl-2'-deoxytaxol-N-benzoyltransferase. Single arrow represents a one-step reaction, while multiple arrows represent multi-step reactions. Dashed arrow: putative pathway; Blue dashed box: key precursors; Red dashed box: the final product taxol; Red color: newly added structure in each step; Blue color: putative structure.

2.2. Biosynthesis of the Baccatin III

During the tailoring steps in paclitaxel biosynthesis, cytochrome P450 enzymes mediate most hydroxylation reactions. Several P450 enzymes have been identified that catalyze hydroxylation at the C2, C5, C7, C10, and C13 positions. Recent studies have reported progress in elucidating the functions of the C4 β -C20 epoxidase, C1 hydroxylase (T1 β OH), different C9 hydroxylases (T9 α OHs), C9 oxygenase, taxane-2' α -hydroxylase (T2' α OH), 3'-N-debenzoyl-2'-deoxytaxol N-benzoyltransferase (DBTNBT), and so on [12,15]. After years of effort, especially the explosive innovative discoveries in the past two years, the catalytic components involved in the pathway from taxa-4(5),11(12)-diene to paclitaxel have been almost completely identified (Table 1). It has long been assumed that among all hydroxylation reactions, the first to occur is C5 hydroxylation, catalyzed by taxa-4(5),11(12)-diene-5 α -hydroxylase T5 α OH [21]. Taxa-4(20),11(12)-diene is directly converted by T5 α OH to yield taxa-4(20),11(12)-dien-5 α -ol (T5 α -ol) [22]. When taxa-4(5),11(12)-diene serves as the substrate, T5 α OH converts it into T5 α -ol via C5 hydroxylation coupled with double-bond rearrangement. Additionally, T5 α OH can catalyze the formation of multiple mono- and di-oxidized byproducts, including OCT (5(12)-oxa-3(11)-cyclotaxane) and iso-OCT (5(11)-oxa-3(11)-cyclotaxane) [21]. Recently, the FoTO1 was found to be crucial for promoting the formation of the desired product T5 α -ol during the first oxidation, resolving a long-standing bottleneck in

the paclitaxel pathway reconstitution [11]. Subsequently, owing to the high catalytic promiscuity of P450 enzymes and acyltransferases, particularly in oxetane ring formation, multiple catalytic mechanisms have been proposed. The turning point occurred when an α -ketoglutarate/Fe (II)-dependent dioxygenase (ODD), named as TB328, was identified as the catalyst for C4 β -C20 epoxidation. This discovery led to the hypothesis that an ODD enzyme mediated reaction accelerates the taxa-4(5),11(12)-diene oxidation cascade [15]. Subsequently, the formation mechanism of oxetane was elucidated by different enzymes. Specifically, recent reports demonstrate that the P450 enzyme T5 α OH can also orchestrate oxetane ring formation through a two-step epoxidation mechanism, fundamentally revising earlier assumptions regarding its catalytic limits [23]. In 2024, enzymatic activity assays performed on 58 key candidate genes in a tobacco host system identified a bifunctional P450 enzyme, named TOT1, that participates in the oxetane ring formation of paclitaxel [13]. While recent systematic reviews have chronicled these conceptual shifts [24]. CYP725A55 [13] and TmCYP1 [14] are also the same P450 enzyme that catalyzes the oxetane ester formation via a cascade oxidation-concerted acyl rearrangement mechanism. These divergent results of oxetane ring formation in paclitaxel biosynthesis also suggest that the pathway to paclitaxel may involve a complex network instead of a linear network.

In previous studies, two alternative pathways have been proposed for the downstream reactions following the C5 hydroxylation of taxa-4(5),11(12)-diene: In the first proposed route, T5 α -ol undergoes acetylation at the 5 α -hydroxyl group, catalyzed by taxa-4(5),11(12)-diene-5 α -ol-O-acetyltransferase (TAT), forming Taxa-4(20),11(12)-dien-5 α -yl-acetate. This intermediate is then hydroxylated at the C10 position by taxane-10 β -hydroxylase (T10 β OH), producing Taxa-4(20),11(12)-dien-5 α -acetoxy-10 β -ol. Alternatively, the CYP725A family taxane 13 α -hydroxylase (T13 α OH) catalyzes the hydroxylation of T5 α -ol at the C13 position, yielding Taxa-4(20),11(12)-diene-5 α -13 α -diol. Further modifications occur through hydroxylation at C1, C2, C7, and C9, acylation at C2, C5, and C10, and ketonization at C9, although the precise sequence of these steps remains unclear. It was proposed that TB328 and P450 enzyme T5 α OH act synergistically to generate 4 β -C20-epoxy-taxa-4(5),11(12)-diene-4 α -ol, which is subsequently modified through C9 hydroxylation and oxidation. Using the same transient expression system and dropout experiments, taxane-1 β -hydroxylase (T1 β OH), taxane-9 α -hydroxylase (T9 α OH), and taxane-9 α -oxidase were further identified [15]. However, the precise sequences of the downstream reactions remain unresolved. Subsequently, the intermediate 2-debenzoyltaxane undergoes regioselective benzylation catalyzed by taxane-2 α -O-benzoyltransferase (TBT) to yield 10-deacetylbaaccatin III (10-DAB). This is followed by the final modification of the taxane core, in which 10-deacetylbaaccatin III-10-O-acetyltransferase (DBAT) catalyzes acetylation of the C10 hydroxyl group of 10-DAB, resulting in the formation of baaccatin III [25]. The early steps of the paclitaxel biosynthetic pathway were reconstructed in a tobacco system, demonstrating that taxa-4(20),11(12)-dien-5 α -ol can be sequentially converted by early paclitaxel pathway enzymes either in the order TAT-T10 β OH-DBAT-T13 α OH or T13 α OH-TAT-T10 β OH-DBAT, producing 5 α ,10 β -diacetoxy-13 α -ol as an intermediate for subsequent reactions, ultimately leading to the formation of the 5 α ,10 β -diacetoxy-13-one intermediate [26]. Based on the newly discovered oxetane ring-forming enzyme, the research groups of Jianbin Yan and Zhihua Zhou constructed biosynthetic pathways for baaccatin III and 5 α ,10 β -diacetoxy-13-one in tobacco and *Saccharomyces cerevisiae*, respectively. In addition, both teams identified the enzyme T9 α OH1, which is responsible for hydroxylation at the C9 position of the taxane core [12,13]. Using single-nucleus transcriptomic sequencing and a multi-gene perturbation strategy, the group identified key catalysts for paclitaxel, including T9 α OH-750C, the scaffold protein FoTO1, two T1 β OH variants (T1 β OH-184 and T1 β OH-686), and T9ox. In contrast to previous findings, they also discovered T7AT, which catalyzes acetylation or benzylation at the C-7 β position, as well as T9dA and T7dA, which remove acetyl groups at the C-9 and C-7 positions, respectively. T7AT is particularly important as it is required for the function of TOT and facilitates the formation of the oxirane ring, a crucial step in the pathway [11]. In 2026, the baaccatin III biosynthetic

pathway was further elucidated. Through the transcriptomic analysis of *T. media* cell cultures, four previously unreported deacetylases (T7dA1, T79dA, T13dA1, and T13dA2) were identified belonging to the carboxylesterase family, which plays a pivotal role in the deacetylation of plant-derived natural products. Substrate-feeding experiments utilizing a *N. benthamiana* transient expression system demonstrated that T7dA1 exhibits higher catalytic activity than the previously reported T7dA. Furthermore, these assays revealed that T79dA functions as a bifunctional deacetylase capable of removing acetyl groups at the C7 and C9 positions [27]. Most importantly, the discovery of the C13-deacetylases T13dA1 and T13dA2 uncovered a cryptic “C13 α -O-acetylation-deacetylation module” within the paclitaxel biosynthetic pathway. This critical finding repositioned classic taxoids, such as taxusin and baccatin VI, as bona fide on-pathway intermediates rather than metabolic byproducts. Although TAX19 has been characterized as a native taxane C13 α -O-acetyltransferase in *Taxus*, the baccatin III biosynthetic route necessitates a hydroxyl group at the C13 α position [28]. This structural requirement further substantiates the existence of a dynamic “C13 α -O-acetylation-deacetylation” process during biosynthesis. Within this framework, 13-hydroxytaxusin is converted to taxusin via the action of TAX19. A subsequent catalytic cascade involving T2 α OH, TBT, T7 β OH, T7AT, TOT, and T1 β OH then transforms taxusin into baccatin VI. From this point, baccatin VI can proceed through two distinct routes to generate baccatin III. In Pathway I, the sequence initiates with deacetylation at the C7 and C9 positions. *In vitro* steady-state kinetics confirmed the specific catalytic order of T13dA and T9ox, demonstrating that baccatin VI yields baccatin III through the sequential action of T7dA1, T9dA, T13dA, and T9ox. Conversely, Pathway II begins with C9 ketonization orchestrated by T9dA and T9ox. Following this step, the intermediate undergoes deacetylation at the C7 and C13 positions, catalyzed by T7dA1 and T13dA1 respectively, to ultimately form baccatin III [27]. In summary, although different research groups have drawn divergent conclusions regarding the catalytic sequence in the baccatin III biosynthetic pathway, recent studies on oxetane ring formation, the nuclear transport factor 2 (NTF2)-like protein (FoTO1), the discovery of new functional enzymes, and the construction of heterologous taxane biosynthetic pathways have collectively expanded our understanding of the functional diversity of paclitaxel P450 enzymes. Additionally, such observations offer robust proof that paclitaxel biosynthesis is rooted in a coordinated metabolic network rather than a traditional, strictly linear catalytic process.

Furthermore, within *Taxus* plants, once the cyclized taxa-4(5),11(12)-diene core is formed, it can be modified along multiple branching pathways through hydroxylation or acylation at other positions to generate diverse taxane compounds. A P450 monooxygenase, taxane-14 β -hydroxylase (T14 β OH), which functions in an alternative branch of the taxane biosynthetic pathway, was subsequently identified [29]. Studies have shown that the expression of T14 β OH is negatively correlated with paclitaxel content, suggesting that multiple biosynthetic routes for taxanes may exist in *Taxus*, forming a complex synthetic network. After the formation of the tricyclic diterpene core, various enzymatic modifications lead to the production of diverse taxane compounds. Subsequent studies have shown that, in addition to the 19 reactions required to convert GGPP to paclitaxel, several branching pathways involving alternative hydroxylation and acylation reactions lead to the formation of nonproductive taxanes, which is a major cause of the accumulation of numerous intermediate byproducts [25].

2.3. Biosynthesis, Attachment, and Modification of the β -Phenylalanyl Side Chain

Initially, phenylalanine aminomutase (PAM) catalyzes the isomerization of α -phenylalanine to β -phenylalanine [30]. Subsequently, β -phenylalanyl-CoA ligase (PCL) activates this side chain to form β -phenylalanyl-CoA [31]. In 2023, efforts to characterize side-chain attachment successfully identified the native β -phenylalanyl-CoA ligase sequence utilizing a tobacco transient expression system [15]. To further optimize this catalytic step, subsequent comparative assays in 2025 evaluated three distinct CoA ligases: the native TBPCCL from *Taxus baccata*, AAE16 from *Taxus chinensis*, and an engineered fungal variant,

CoAL(A312G), derived from *Penicillium chrysogenum* [16]. These functional comparisons demonstrated that CoAL(A312G) possesses significantly superior catalytic activity relative to both plant-derived enzymes. Previous studies have demonstrated that baccatin III:3-amino-3-phenylpropanoyltransferase (BAPT) catalyzes the acylation of baccatin III at the C13 position using β -phenylalanyl-CoA as the acyl donor, generating β -phenylalanyl baccatin III [32]. This intermediate is subsequently hydroxylated at the C2' position of the side chain by taxane-2' α -hydroxylase (T2' α OH) to yield 3'-N-debenzoyltaxol. Finally, 3'-N-debenzoyl-2'-deoxytaxol N-benzoyltransferase (DBTNBT) catalyzes the benzoylation of the side-chain C3' nitrogen atom, producing paclitaxel [32]. In 2025, Sotirios C. Kampranis' team revised the final two key steps of side-chain biosynthesis in the paclitaxel pathway, specifically C2' α -hydroxylation and 3'-N-benzoylation. They identified taxoid-2'-oxoglutarate-dependent dioxygenase (T2'OGD) as the enzyme responsible for C2' hydroxylation and taxoid-3'-N-benzoyltransferase (T3'NBT) as the enzyme responsible for 3'-N-benzoylation, which was different from the previously proposed roles of T2' α OH (TB506) and DBTNBT. They also investigated the sequential order from baccatin III to paclitaxel. First, the C13 hydroxyl group of baccatin III is conjugated with β -phenylalanyl-CoA by BAPT to form the intermediate N-debenzoyl-2'-deoxytaxol. Subsequently, T2'OGD hydroxylates the C2' position of this intermediate, generating N-debenzoyltaxol. Finally, T3'NBT transfers a benzoyl group to the N3' position of the side chain, yielding the final product paclitaxel. Moreover, the study revealed that the last two steps of paclitaxel biosynthesis do not necessarily require baccatin III as the substrate. Instead, 10-deacetylbaccatin III can also serve as a substrate for T2'OGD, undergoing C2' hydroxylation and subsequent N3' benzoylation, followed by DBAT-mediated acetylation to produce paclitaxel. These findings demonstrate that while the action of BAPT is not strictly fixed in order, the sequence of T2'OGD and T3'NBT is invariant, as N3' benzoylation can only occur after C2' hydroxylation has been completed [16].

Table 1. The identified paclitaxel biosynthetic enzymes and references.

	Name	Abbreviation	Accession Number	References
1	Geranylgeranyl diphosphate synthase	GGPPS	AF081514	[33]
2	Taxa-4(5),11(12)-diene synthase	TS	AY364469	[34]
3	Taxa-4(5),11(12)-diene-5 α -hydroxylase	T5 α OH	AY289209	[35]
4	Taxa-4(5),11(12)-diene-13 α -hydroxylase	T13 α OH	AY056019	[36]
5	Taxa-4(5),11(12)-diene-5 α -ol-O-acetyl transferase	TAT	AF190130	[37]
6	Taxa-4(5),11(12)-diene-10 β -hydroxylase	T10 β OH	AF318211/ AY563635	[31,38]
7	Taxa-4(5),11(12)-diene-7 β -hydroxylase	T7 β OH	AY307951	[38]
8	Taxa-4(5),11(12)-diene-2 α -hydroxylase	T2 α OH	AY518383	[39]
9	Taxane-2 α -O-benzoyl transferase	TBT	AF297618	[40]
10	10-deacetylbaccatin III-10-O-acetyl transferase	DBAT	AF193765	[41]
11	Phenylalanine aminomutase	PAM	AY582743	[30]
12	β -phenylalanoyl-CoA ligase	PCL	KM593667	[31]
13	Baccatin III:3-amino, 13-phenylpropanoyltransferase	BAPT	AY082804	[31]
14	Taxane-2' α -hydroxylase	T2' α OH	KP178208	[32]
15	N-benzoyl transferase	DBTNBT	AF466397	[42]
16	Taxa-4(5),11(12)-diene-14 β -hydroxylase	T14 β OH	AY188177	[29]
17	Cytochrome P450 reductase	TcCPR	AY571340	[29]
18	C4 β -C20 epoxidase	TB328		[15]
19	Taxane 1 β -hydroxylase	T1 β OH		[15]
20	Taxane 9 α -hydroxylase	T9 α OH		[15]
21	Taxane 9 α -dioxygenase	T9OX		[15]
22	Phenylalanine-CoA ligase	PCL		[15]

23	Taxane oxetanase	TOT	[12]
24	Taxane 9 α -hydroxylase	T9 α OH1	[12]
25	Taxane 9 α -hydroxylase	CYP725A37 PP197199/PP197200	[13]
26	Taxane oxetanase	CYP725A55	PP197201 [13]
27	Acyltransferase	AT5	PP197202 [13]
28	CoA ligase	CoAL(A312G)	[16]
29	Taxoid-2'-oxoglutarate-dependent dioxygenase	T2'OGD	PQ015324 [16]
30	Taxoid-3'-N-benzoyltransferase	T3'NBT	PQ015327 [16]
31	Taxane 9 α -hydroxylase	T9 α OH-750C	[11]
32	Taxane 1 β -hydroxylase	T1 β OH-184	[11]
33	Taxane 1 β -hydroxylase	T1 β OH-686	[11]
34	Taxane 7 β -O-acyltransferase	T7AT	[11]
35	DeAc898	T9dA	[11]
36	DeAc1023	T7dA	[11]
37	Taxane C7 β -O-deacetylase	T7dA1	PX848829 [27]
38	Taxane bifunctional deacetylase	T79dA	PX848830 [27]
39	Taxane C13-O-deacetylase	T13dA1	PX848827 [27]
40	Taxane C13-O-deacetylase	T13dA2	PX848828 [27]
41	Taxoid acetyltransferase	TAX19	AY628434 [28]

3. Multi-Omics Approaches Facilitating the Evolutionary Origins Illustration of Paclitaxel Biosynthesis

In recent years, omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, have been extensively applied to paclitaxel biosynthesis, functional gene discovery, transcription factor analysis, and the detection of associated metabolites. These approaches provide robust technical support for molecular biology studies and the metabolic pathway analysis of paclitaxel. Moreover, the generation of large-scale omics data and integrative multi-omics analyses has laid a solid foundation for investigating the biosynthesis and regulatory mechanisms of secondary metabolites in *Taxus*.

3.1. Applications of Genomic Analysis in Paclitaxel Biosynthesis

In recent years, three Chinese research teams have successively released the genomic data of *Taxus wallichiana* var. *mairei* [6], Himalayan yew (*Taxus wallichiana*) [7], and *Taxus yunnanensis* [8]. These studies have systematically investigated the evolutionary origins of the paclitaxel biosynthetic pathway and its key gene clusters, providing valuable insights into the formation and regulatory mechanisms of paclitaxel biosynthesis. Currently, the cloned hydroxylases involved in paclitaxel biosynthesis from *Taxus* species are classified into the CYP725A subfamily [7]. Through genome analysis of *Taxus mairei*, researchers revealed the genomic distribution and regulatory patterns of the cytochrome P450 family and discovered that paclitaxel biosynthetic genes are clustered on chromosome 9 of *Taxus*. Furthermore, by constructing a co-regulatory gene network, they identified 17 CYP725A genes, three transferases, and 10 transcription factors [6]. In addition, chromosome-level whole-genome sequencing of *Taxus wallichiana* (Himalayan yew) enabled the resolution of key gene clusters responsible for paclitaxel biosynthesis, leading to the identification of 31 candidate P450 genes. Within this cluster, the isoenzymes TS-2 and T5 α OH-2, homologous to TS and T5 α OH, were identified. Furthermore, the study demonstrated that tandem gene duplication is the primary driving force behind the evolution of gene families involved in paclitaxel biosynthesis in *Taxus*, providing essential genetic resources for the comprehensive elucidation of the paclitaxel biosynthetic pathway [7]. Genomic analysis of *Taxus yunnanensis* (Yunnan yew) revealed that the gene clusters involved in paclitaxel biosynthesis are predominantly located on chromosome 12 and that the hydroxylase gene families associated with paclitaxel biosynthesis have undergone significant expansion, thereby laying a solid foundation for understanding the formation of the paclitaxel biosynthetic pathway

[8]. Relevant genomic studies will enhance our understanding of the paclitaxel biosynthetic pathway and its genetic basis, contributing to the resolution of key scientific questions in gymnosperm genome evolution. The candidate genes and transcription factors identified through these efforts provide valuable genetic resources and molecular targets for paclitaxel biosynthesis research, facilitating the design and application of heterologous paclitaxel production.

To investigate the conservation of the paclitaxel biosynthesis pathway within closely related Taxaceae species, Shen's team generated a chromosome-level genome assembly of *Pseudotaxus chienii* and performed matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) analysis. This approach yielded a series of spatially resolved metabolite maps comprising 9999 data points, revealing tissue-specific accumulation patterns of bioactive taxanes. Comparative genomic analysis across *P. chienii*, *Taxus wallichiana*, and *Torreya grandis* further indicated that disruption of TBT and TOT1 (CYP1) enzymatic steps may redirect metabolic flux toward taxusin or related analogs rather than paclitaxel. Notably, no taxa-4(5),11(12)-diene synthase (TS) homolog was identified in *T. grandis*, suggesting the absence of a functional taxane biosynthesis pathway in this species. Collectively, this genomic study of *P. chienii* provides important insights into the evolutionary origins and diversification of taxane biosynthesis [10].

3.2. Application of Transcriptome Analysis in Paclitaxel Biosynthesis

With the advancement of high-throughput sequencing technologies, a significant number of *Taxus* transcriptome datasets have been released. During 2024, a total of 1254 transcriptome datasets could be available in GenBank. Transcriptome-based analyses have identified several candidate genes and transcription factors involved in the paclitaxel biosynthesis pathway [43]. Moreover, comparative transcriptome analyses have been employed to investigate the tissue-specific distribution and differential accumulation of taxanes across various *Taxus* species [44]. Recent mining of these publicly available resources has uncovered several previously uncharacterized catalytic components, including C4 β -C20 epoxidase, T9 α OH, PCL [31], and T2' α OH [15]. In addition, using RNA-Seq technology, researchers have investigated the regulatory mechanisms of paclitaxel and other taxanes, identifying several transcription factors that modulate paclitaxel biosynthesis. Analyses and functional studies of transcription factor families, including ERF, bHLH, WRKY, MYC, and MYB, have identified regulators of paclitaxel biosynthetic gene expression, thereby contributing to increased paclitaxel accumulation in transgenic *Taxus* cell lines [45]. The discovery of these functional genes and transcription factors provides critical insights for advancing the genetic engineering of paclitaxel biosynthesis and elucidating the regulatory mechanisms attributing its production.

3.3. Application of Multi-Omics Approaches in Paclitaxel Biosynthesis

Integrative multi-omics analyses allow the capture of dynamic regulations in *Taxus* plants and the paclitaxel biosynthetic pathway from multiple perspectives, thus facilitating the exploration of the complex mechanisms underlying paclitaxel biosynthesis. Combined transcriptome and metabolome analyses help elucidate the regulatory networks of gene expression and metabolic pathways responsible for metabolite synthesis in *Taxus*, laying a foundation for delineation of taxane biosynthesis routes. Similarly, integrating proteomic and metabolomic data provides deeper insights into the mechanisms underlying gene expression regulation. Yu et al. systematically analyzed *Taxus* stem tissues using proteomic and metabolomic approaches. Metabolomic profiling revealed tissue-specific accumulation of metabolites in the stems, while proteomic analysis identified 10 key enzymes involved in paclitaxel biosynthesis. Additionally, they found that TmMYB3, specifically expressed in the phloem, regulates paclitaxel biosynthesis by activating the expression of TBT and TS within the pathway, highlighting its critical role in transcriptional regulation of paclitaxel production. The authors conducted a quantitative analysis of paclitaxel, 10-deacetylpaclitaxel,

baccatin III, and 10-DAB in four major stem tissues using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The results revealed that paclitaxel and 10-DAB predominantly accumulated in the phloem, baccatin III mainly in the pith, and 3'-N-debenzoyltaxol (DAP) primarily in the cortex and phloem, while all four metabolites exhibited the lowest levels in the xylem [46]. Recently, the same authors employed advanced mass spectrometry imaging techniques to generate spatial distribution maps of taxane compounds in young *Taxus* stems. Furthermore, the study revealed that T14 β OH, T5 α OH, and TS are predominantly expressed in endodermal cell populations, T10 β OH and DBTNBT are primarily expressed in xylem parenchyma cells, and DBAT is expressed in epidermal cell populations. Through single-cell expression profiling, numerous cell type-specific transcription factors, including members of the MYB, TEM, RAV, and NAC families, were identified, and their downstream target genes were predicted. This work established a cell type-resolved expression atlas of young *Taxus* stems at single-cell resolution, providing a foundation for further studies on the cell-specific regulation of taxane biosynthesis [47]. Liang and colleagues, based on genomic and transcriptomic data from *Taxus yunnanensis*, *Taxus wallichiana* var. *mairei*, and related species, conducted gene co-expression analyses across multiple tissues (leaves, roots, stems, and arils) under various treatments, including methyl jasmonate induction. Through this approach, they successfully identified the two key enzymes catalyzing the final steps of paclitaxel biosynthesis: taxoid-2'-oxoglutarate-dependent dioxygenase (T2'OGD) and taxoid-3'-N-benzoyltransferase (T3'NBT) [16]. Recently, a strategy called "multiplexed perturbation \times single nuclei" (mpXsn) was developed, in which 136 different treatments, including hormones, pathogens, and metabolic intermediates, were applied to *Taxus* tissues. Following the treatments, the tissues underwent single-nucleus RNA sequencing, enabling the construction of a highly accurate gene co-expression network. This network provided a clearer understanding of gene interactions and their roles in paclitaxel biosynthesis. A key strength of the mpXsn strategy is its ability to partition known paclitaxel biosynthesis genes into three distinct expression modules corresponding to the early, middle, and late stages of the biosynthetic pathway. From this, eight important new genes, including T9 α OH-750C, T7AT, and T1 β OH, were identified. In this study, mpXsn served as a powerful discovery tool, with metabolomics used to assess phenotypic outcomes and validate findings, while proteomics provided insights into the underlying mechanisms [11]. All results were interpreted within the larger genomic context. This multi-omics approach successfully addressed the challenges posed by the large and complex genome of *Taxus*, providing valuable insights into paclitaxel biosynthesis.

4. Advances in Microbial and Plant Chassis Engineering for the Biosynthesis of Paclitaxel

As a disruptive innovation in biotechnology, synthetic biology offers novel solutions to critical challenges in resource sustainability, environmental protection, and related fields. The biosynthesis of plant natural products has emerged as a research hotspot of synthetic biology. To date, intermediate pathways of paclitaxel and the heterologous production of several precursors have been successfully reconstructed in diverse chassis organisms, including *Escherichia coli*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, tobacco cells, and endophytic fungi (Table 2).

4.1. Advances in Paclitaxel Biosynthesis Using Prokaryotic Chassis

Early studies directly overexpressed IDI, GGPPS, and TS in *E. coli*, achieving a taxa-4(5),11(12)-diene yield of approximately 0.5 mg/L [48]. Subsequent overexpression of the endogenous MEP pathway in *E. coli* further increased taxa-4(5),11(12)-diene production to 1.3 mg/L. A multivariate modular metabolic engineering strategy partitioned the paclitaxel biosynthetic pathway into two functional modules for systematic precursor enhancement: an upstream module containing four rate-limiting genes (*dxs*, *idi*, *ispD*, and *ispF*) for IPP production, and a downstream module containing two heterologous genes (GGPPS and

TS) for taxa-4(5),11(12)-diene generation. By optimizing both the native MEP pathway and the taxa-4(5),11(12)-diene biosynthetic pathway, the researchers successfully overexpressed TS and T5 α OH in *E. coli*, achieving an approximately 15,000-fold increase in taxa-4(5),11(12)-diene production, reaching 1 g/L—the highest reported yield to date. Simultaneously, taxa-4(5),11(12)-dien-5 α -ol (T5 α -ol) production increased 2400-fold, reaching 58 ± 3 mg/L [49], thereby laying the foundation for subsequent P450 mediated oxidation to downstream metabolites. The study also demonstrated that when exogenous indole concentrations exceeded approximately 100 mg/L, taxa-4(5),11(12)-diene synthesis was severely inhibited, and further increases in indole concentration suppressed cell growth, with the inhibitory effect being highly strain-dependent. The biochemical mechanism underlying the interaction between indole and the isoprenoid pathway remains unclear; however, the results suggest that indole may act synergistically with isoprenoid-derived terpenoids to inhibit cell growth. Subsequently, the same research group [50] combined the GGPPS and TS genes with *E. coli* native MEP pathway to investigate the effects of different promoters and cellular backgrounds on taxa-4(5),11(12)-diene production. They found that JM109 (*DE3*) produced approximately 2.5 times more taxa-4(5),11(12)-diene than the BL21 (*DE3*) strain. Metabolic engineering strategies incorporating the mevalonate (MVA) pathway alongside the TS gene into *E. coli* yielded a modest taxa-4(5),11(12)-diene output of only 11.3 ± 0.5 mg/L [8]. Prompted by these limitations in bacterial hosts, parallel studies shifted the production platform from bacteria to filamentous fungi. Initial optimizations in *Alternaria alternata* TPF6 evaluated the relative strengths of six heterologous promoters. The integration of these characterized promoters for the targeted overexpression of heterologous IDI, tHMGR (truncated 3-hydroxy-3-methylglutaryl-CoA reductase), and TS ultimately established a stable fungal biosynthesis system, achieving a taxa-4(5),11(12)-diene titer of 61.9 ± 6.3 μ g/L [19].

Recent engineering efforts directed toward the *de novo* synthesis of paclitaxel precursors established a heterologous MVA pathway in *E. coli*. The researchers first screened key enzymes in the pathway, including TS and cytochrome P450 reductase (CPR), and optimized both the linker between T5 α OH and CPR fusion proteins and the fermentation conditions. Ultimately, the oxidized taxane yield reached 27 mg/L, with T5 α -ol production reaching 7 mg/L, representing a 12-fold and 23-fold increase, respectively. In a parallel approach, co-expression of GGPPS and TS in *E. coli* achieved a taxa-4(5),11(12)-diene yield of 93.5 mg/L in shake-flask cultures. Expansion of the microbial production platform to *Bacillus subtilis* involved overexpressing a plant-derived TS gene alongside a customized synthetic operon. This operon carried genes from *B. subtilis*'s native MEP pathway, as well as IspA (encoding geranyl-geranyl and farnesyl diphosphate synthase), which enabled the production of taxa-4(5),11(12)-diene in *B. subtilis* 168 [51]. Furthermore, by overexpressing the *crtE* gene (encoding GGPPS) to enhance GGPP supply, the strain achieved a substantial increase in taxa-4(5),11(12)-diene production. Compared with a strain expressing only TS and relying on *B. subtilis*'s native pathway, the overexpression of MEP pathway enzymes, IspA and GGPPS resulted in an 83-fold increase in taxa-4(5),11(12)-diene yield. The engineered strain produced a total taxa-4(5),11(12)-diene yield of 17.8 mg/L, demonstrating that *B. subtilis* can serve as a promising platform for paclitaxel production. Subsequently, taxa-4(5),11(12)-diene undergoes the first oxidation step catalyzed by T5 α OH. However, in *E. coli*, the absence of an internal membrane system and the lipophilic N-terminal region of P450 reduce its solubility, making the successful expression of P450 enzymes in *E. coli* particularly challenging. N-terminal engineering of the P450 enzyme, coupled with the systematic optimization of its coupling with CPR, successfully facilitated high-level T5 α OH expression in *E. coli* and achieved a hydroxylated taxane yield of 570 ± 45 mg/L [52]. Heterologous introduction of the DBAT gene into *E. coli*, supplemented by the feeding of exogenous 10-DAB, further enabled the successful semi-synthesis of the key intermediate baccatin III.

Due to the absence of efficient isoprenoid biosynthetic pathways, the use of *E. coli* and other prokaryotic hosts for the heterologous production of plant metabolites presents inherent limitations. Additionally, prokaryotic hosts often produce target proteins in insoluble and nonfunctional forms, and the

supply of NADPH, a crucial cofactor for plant cytochrome P450 enzymes, is severely limited in prokaryotic systems. Furthermore, prokaryotes lack the compartmentalization found in eukaryotic cells, which creates distinct intracellular environments in eukaryotes, enabling the spatial and temporal partitioning of intermediates during final product formation. Consequently, unicellular eukaryotes, such as yeast, with their membrane-bound organelles and cellular compartmentalization, have attracted considerable attention for heterologous production [53].

4.2. Advances in Paclitaxel Biosynthesis Using Yeast Chassis

Microbial co-culture platforms integrating *E. coli* and *S. cerevisiae* successfully enabled the biosynthesis of oxygenated taxanes. In this system, *E. coli* was utilized for its rapid growth capacity and efficient taxa-4(5),11(12)-diene production, while *S. cerevisiae* was chosen for its abundant intracellular membrane structures that enable the functional expression of cytochrome P450 enzymes. However, ethanol produced by *S. cerevisiae* acts as a growth inhibitor for *E. coli*. To overcome this limitation, the researchers employed xylose as the carbon source, which can only be metabolized by *E. coli*. During xylose consumption, *E. coli* generates acetate, which can be utilized by *S. cerevisiae* without leading to ethanol production. Ultimately, this strategy resulted in the production of 33 mg/L of oxygenated taxanes. DeJong et al. successfully introduced eight genes involved in the paclitaxel biosynthetic pathway (GGPPS, TS, T5 α OH, T10 β OH, T13 α OH, TAT, TBT, and DBAT) into *S. cerevisiae* using two plasmids. The expression of these genes was verified through both *in vivo* and *in vitro* experiments, and the production of GGPP and taxa-4(5),11(12)-diene was detected in the system. This work demonstrated the functional activity of fusion proteins in yeast and confirmed that the native isoprenoid precursors IPP and DMAPP are sufficient to initiate the terpenoid biosynthetic pathway. However, the yield of T5 α -ol was very low. Engels et al. successfully introduced heterologous genes encoding isoprenoid biosynthetic enzymes along with regulatory factors that suppress competing pathways into *S. cerevisiae*. When yeast co-expressed HMGR, the mutated regulatory protein UPC2-1, GGPPS, and TS, the production of taxa-4(5),11(12)-diene reached 8.7 mg/L. Subsequently, Ding et al. identified the most catalytically efficient GGPPS variant by protein modeling and molecular docking, and co-expressed it with ERG20, tHMGR, and TS in *S. cerevisiae*, resulting in a taxa-4(5),11(12)-diene titer of 72.8 mg/L [54].

Integration of a tagged and multi-copy TS gene driven by the GAL1 promoter resulted in a 22-fold enhancement in taxa-4(5),11(12)-diene titer. Subsequent fermentation of this engineered platform produced 57 ± 3 mg/L at 30 °C, and the yield was further elevated to 129 mg/L by reducing the cultivation temperature to 20 °C [55]. Further genetic modifications to strain LRS5 involved the introduction of genes encoding T5 α OH, a homologous CPR, and the TAT gene from *Taxus cuspidata*, yielding the advanced strain LRS6. Through optimization of fermentation conditions and pH control, the researchers achieved a 2.7-fold increase in oxygenated taxane production, reaching 78 mg/L [56]. Subsequent efforts to maximize T5 α OH activity at both enzymatic and whole-cell levels in *S. cerevisiae* revealed that the uncoupling of T5 α OH and CPR interactions potentially facilitates the formation of oxygenated taxanes. Through the construction of fusion proteins to enhance T5 α -ol production, they achieved a maximum oxygenated taxane yield of approximately 361.4 ± 52.4 mg/L using 10 mL resting cell cultures, with T5 α -ol reaching 38.1 ± 8.4 mg/L [57]. Integration of a push-pull strategy with the endogenous MVA pathway in yeast successfully maximized precursor supply while minimizing competitive by-pathway metabolism. Enhancing the soluble expression of TS via SUMO fusion, coupled with the targeted overexpression of tHMG1, GGS1, and TS, ultimately yielded a taxa-4(5),11(12)-diene titer of 101.4 mg/L during fed-batch fermentation. Walls and colleagues addressed nutrient limitation by enhancing culture nutrition, and under optimized conditions in a 1 L bioreactor, they achieved a maximum taxa-4(5),11(12)-diene titer of 71 ± 8 mg/L after 95 h of cultivation. The main products of T5 α OH, iso-OCT, OCT, and T5 α -ol-accumulated to 16 ± 3 , 44 ± 3 , and 42 ± 4 mg/L, respectively, while the TAT product T5 α -Ac reached a maximum of 21 ± 0.3 mg/L, nearly

sixfold higher than previously reported levels [56]. However, due to the poor expression and low catalytic activity of T5 α OH in heterologous hosts, its primary products remained OCT and its isomer iso-OCT, with less than 10% of the taxa-4(5),11(12)-diene precursor ultimately converted to the target T5 α -ol. As observed in *E. coli*, the high-level catalytic activity of P450 enzymes in yeast is kinetically dependent on efficient intracellular NADPH supply. Additionally, the formation of by-products and other endogenous metabolites in yeast interferes with the heterologous production of paclitaxel.

In 2024, the biosynthetic pathway for highly oxidized paclitaxel intermediates was reconstructed in *S. cerevisiae* by feeding T5 α -ol. This work systematically elucidated the complete biosynthetic route from the C5 mono-oxygenated product of taxa-4(5),11(12)-diene to the highly oxidized 1 β -dehydroxybaccatin VI, advancing a comprehensive understanding of paclitaxel biosynthesis. The researchers identified the paclitaxel 9 α -hydroxylase responsible for the fourth hydroxylation step, as well as CYP725A55, which catalyzes oxetane ring formation via a cascade oxidation coupled with acyl rearrangement mechanism, and the acetyltransferase responsible for C7-OAc formation. Furthermore, the study highlighted the need to further clarify the complete biosynthetic pathway from 1 β -dehydroxybaccatin VI to baccatin III, as well as the parallel biosynthetic pathways leading to paclitaxel, and emphasized the importance of confirming the necessity of taxane-1 β -hydroxylase and taxane-9 α -oxidase in pathway construction. This systematic work provides a solid foundation for fully elucidating paclitaxel biosynthesis and its heterologous production in yeast cells.

In 2025, the research group of Sotirios C. Kampranis employed a tobacco-based system to successfully identify and validate the catalytic activities of CoAL(A312G), T2'OGD, and T3'NBT [16]. Subsequently, they reconstructed the paclitaxel side-chain biosynthetic pathway in *Saccharomyces cerevisiae*. However, due to poor protein stability and solubility of BAPT in yeast cells, the intermediate N-debenzoyl-2'-deoxytaxol could not be detected. To address this limitation, the team applied Protein-Sol prediction, site-directed mutagenesis, and computational approaches to generate a novel BAPT variant (BAPTm). Introduction of BAPTm increased the yield of N-debenzoyl-2'-deoxytaxol by 27%. Furthermore, by fusing maltose-binding protein (MBP) at the N-terminus via a tetra-amino acid linker (IGGG), the solubility of BAPTm (designated MBPig3BAPTm) was further improved. Ultimately, the integration of CoAL(A312G), MBPig3BAPTm, T2'OGD, and T3'NBT into *S. cerevisiae*, together with supplementation of baccatin III and β -phenylalanine, enabled the successful detection of paclitaxel, achieving a final titer of 0.59 ± 0.03 μ g/L.

4.3. Progress in Paclitaxel Biosynthesis Using Plant-Based Chassis Systems

Plants are capable of harnessing sunlight and atmospheric CO₂ to produce secondary metabolites [58]. Leveraging the abundant endogenous IPP and DMAPP in plant cells, parts of the paclitaxel biosynthetic pathway have been successfully introduced into *Arabidopsis thaliana* [59]. Following overexpression of the TS gene under the constitutive CaMV 35S promoter, taxa-4(5),11(12)-diene was successfully detected in homozygous plants, demonstrating functional expression of the recombinant protein in *A. thaliana*. However, overexpression of the relevant genes adversely affected plant growth, including reduced hypocotyl length, leaf chlorosis, slowed growth, and delayed flowering. These effects were hypothesized to result either from the cytotoxicity of taxa-4(5),11(12)-diene or from TS expression interfering with the biosynthesis of other essential terpenoids, such as gibberellins or carotenoids, during development. To mitigate these effects, the TS promoter was replaced with a glucocorticoid-inducible promoter, which prevented GGPP flux from being directed into the taxa-4(5),11(12)-diene pathway during plant growth and development, thereby reducing the detrimental phenotypic impacts of TS expression. Nevertheless, taxa-4(5),11(12)-diene production still remained low.

Studies have shown that tobacco is also an excellent host for heterologous paclitaxel production. The TS and T5 α OH genes were introduced into tobacco and achieved trichome-specific expression. However, the engineered plants did not produce the desired T5 α -ol but instead accumulated the byproduct OCT [60].

Implementation of a chloroplast compartmentalization strategy in *N. benthamiana* spatially segregated TS, T5 α OH, and CPR. This was combined with an optimized supply of isoprenoid precursors, resulting in the production of taxa-4(5),11(12)-diene and T5 α -ol at 56.5 μ g/g and 1.3 μ g/g of fresh leaf tissue, respectively [61]. Parallel development of transplastomic tobacco lines initially resulted in minimal taxa-4(5),11(12)-diene accumulation when TS was co-expressed in both chloroplasts and the cytosol. Remarkably, when TS was expressed with an added chloroplast transit peptide, taxa-4(5),11(12)-diene levels in chloroplasts increased significantly, reaching 87.8 μ g/g of cell dry weight. These results highlight the importance of transporting intermediates into chloroplasts and their subsequent post-translational modifications for achieving high-level taxa-4(5),11(12)-diene production [62].

Integration of transcriptomics, cell biology, metabolomics, and single-enzyme knockout experiments identified the minimal gene set required for heterologous paclitaxel biosynthesis. By transiently expressing a truncated plastid-targeted diterpene synthase (nsTXS) and geranylgeranyl diphosphate synthase (nsGGPS) in *N. benthamiana*, along with the cytosolic mevalonate (MVA) pathway's rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), they established a functional biosynthetic pathway for taxa-4(5),11(12)-diene. This led to the production of approximately 100 μ g of taxa-4(5),11(12)-diene per gram of fresh leaf tissue. By incorporating previously characterized enzymes along with newly identified catalysts—including a C4 β -C20 epoxidase, taxane 1 β -hydroxylase (T1 β OH), taxane 9 α -hydroxylase (T9 α OH), taxane 9 α -dioxygenase, and phenylalanine-CoA ligase (PCL)—the team successfully synthesized the key intermediate baccatin III and reconstituted its conversion to paclitaxel in *N. benthamiana* [15]. In April 2025, Sotirios C. Kampranis's team identified a novel acyl-CoA ligase, CoAL(A312G), which functions in coupling β -phenylalanoyl-CoA, an inherently unstable intermediate, with baccatin III via BAPT to yield N-debenzoyl-2'-deoxytaxol. Co-expression of CoAL(A312G) with BAPT and PAM significantly enhanced the biosynthetic efficiency, achieving an exogenous baccatin III conversion rate exceeding 60%. The study also pinpointed T2'OGD and T3'NBT as key enzymes responsible for the final steps of paclitaxel biosynthesis. When PAM, CoAL(A312G), BAPT, T2'OGD, and T3'NBT were co-expressed in tobacco leaves, paclitaxel accumulation reached 1.99 ± 0.19 μ g per gram of fresh leaf weight. Notably, the C4 β -C20 epoxidase, an α -ketoglutarate-dependent dioxygenase, overcame the first biosynthetic bottleneck in paclitaxel metabolic engineering. While functional expression was not achieved in *E. coli* or *S. cerevisiae*, the tobacco chassis facilitated its activity, enabling baccatin III production. These results define a minimal pathway for biosynthesis, suggesting a metabolic network rather than a strictly linear sequence.

Notably, the newly identified C4 β -C20 epoxidase, an α -ketoglutarate-dependent dioxygenase, overcomes the first biosynthetic bottleneck in paclitaxel metabolic engineering. Cofactors or chaperone proteins within the tobacco chassis likely facilitated the functional expression of the C4 β -C20 epoxidase, enabling the production of baccatin III in plant cells, whereas functional expression was not achieved in *E. coli* or yeast. Although the yields of 10-deacetylbaccatin III and baccatin III in this heterologous system are lower than those in the native paclitaxel-producing environment, these results define a complete, minimal pathway for their biosynthesis. Furthermore, the researchers observed that this pathway likely represents a metabolic network rather than a strictly linear sequence.

In January 2024, a streamlined baccatin III biosynthetic route was identified. By transiently expressing TOT and T9 α OH-1 alongside seven other known biosynthetic genes (TXS, T5 α OH, T13 α OH, T2 α OH, T7 β OH, TAT, and TBT) in *N. benthamiana*, the researchers successfully confirmed baccatin III production. Their biochemical analyses showed that these core genes are co-regulated by the plant hormone jasmonate, with highly correlated expression patterns, indicative of tight synergistic control within the cell. Subcellular localization experiments demonstrated that GGPP is first converted to taxa-4(5),11(12)-diene in chloroplasts by TXS. Taxa-4(5),11(12)-diene is then transferred to the cytosol via plastid-endoplasmic reticulum (ER) contact sites, where six ER-anchored oxidases (T2 α OH, T5 α OH, T7 β OH, T9 α OH, T13 α OH,

and TOT) collaborate with two cytosolic acyltransferases (TAT and TBT) to produce baccatin III [12]. Systematic reconstruction of the early paclitaxel biosynthetic network recently enabled the structural elucidation of four novel T5 α OH products. Chemical profiling reveals that a significant portion of these newly isolated compounds represent over-oxidized derivatives originating from the primary mono-oxygenated metabolites [26]. Their engineered system successfully reconstructed a six-step biosynthetic pathway for the production of 5 α ,10 β -diacetoxy-taxa-4(5),11(12)-diene-13 α -ol, which can be isolated. Additionally, the team updated the overall biosynthetic pathway for baccatin III, identifying the nuclear transport factor 2 (NTF2) domain-containing protein, FoTO1, as a cofactor that selectively increases taxa-4(5),11(12)-diene-5 α -ol production to over 90%. By heterologously expressing seven newly identified genes (FoTO1, T9 α OH-750C, T7AT, T1 β OH-184, T1 β OH-686, T9dA, T7dA) and nine previously known genes (TS, T5 α OH, TAT, T10 β OH, DBAT, T13 α OH, T2 α OH, TBT, TOT) in *N. benthamiana*, the team achieved the complete biosynthesis of baccatin III from GGPP, with yields ranging from 10 to 30 μ g/g dry weight (DW), comparable to the natural content found in *Taxus* needles. This achievement fills several critical gaps in the paclitaxel biosynthesis pathway and lays the groundwork for the sustainable production of paclitaxel. Recent studies on the baccatin III biosynthetic pathway have identified taxusin and baccatin VI as key intermediates. The discovery of the enzyme T13dA, alongside other deacetylases, has clarified the metabolic network leading to baccatin III. The integration of a C13 α -O-acetylation-deacetylation module has facilitated the de novo production of taxanes in plant hosts. By introducing the genes T13dA1, T79dA, and T7dA1, researchers reconstructed 18-gene and 19-gene baccatin III biosynthetic pathways in *Nicotiana benthamiana*. With the utilization of the C9-hydroxylase T9 α H-750C, the engineered 19-gene pathway produced a baccatin III yield of 23 μ g/g dry weight (DW). This yield is comparable to that of the previously established 17-gene pathway. However, unlike the 17-gene route that lacks the C13-deacetylation module, the 18-gene and 19-gene pathways emulate the endogenous metabolic network of *Taxus*. The inclusion of the acetylation-deacetylation mechanism directs the metabolic flux through the intermediates taxusin and baccatin VI. This pathway configuration circumvents potential catalytic bottlenecks associated with simplified linear routes. Consequently, these reconstructed pathways validate the biological function of the acetylation-deacetylation cycle and provide a functional framework for the future metabolic engineering of taxanes in plant chassis [27].

Meanwhile, significant advancements have been made in the development of transformation systems for *Taxus* species. Recently, the team led by Mercedes Bonfill reported the establishment of distinct transformed lines of *Taxus baccata*, mediated by *Rhizobium rhizogenes* A4. Although transgenic *Taxus* roots exhibit relatively slow growth, a dedifferentiation strategy was employed to obtain callus cultures, followed by the establishment of suspension cell systems. To enhance paclitaxel production, two key limiting genes, BAPT and DBTNBT, were overexpressed in a two-stage culture system under dual elicitation treatments. Compared to wild-type lines, paclitaxel content in DBTNBT-overexpressing lines increased more than fourfold, while BAPT-overexpressing lines exhibited a twofold increase. This marks the first successful transformation of *Taxus* seedlings via direct inoculation with two engineered *R. rhizogenes* A4 strains carrying paclitaxel biosynthetic genes. Earlier protocols utilizing the wild-type *R. rhizogenes* A4 strain successfully induced hairy root formation in *Taxus* through a strictly *in vitro* infection process [63]. Transcriptional analysis of the paclitaxel biosynthetic genes further revealed that GGPPS, TS, and DBAT were the most responsive to DBTNBT overexpression and dual elicitation, with their expression exhibiting a gradual and sustained increase.

Table 2. Heterologous expression systems for paclitaxel.

Synthetic Systems	Products	Yield	Research Methods	Publication Date	References
<i>Escherichia coli</i>	Taxa-4(5),11(12)-diene	1.3 mg/L	Overexpression of IDI, GGPPS, TS, and DXP	2001	[48]
<i>Escherichia coli</i>	Taxa-4(5),11(12)-diene	1 g/L	Multivariate modular metabolic engineering with overexpression of TS and T5 α OH	2010	[64]
<i>Escherichia coli</i>	Taxa-4(5),11(12)-diene-5 α -ol	58 \pm 3 mg/L			
<i>Escherichia coli</i>	Taxa-4(5),11(12)-diene	4.5 mg/g DW	JM109(<i>DE3</i>) strain, 22 $^{\circ}$ C	2012	[65]
<i>Cladosporium TPF6</i>	Taxa-4(5),11(12)-diene	61.9 \pm 6.3 μ g/L	alcA promoter, overexpression of IDI and tHMGR	2017	[19]
<i>Escherichia coli</i>	Oxidized taxanes	27 mg/L	TbrTS, <i>Taxus</i> CPR, T5 α OH-GSTGS-CPR, introduction of a heterologous MVA pathway	2022	[66]
<i>Escherichia coli</i>	Taxa-4(5),11(12)-diene-5 α -ol	7 mg/L			
<i>Escherichia coli</i>	Taxa-4(5),11(12)-diene	93.5 mg/L	Fusion expression of GGPP and TS	2022	[67]
<i>Escherichia coli</i>	Oxidized taxanes	570 \pm 45 mg/L	N-terminal modification of P450 enzyme	2016	[52]
<i>Bacillus subtilis</i>	Taxa-4(5),11(12)-diene	17.8 mg/L	Overexpression of the MEP pathway, GGPPS, and TS	2019	[51]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene	8.7 mg/L	Co-expression of tHMGR, the mutant regulatory protein UPC2-1, GGPPS, and TS	2008	[68]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene	72.8 mg/L	YSG50 strain, GGPPSbc	2014	[54]
<i>Saccharomyces cerevisiae</i>	Oxidized taxanes	33 mg/L	Co-culture of <i>Escherichia coli</i> and <i>Saccharomyces cerevisiae</i>	2015	[49]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene	129 mg/L	Multi-copy TS with an MBP tag, 20 $^{\circ}$ C	2020	[55]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -ol	20 mg/L	Fermentation process optimization, pH adjustment	2020	[56]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -yl acetate	3.7 mg/L			
<i>Saccharomyces cerevisiae</i>	Oxidized taxanes	78 mg/L			
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -ol	42 mg/L	2 \times YP medium, statistically determined screening design	2022	[69]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -yl acetate	22 mg/L			
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -ol	38.1 \pm 8.4 mg/L	Promoter pHXT7, fusion expression of T5OH and CPR, assayed in resting cells under neutral pH conditions	2022	[57]
<i>Saccharomyces cerevisiae</i>	Oxidized taxanes	361.4 \pm 52.4 mg/L			
<i>Yarrowia lipolytica</i>	Taxa-4(5),11(12)-diene	101.4 mg/L	Fusion expression of SUMO with TS, with overexpression of tHMG1, GGS1, and TS	2023	[70]
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene	215 mg/L	Computational metabolic engineering	2023	[71]

<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -ol	43.65 mg/L			
<i>Saccharomyces cerevisiae</i>	Taxa-4(5),11(12)-diene-5 α -yl acetate	26.2 mg/L			
<i>Saccharomyces cerevisiae</i>	1 β -dehydroxybaccatin VI	--	Co-expression of 12 identified genes in cells with feeding of taxadien-5 α -ol	2024	[13]
<i>Arabidopsis thaliana</i>	Taxa-4(5),11(12)-diene	600 ng/g DW	Glucocorticoid-inducible expression of TS	2004	[59]
<i>Nicotiana benthamiana</i>	Taxa-4(5),11(12)-diene	50 μ g/g DW	Expression of TS with MeJA induction and silencing of PSY and PDS	2014	[72]
<i>Nicotiana benthamiana</i>	Taxa-4(5),11(12)-diene	56.6 \pm 3.2 μ g/g fresh weight	Compartmentalization strategy with co-expression of DXS and GGPPS	2019	[61]
<i>Nicotiana benthamiana</i>	Taxadien-5 α -ol	1.3 \pm 0.5 μ g/g fresh weight			
<i>Nicotiana tabacum</i> cv. Xanthi	Taxa-4(5),11(12)-diene	87.8 μ g/g DW	N-terminal chloroplast transit peptide fusion of TS	2021	[62]
<i>Nicotiana benthamiana</i>	Baccatin III	154.87 ng/g fresh weight	Transient co-expression of C4 β -C20 epoxidase, T9 α OH, T1 β OH, and T9OX along with nine other known genes	2023	[15]
<i>Nicotiana benthamiana</i>	Paclitaxel	64.29 ng/g fresh weight	Transient co-expression of PCL with BAPT, PAM, DBTNBT, and T2'OH	2023	[15]
<i>Nicotiana benthamiana</i>	Taxa-4(5),11(12)-diene-5 α -ol	--	Attenuation of T5 α OH expression using the weak constitutive NOS promoter	2024	[26]
<i>Nicotiana benthamiana</i>	5 α ,10 β -Diacetoxy-taxa-4(20),11-diene-13 α -ol	42 μ g/g DW	(NOS)T10 β OH, DBAT and (NOS)T13 α OH	2024	[26]
<i>Nicotiana benthamiana</i>	Baccatin III	50 ng/g DW	Transient co-expression of TOT and T9 α OH-1 along with seven other known biosynthetic genes	2024	[12]
<i>Nicotiana benthamiana</i>	Baccatin III	10–30 μ g/g DW	Transient expression of 7 newly discovered genes and 9 additional previously known genes.	2025	[73]
<i>Nicotiana benthamiana</i>	Baccatin III	23 μ g/g DW	Transient co-expression of 18-gene/19-gene pathway including C13-acetylation-deacetylation module	2026	[27]

4.4. Research Progress on Paclitaxel Biosynthesis by *Taxus*-Associated Endophytic Fungi

Additionally, it has been discovered that certain endophytic fungi associated with *Taxus* species are capable of producing paclitaxel. Initial screening and mutagenic optimization of one such isolate successfully elevated its native paclitaxel titer from 125.7 μ g/L to 314.07 μ g/L. Combinatorial treatments utilizing physical and chemical mutagenesis generated alternative strains producing 448.52 μ g/L paclitaxel, with subsequent protoplast fusion events further improving the yield to 468.62 μ g/L. Wang et al. [74] were the first to apply the REMI method to obtain a paclitaxel-producing fungal strain (BT2) with hygromycin B resistance. Similarly, genomic integration of a hygromycin B phosphotransferase gene, strictly driven by

the *trpC* promoter, achieved protoplast regeneration frequencies exceeding 6% in targeted endophytes. These approaches have successfully facilitated the transfer of paclitaxel biosynthetic genes into engineered fungi to enhance paclitaxel production. Collectively, these genetic manipulation strategies facilitated the preliminary transfer of selective markers and vector elements into fungal platforms. *Agrobacterium*-mediated transformation of *Cladosporium cladosporioides* subsequently yielded the MD2 strain, a process strictly reliant on optimized co-culture conditions for stable integration. Liu et al. developed a highly efficient *Agrobacterium*-mediated transformation method for the paclitaxel-producing endophytic fungus *Ozonium* sp. EFY21 and optimized several factors affecting transformation efficiency and transformant stability. However, none of these studies have integrated the biosynthetic genes for paclitaxel into the genomes of the transformed fungi. Targeted genetic editing, such as the CRISPR/Cas9 system, was used to disrupt sterol metabolism in filamentous fungi by knocking out both squalene synthase and cycloartenol synthase. This concurrent knockout successfully redirected metabolic flux, thereby enhancing paclitaxel accumulation [75].

A comparative assessment of paclitaxel yields across different endophytic fungal strains reveals substantial variability, with the highest reported yield of 468.62 $\mu\text{g/L}$ still orders of magnitude lower than that required for industrial applications. Several key bottlenecks limit the commercial application of this approach, including: (1) strain instability, with paclitaxel production often declining after repeated subculturing; (2) intrinsically low yields that remain insufficient for cost-effective production; and (3) challenges in fermentation scalability, as endophytic fungi typically exhibit slow growth and complex nutritional requirements; (4) random mutagenesis and protoplast fusion offer moderate yield improvements but lack predictability and sustainability. *Agrobacterium*-mediated transformation has not yet achieved stable integration of complete paclitaxel pathways. CRISPR/Cas9-based metabolic reprogramming is promising but remains at an early stage. Overall, current research on *Taxus* endophytic fungi remains relatively limited. Compared to *E. coli*, *S. cerevisiae*, and tobacco chassis, the genetic manipulation and cultivation systems for *Taxus*-derived endophytic fungi remain underdeveloped. With the continued advancement of omics-driven data mining, synthetic biology, and analytical technologies, future studies on endophytic fungi are expected to provide further insights into the biosynthesis of paclitaxel. However, given the current yield and stability constraints, endophytic fungal platforms are more likely to serve as valuable models for understanding paclitaxel biosynthesis rather than as immediate industrial production hosts.

4.5. Engineering Cyanobacteria to Produce Paclitaxel Precursors from CO_2

Algae have emerged as advanced cellular factories, demonstrating substantial progress in the production of high-value compounds such as biofuels, pharmaceuticals, and nutraceuticals. Their rapid growth, adaptability to diverse environments, and inherent capacity for photosynthetic carbon fixation position them as promising platforms for sustainable biotechnological applications. Advances in genetic engineering and metabolic optimization have further enabled the tailored design of algal systems for efficient synthesis of complex molecules, providing environmentally sustainable alternatives to conventional manufacturing processes.

Although synthetic biology has facilitated the *de novo* synthesis of paclitaxel precursors in various microbial hosts, the complete biosynthesis of paclitaxel remains challenging due to limited oxidation efficiency in heterotrophic microorganisms. Ma's team engineered *Synechocystis* sp. PCC 6803 with modular metabolic pathways comprising enzymes from the MEP pathway and the paclitaxel biosynthetic cascade to produce T5 α -ol. The optimized strain DIGT-P560 achieved production titers of up to 17.43 mg/L of oxygenated taxanes and 4.32 mg/L of T5 α -ol. Transcriptomic analysis of DIGT-P560 further indicated that establishing a flux toward oxygenated taxanes may enhance photosynthetic electron transfer efficiency and central metabolism in the engineered strain, thereby alleviating metabolic disturbances caused by the introduction of exogenous genes. This work represents the first demonstration of photosynthetic production

of taxa-4(5),11(12)-diene-5 α -ol from CO₂ in cyanobacteria, underscoring the potential of engineered cyanobacteria as biosolar cell factories for high-value terpenoid production and offering new avenues for rational strain engineering and optimization [76].

5. Advances in the Engineering and Catalytic Mechanisms of Key Enzymes Involved in Paclitaxel Biosynthesis

In the early stages of paclitaxel biosynthesis, although taxa-4(5),11(12)-diene synthase (TS) exhibits low catalytic efficiency and produces multiple byproducts [77], it is not considered the primary rate-limiting step in the pathway. Instead, the hydroxylation of taxa-4(5),11(12)-diene at the C5 position, catalyzed by taxa-4(5),11(12)-diene-5 α -hydroxylase (T5 α OH), has been identified as the critical bottleneck and principal rate-limiting step in paclitaxel biosynthesis. Directed evolution, a widely applied enzyme engineering strategy, emulates the principles of genetic mutation and natural selection, introducing iterative mutations and screening cycles within comparatively short timeframes. Despite notable progress, the engineering of T5 α OH and other cytochrome P450 enzymes in the paclitaxel pathway through directed evolution remains highly challenging, primarily owing to limited knowledge of their catalytic mechanisms and the absence of efficient high-throughput screening strategies.

Rational and semi-rational enzyme engineering strategies, which depend on sequence, structural, and functional information, enhance the likelihood of beneficial mutations and significantly reduce experimental workload. Amino acid substitutions within the catalytic active site can enhance enzymatic activity; however, achieving such improvements requires precise conformational control to sustain a delicate balance between thermodynamic flexibility and structural rigidity [78]. The integration of computational approaches with experimental validation has proven highly valuable for elucidating catalytic mechanisms and guiding rational or semi-rational protein engineering, particularly in improving substrate recognition and catalytic efficiency of P450 enzymes. Recently, substantial breakthroughs in pathway design within plant hosts have provided promising solutions to the persistent challenges associated with the chemical catalysis by P450 enzymes in paclitaxel biosynthesis [53].

5.1. Engineering of the Key Enzyme TS

TS consists of 862 amino acid residues, including an N-terminal plastid-targeting signal of approximately 80 residues and a catalytic active site structured by three helical regions [79]. The C-terminal domain (S553–V862) corresponds to a class I terpene cyclase, whereas the N-terminal domain (M107–I135) and the “insertion” domain (S349–Q552) constitute a degenerate class II terpene cyclase [80]. Class I terpene cyclase employs a trinuclear metal cluster that coordinates the conserved motifs DDXXD and (N,D)DXX(S,T)XXXE, thereby facilitating diphosphate ionization of isoprenoid substrates to generate carbocation intermediates and initiate catalysis.

In contrast, class II terpene cyclase utilizes aspartate residues within the DXDD motif to catalyze protonation of isoprenoid double bonds or oxirane moieties, thereby initiating carbocation formation. Despite differences in protein folding and substrate activation mechanisms between the two cyclase classes, the structural architecture of TS suggests an evolutionary relationship in terpene biosynthesis [79]. The catalytic activity of TS is mediated primarily by its C-terminal domain. Initially, a cluster of three Mg²⁺ ions binds GGPP and promotes diphosphate ionization. The subsequent diphosphate hydrolysis generates a reactive carbocation intermediate, while the enzyme’s active-site pocket shifts conformationally from an open to a closed state. This conformational transition enables the carbocation to undergo a cascade of rearrangements and cyclization reactions, ultimately yielding multiple taxa-4(5),11(12)-diene isomers [81].

The reaction pathway involving transient intermediates has increasingly emerged as a focal point of TS research. Kinetic and thermodynamic computational analyses have revealed that carbocation

rearrangements catalyzed by TS are strongly influenced by the enzyme's microenvironment and intrinsic conformational dynamics. Factors such as strong electrostatic interactions dependent on cation positioning, orientation, and conformational shifts, the spatial architecture of the active site, and the presence of water molecules in the catalytic pocket collectively modulate the reaction energy barrier, resulting in distinct product configurations [81]. A notable feature of TS is its active-site cavity, which is considerably larger than the final product, taxa-4(5),11(12)-diene. This spatial redundancy is considered a primary factor contributing to the enzyme's product promiscuity. In parallel, extensive mutagenesis studies have identified additional structural determinants critical to catalysis in TS. Beyond the previously identified Y688 residue, tryptophan at position 753 plays a pivotal role in stabilizing carbocation intermediates via π -cation interactions. Remarkably, substitution of this residue with histidine results in a complete shift of the product profile, converting taxa-4(5),11(12)-diene into the alternative diterpene cembrene A [82,83].

TS is a key enzyme in paclitaxel biosynthesis, directing metabolic flux into the taxane pathway through cyclization activity. During fermentation, TS cyclizes geranylgeranyl pyrophosphate (GGPP) to yield 93.2% taxa-4(5),11(12)-diene, with minor byproducts of 4.7% taxa-4(20),11(12)-diene and additional diterpene scaffolds. Subsequently, only 10% of taxa-4(5),11(12)-diene is converted by taxa-4(5),11(12)-diene-5 α -hydroxylase (T5 α OH) into the target product, taxa-4(5),11(12)-diene-4(20),11(12)-5 α -ol [22]. Evidence suggests that T5 α OH has a higher substrate affinity for taxa-4(20),11(12)-diene than for taxa-4(5),11(12)-diene. Targeted mutagenesis near the TS active site revealed that substitution of tyrosine 688 with leucine TS (Y688L) significantly enhanced production of taxa 4(20),11(12) diene. When the TS (Y688L) variant was introduced into a T5 α OH-expressing strain, taxa-4(5),11(12)-diene-5 α -ol yield increased 2.4-fold. This study indicates that engineering TS to favor taxa-4(20),11(12)-diene formation can channel substrates directly into T5 α OH-mediated P450 hydroxylation, bypassing the epoxide cleavage pathway and markedly increasing the proportion of the target product [22]. With the elucidation of the TS crystal structure [79], systematic mutagenesis of key residues has been performed. Based on structural modeling, several TS variants were engineered, including S713T, W753H, W753H/C830A, V584K/L/M, S587A, V714A/I, C803A/S, and Y841F. All variants retained catalytic activity, with S713T preserving 97.4% of wild-type enzymatic activity [84].

5.2. Advances in the Study of the Catalytic Mechanism of Taxa-4(5),11(12)-Diene-5 α -Hydroxylase (T5 α OH)

Before the foundation of FoTO1, investigations into the catalytic mechanism of T5 α OH have mainly focused on two proposed pathways: the radical-rebound mechanism and the epoxidation mechanism [82,83]. Yadav et al. [85] suggested that competitive proton abstraction by the ferryl-oxo species at the C3, C13, and C20 positions contributes to the diversity of T5 α OH oxidation products. Edgar and colleagues [22,23] hypothesized that taxa-4(5),11(12)-diene is initially converted by T5 α OH into an unstable epoxide intermediate, which subsequently undergoes non-specific cleavage to yield various oxidized products, including taxa-4(5),11(12)-diene-5 α -ol, in accordance with the epoxidation mechanism. Meanwhile, the isomeric form taxa-4(20),11(12)-diene bypasses the epoxide cleavage step and produces the target product taxa-4(5),11(12)-diene-5 α -ol directly via the canonical P450 radical-rebound mechanism. Barton et al. further demonstrated that taxa-4(5),11(12)-diene-4(5)-epoxide is acid-sensitive and undergoes multiple rearrangements under varying chemical conditions, particularly in the presence of iron-porphyrin, thereby highlighting challenges to its use as a viable intermediate [83]. Since the initial discovery of T5 α OH in *Taxus* in 1996, numerous strategies have been implemented, including N-terminal engineering, optimization of the electron transfer chain, ribosomal switch regulation, resting-cell cultivation, endoplasmic reticulum remodeling, and subcellular compartmentalization [53]. In parallel, extensive optimization of catalytic conditions, including temperature, O₂ concentration, pH, and cofactors, has also been performed [56,60]. However, significant breakthroughs in the molecular-level engineering of T5 α OH are still lacking. Yadav et al. developed a homology model of T5 α OH based on six P450 structures and

designed 53 variants. Among these, six mutants showed enhanced performance in either conversion efficiency or T5 α -ol selectivity. Notably, the K131R mutant increased conversion at the expense of selectivity, whereas the V374L mutant enhanced selectivity but reduced conversion. Building on in-depth analyses of the paclitaxel biosynthetic gene cluster, our research group conducted large-scale functional screening of candidate sequences from the CYP725A subfamily of Himalayan yew. Two homologs, T5 α OH-1 and T5 α OH-2, were identified and experimentally confirmed to catalyze C5 hydroxylation of taxa-4(5),11(12)-diene. Nevertheless, their catalytic performance did not exceed that of previously reported T5 α OH [7].

In 2024, our research group, in collaboration with Binju Wang's group and Zhenzhan Chang's group, employed X-ray crystallography, molecular dynamics (MD) simulations, quantum mechanics/molecular mechanics (QM/MM) calculations, and quantum mechanics (QM) computations to elucidate the crystal structure of the CYP725A4-taxa-4(5),11(12)-diene complex. Through comprehensive computational analyses, the catalytic mechanism of CYP725A4 in the native paclitaxel biosynthesis pathway was elucidated [86]. The results indicate that oxidation of taxa-4(5),11(12)-diene generates a zwitterionic intermediate, which can proceed along two competing transformation routes. In one pathway, an epoxide is formed and subsequently undergoes a water-mediated rearrangement to yield the target product, taxa-4(5),11(12)-diene-5 α -ol. In the alternative pathway, protonation of the oxygen atom in the zwitterionic intermediate facilitates hydrogen shifts and carbon–oxygen coupling, leading to the formation of the side products OCT/iso-OCT. In contrast, taxa-4(20),11(12)-diene undergoes direct C5 hydroxylation, yielding taxa-4(5),11(12)-diene-5 α -ol without formation of the epoxide intermediate. These crystallographic and computational studies provide valuable insights into the catalytic mechanism of CYP725A4 and lay the groundwork for its further engineering to enhance performance in paclitaxel biosynthesis.

5.3. Enzyme Engineering of T10 β OH

In a recent study, the structural model of the N-terminally truncated enzyme t20T10 β OH was predicted using AlphaFold2. The model features an N-terminal transmembrane domain connected to a catalytic core comprising 12 α -helices and antiparallel β -sheets, with the heme cofactor positioned between helices I and L. Building on this structure, the PROSS tool was employed to predict 45 single-point mutations with the potential to improve protein folding, each of which was subsequently evaluated energetically using FoldX. Experimental validation identified three beneficial mutations (I75F, L226K, and S345V) which enhanced substrate conversion by 2.1-, 1.4-, and 1.4-fold, respectively, compared to the wild-type enzyme. Mechanistic analysis suggested that I75F increases hydrophobicity at the substrate channel entrance to facilitate substrate access, L226K modulates enzyme conformation through altered surface charge distribution, and S345V stabilizes the protein core via enhanced hydrophobic interactions. Further combination of these mutations yielded double and triple mutants, among which the triple mutant I75F/L226K/S345V achieved the highest substrate conversion (2.8-fold increase) and exhibited a 9.5-fold elevation in cytochrome P450 T10 β OH heterologous expression. This study provides a valuable engineering framework for enhancing the *de novo* biosynthesis of T10 β OH and related taxanes [87].

5.4. Designing an Oxidase Toolbox for Site-Directed Oxidation of Taxanes

Although the biosynthetic pathway of paclitaxel has been progressively elucidated, its overall efficiency remains constrained by the limited activity and product selectivity of the native hydroxylases involved. To address this limitation, the researchers focused on identifying non-native oxidases capable of functionalizing taxa-4(5),11(12)-diene as alternatives to cytochrome P450 enzymes. Among 29 screened fungal oxidases, TteUPO was selected due to its superior activity toward taxa-4(5),11(12)-diene and high protein expression level. Based on this candidate, an artificial enzyme toolbox was developed using

engineered variants of TteUPO, enabling site-specific oxidation of taxanes at multiple positions (C-4, C-6, C-10, C-11, C-12, and C-13). A collection of TteUPO mutants with distinct regioselectivities toward the taxa-4(5),11(12)-diene skeleton was constructed and utilized for pathway engineering. Moreover, by integrating the taxa-4(5),11(12)-diene biosynthetic pathway and enabling heterologous expression of a selected TteUPO mutant in *E. coli*, the *de novo* biosynthesis of oxidized taxa-4(5),11(12)-diene derivatives was achieved in this engineered chassis. Together, these findings provide an innovative strategy to establish efficient synthetic routes toward paclitaxel and to generate structurally diverse analogs for medicinal evaluation, thereby establishing a versatile platform for developing artificial synthetic pathways to paclitaxel and related taxanes [17].

5.5. Advances in the Mechanistic Study of Oxetane Ring Formation

Recent breakthroughs have clarified the mechanism of oxetane ring formation. Unlike other candidate enzymes implicated in oxetane formation, TB328 is not a P450 enzyme but rather an α -ketoglutarate/Fe(II)-dependent dioxygenase (ODD). Through transient expression in *N. benthamiana*, the researchers demonstrated that TB328 catalyzes the conversion of taxusin into taxusin-4 β ,20-epoxide, thereby enabling the *de novo* biosynthesis of baccatin III in the tobacco chassis. However, attempts to express TB328 in *E. coli* or *S. cerevisiae* strains engineered for taxa-4(5),11(12)-diene production were not successful. Furthermore, the detailed reaction mechanism remains unresolved because the low yield of the oxidized product prevents reliable NMR characterization [15].

In 2023, the research team led by Sotirios C. Kampranis published a study entitled challenged prevailing hypotheses that the C5 position of taxa-4(5),11(12)-diene-5 α -ol is first acetylated and then oxidized through multiple steps to form the oxetane ring. The researchers observed taxane compounds in *Taxus* species that contained oxetane rings without prior acetylation, which led them to propose a “cyclization-first, acetylation-later” mechanism [23]. In their study, candidate genes were screened through genomic and transcriptomic analyses, and validation was conducted using two heterologous expression systems (*S. cerevisiae* and *N. benthamiana*) in combination with structural characterization of the resulting products. They ultimately identified CYP725A4 (also known as T5 α OH) as the enzyme responsible for oxetane ring formation. The authors discovered that CYP725A4 catalyzes oxidation at multiple positions on the taxa-4(5),11(12)-diene precursor, potentially forming the oxetane ring through a two-step epoxidation mechanism, thereby revising the previous understanding of its oxidative products and catalytic mechanism. Moreover, the study demonstrated that both taxa-4(5),11(12)-diene and taxa-4(20),11(12)-diene serve as starting substrates for the generation of the target epoxidized intermediate, suggesting the presence of multiple branched biosynthetic pathways leading to paclitaxel [23].

In 2024, P450 enzyme TOT1 was found to play a key role in the formation of the oxetane ring. It did not catalyze the direct conversion of a three-membered ring intermediate into a four-membered ring [12]. Instead, the enzyme simultaneously converts the double-bond structure into both three- and four-membered ring systems. Using density functional theory (DFT) calculations in combination with substrate selectivity assays, the study demonstrated that both epoxide and oxetane products arise from a carbocation intermediate within TOT1. However, oxetane formation was shown to be energetically and kinetically more favorable, providing a molecular explanation for its predominance and indicating that the epoxide is not a necessary intermediate in this process [12]. Almost concurrently, the catalytic mechanism of TOT1's isoenzyme, CYP725A55, for the same reaction was elucidated by Zhihua Zhou's team and Jungui Dai's team. Isotope labeling experiments confirmed that the oxetane ring is likely formed through a direct oxidation-acyl rearrangement process, consistent with the predictions of Yan's team [13]. These findings overturn three decades of conventional understanding of this structural motif and fill a critical gap in elucidating the molecular mechanism of ring-expansion reactions that generate oxygen-containing four-membered rings, a process previously observed only in plants.

5.6. Engineering of DBAT in the Paclitaxel Biosynthetic Pathway

In recent years, owing to the challenges in modifying the substrate specificity and enhancing the catalytic activity of T5 α OH, increasing attention has been directed toward engineering DBAT, a downstream enzyme in the paclitaxel biosynthetic pathway. Alanine scanning was employed to identify the active site of DBAT, leading to the design of a double mutant (G38R/F301V) that exhibited a sixfold increase in catalytic efficiency relative to the wild type. A single-point mutant (G38R) was found to exhibit a 2.15-fold improvement in catalytic efficiency [38,88]. Molecular docking analysis further revealed the critical role of His162 in DBAT catalysis, and alanine scanning indicated that substitution of four residues (H162, R363, G361, and I164) with alanine completely abolished enzymatic activity. Application of a semi-rational design strategy generated a double mutant (I43S/D390R) that not only enhanced catalytic efficiency but also facilitated more effective utilization of vinyl acetate, a more economical acyl donor than acetyl-CoA [89].

5.7. Engineering of Key Enzymes for the Conversion of Paclitaxel Natural Analogues

At present, paclitaxel obtained from nursery-cultivated *Taxus* species remains one of the primary sources for clinical use. However, its natural abundance is extremely low, ranging from 0 to 0.069%, with the highest concentrations occurring in the bark. By contrast, the content of its natural analog, 7- β -xylosyl-10-deacetylpaclitaxel (XDT), is generally much higher than that of paclitaxel. Typically, removal of the xylosyl moiety from XDT yields 10-deacetylpaclitaxel (DT), which can subsequently be converted to paclitaxel through acetylation at the C10 position. Chen et al. conducted single and multiple site-directed mutagenesis at 11 residues of the fungal glycosidases LXYL-P1-1 and LXYL-P1-2 to identify amino acid positions associated with enhanced activity, ultimately obtaining variants with improved catalytic performance. Among the 17 mutants, the E12 variant (A72T/V91S) displayed the highest β -xylosidase and β -glucosidase activities, exceeding those of LXYL-P1-2 by 2.8- and 3-fold, respectively [90]. During paclitaxel extraction, these analogs are often discarded, leading to resource waste and potential environmental contamination. The utilization of XDT as a substrate for paclitaxel semi-synthesis holds substantial practical value in reducing bioconversion costs.

In summary, engineering of key enzymes within the paclitaxel biosynthetic pathway constitutes an effective strategy. Such modifications can enhance substrate utilization and selectivity and improve catalytic efficiency, particularly through the targeted design of critical enzymes. These advances provide a solid foundation for future heterologous biosynthesis and industrial-scale production of paclitaxel.

5.8. Recent Study on Catalytic Mechanistic Analysis of FoTO1

Regarding recent studies on the catalytic mechanism elucidation of FoTO1, which is an NTF2-like superfamily protein in paclitaxel biosynthesis, two preprint articles have recently been published online. Jiang and Liu's team demonstrates that FoTO1 is a dedicated epoxide isomerase that stereospecifically redirects a reactive epoxide intermediate toward productive T5 α -ol formation [91]. Through *in vitro* reconstitution, mutagenesis, and QM/MM calculations, the authors show that a pre-organized D68/D149 catalytic dyad electrostatically activates epoxide ring opening via an isomerization mechanism, in contrast to the water-mediated hydrolysis employed by canonical epoxide hydrolases such as LEH. Modular dissection of the C-terminal extension further reveals a functional partition between catalytic integrity and productive coupling with T5 α OH, mediated by specific hydrophobic contacts that enforce precise geometric complementarity at the binary complex interface. Together, these results demonstrate how electrostatic activation and enzyme association cooperate to control the fate of a highly reactive intermediate in paclitaxel biosynthesis. Sattely and McClune's group systematically elucidates the dual role of FoTO1 [73]. On one hand, FoTO1 functions as an epoxide hydrolase that catalyzes the selective

conversion of taxadiene-4(5)-epoxide to T5 α -ol. On the other hand, through protein-protein interactions with atypical cytochrome P450 enzymes, FoTO1 serves a non-catalytic scaffolding function that organizes a multi-enzyme complex to enhance pathway efficiency. The study also demonstrates that the non-catalytic function of FoTO1 is generalizable across multiple plant diterpenoid biosynthetic pathways, providing new engineering strategies for heterologous production of plant natural products. Collectively, these two complementary studies not only resolve the long-standing catalytic mechanism of FoTO1 but also reveal its unprecedented non-catalytic scaffolding role, marking a significant advance in our understanding of paclitaxel biosynthesis and offering new avenues for pathway engineering in heterologous hosts.

6. The Emerging Technologies Development

To address the bottlenecks in paclitaxel pathway elucidation, enzyme engineering, and heterologous production, a suite of operationally defined emerging technologies has been deployed, with clear input materials, output data, and direct practical applications value.

6.1. *MpXsn: High-Resolution Gene Co-Expression Network for Pathway Discovery*

Recent advances in multi-omics and synthetic biology have provided a suite of emerging technologies that can be operationally deployed to overcome specific bottlenecks in paclitaxel biosynthesis. The multiplexed perturbation \times single-nucleus sequencing (mpXsn) method takes as input *Taxus* tissues treated with 136 distinct perturbations, including hormones, pathway intermediates, and pathogens, followed by single-nucleus RNA sequencing [11]. Its output is a high-resolution gene co-expression network that partitions paclitaxel-associated genes into early, middle, and late expression modules. This technology has been practically applied to discover previously missing genes, especially for the auxiliary protein FoTO1, directly guiding the complete analysis of the paclitaxel synthesis pathway. Thus, mpXsn represents a paradigm shifting discovery tool that transforms correlative transcriptomic data into causal, network guided pathway elucidation.

6.2. *MALDI-IMS: Spatial Metabolomics for Tissue-Specific Taxane Mapping*

MALDI-imaging mass spectrometry (MALDI-IMS) operates on cryosections of *Pseudotsaxus* tissues (such as stems, leaves, and phloem) and generates spatial metabolomic maps comprising up to 10,000 data points that reveal tissue-specific taxane distribution [10]. Practically, these maps identify where paclitaxel and its intermediates accumulate and enabled researchers to select appropriate tissue-specific promoters and design subcellular compartmentalization strategies in engineered plants. MALDI-IMS bridges a critical gap between conventional metabolomics and spatial biology by preserving tissue context, which is essential for understanding the compartmentalized nature of paclitaxel biosynthesis. However, current limitations include relatively low throughput, the need for specialized instrumentation, challenges in absolute quantification, and an inability to reliably distinguish isomeric compounds without tandem MS imaging. Nevertheless, when integrated with some other emerging technologies such as single-nucleus RNA sequencing (snRNA-seq) for cell-type resolution of gene expression, laser microdissection for targeted sampling of specific cell layers, and machine learning algorithms for automated metabolite annotation and spatial pattern recognition, MALDI-IMS can achieve a multi-dimensional understanding of the paclitaxel biosynthetic network, from spatial metabolomics to transcriptional regulation and predictive modeling.

6.3. *Oxidase Toolbox: Site-Directed Oxidation Bypassing P450 Bottlenecks*

The oxidase toolbox based on TteUPO mutants takes taxa-4(5),11(12)-diene as substrate and through engineered fungal unspecific peroxygenase variants expressed in *E. coli*, produces site-specific oxidized taxanes at C4, C6, C10, C11, C12, and C13 positions [17]. This toolbox bypasses the low-activity plant

P450s and enables biosynthesis of oxidized taxanes in prokaryotic chassis while also generating diverse taxane analogs for medicinal chemistry. This represents a paradigm shift in taxane functionalization, as it replaces plant derived membrane-bound P450s with a soluble and bacterial-expressed peroxidase, thereby overcoming long standing bottlenecks in heterologous P450 expression and electron transfer chain assembly. At this stage, the regioselectivity of TteUPO mutants remains less precise than that of native P450s, and further directed evolution is needed to achieve the stereospecific oxidations required for authentic paclitaxel biosynthesis.

6.4. Gene Dropout Experiments

Gene dropout experiments using transient expression in *N. benthamiana* involve the specific deletion of a gene, which achieved by infiltrating tobacco leaves with *Agrobacterium* strains carrying candidate genes for combinatorial infiltration. This method realized the identification of indispensable pathway genes, such as C4 β -C20 epoxidase, T9 α OH, T1 β OH, and PCL [14,15]. This approach has been practically applied to define the minimal gene set required for baccatin III and paclitaxel biosynthesis through systematic gene removal and to validate the order of pathway reactions [12]. Gene dropout experiments have been gradually extended to elucidate the biosynthetic pathways of various complex natural products. This dropout strategy is a powerful functional tool that enables accelerated determination of gene function, transforming correlative transcriptomic or genomic data into causal genetic evidence. When combined with modular pathway design and automated infiltration technologies, gene dropout screening offers an unprecedented capability for rapidly deconvoluting complex plant metabolic networks and accelerating the discovery of minimal biosynthetic routes for pharmaceutical production.

7. Conclusions and Outlooks

Paclitaxel, recognized as one of the most potent natural anticancer agents discovered to date, has undergone a diversified developmental history in its synthesis. At present, the most widely employed approach remains the semi-synthetic route [82]. Commercially available paclitaxel marketed by Bristol Myers Squibb is synthesized via the Holton–Ojima method, employing 10-deacetylbaccatin III as the precursor, with yields of up to 90%. However, the supply of semi-synthetic precursors, such as 10-deacetylbaccatin III or baccatin III, remains dependent on extraction from the scarce yew tree (*Taxus* spp.), severely limiting large-scale production and necessitating optimization via heterologous biosynthesis. Endophytic fungal fermentation has been investigated as an alternative source; however, yields remain low and unstable, with no reports of industrial-scale implementation to date [25]. While the co-evolution of endophytes and *Taxus* species has endowed these microorganisms with a diverse array of metabolic enzyme genes, their inherent genetic instability renders them currently unsuitable for use as industrial chassis cells. Consequently, endophytes are more likely to serve as a potential genetic reservoir in the future, providing critical resources for the mining of novel enzymatic parts. Plant cell culture technology provides another sustainable method for industrial-level paclitaxel production. For example, Phyton utilizes proprietary Plant Cell Fermentation (PCF[®]) technology to directly produce natural active pharmaceutical ingredients from suspension cultures [92]. In recent years, large-scale genome sequencing and analysis of various *Taxus* species have been completed, and the rapid advancement of synthetic biology has established favorable conditions for the heterologous biosynthesis of paclitaxel. This progress has enabled a paradigm shift in paclitaxel biosynthesis research, transitioning from traditional gene mining to systematic chassis reconstruction. With the recent advances in elucidating the oxetane ring formation mechanism, the nuclear transport factor 2 (NTF2)-like protein, FoTO1, and identifying previously unknown catalytic steps, long-standing critical gaps in the synthetic pathway have been bridged, thus rapidly advancing the resolution of the paclitaxel biosynthetic pathway and its heterologous synthesis. However, current research indicates that

although the paclitaxel metabolic pathway has been elucidated, this knowledge has not yet been substantially translated into efficient metabolic flux within engineered chassis. Consequently, enhancing heterologous metabolic flux remains the paramount bottleneck in the field. Currently, advances in microbial and tobacco cell platforms have further facilitated the heterologous biosynthesis of paclitaxel intermediates. However, these chassis cells are fundamentally constrained by a shared core bottleneck: the high functional promiscuity and exceedingly low catalytic efficiency of plant-derived core catalytic elements, particularly cytochrome P450 enzymes and acyltransferases. Consequently, this results in the diversion of metabolic flux and the concomitant accumulation of byproducts. Furthermore, significant discrepancies exist in the efficiency of membrane protein coupling and microenvironmental adaptation across different chassis platforms. Therefore, future research directions should focus on integrating bioinformatics with high-sensitivity multi-species screening systems to systematically investigate the distribution and functional diversity of *Taxus*-derived cytochrome P450 enzymes and acyltransferases. Furthermore, the functional characterization of currently unidentified catalytic parts will further elucidate the reaction steps and complete the paclitaxel biosynthetic landscape. Subsequently, employing methodologies such as multi-species enzyme screening, rational design, and machine learning-assisted enzyme engineering will facilitate the elucidation of the catalytic mechanisms and evolutionary trajectories of key P450 enzymes at the molecular level. By coupling these insights with rational engineering strategies to optimize enzyme activity, the fundamental issue of enzyme promiscuity can be resolved. To address the practical imperatives of industrialized production, the assembly of paclitaxel biosynthetic modules in tobacco cells or engineered eukaryotic microbes must be combined with the simultaneous optimization of critical factors, including cofactor (NADPH, O₂) regeneration, coenzyme compatibility, and precursor supply. This comprehensive approach will alleviate the limitations restricting the heterologous biosynthesis of paclitaxel, ultimately enabling highly efficient heterologous production.

Moreover, integrating chemical synthesis and biosynthesis in a complementary manner could enable greener, more efficient production of paclitaxel [93]. The superiority of this strategy lies in its cross-disciplinary complementarity: it leverages microbial chassis for the construction of the complex taxane polycyclic core skeleton, followed by the application of chemical approaches to append specific side-chain functional groups, ultimately yielding paclitaxel. This synthetic paradigm not only effectively circumvents the inefficiencies inherent in downstream biocatalytic steps but also significantly mitigates the severe environmental impact and high energy consumption associated with traditional total synthesis. In summary, the multifaceted research currently surrounding paclitaxel biosynthesis has not only systematically reconstructed its complex and extensive metabolic network at the theoretical level and elucidated the evolutionary mechanisms of core catalytic elements, but has also laid the critical groundwork for overcoming the catalytic bottlenecks associated with plant-derived cytochrome P450 enzymes. These efforts are of significant scientific importance for deepening the mechanistic understanding of paclitaxel biosynthesis, overcoming bottlenecks associated with key catalytic elements, and advancing the synthetic biology of paclitaxel and its derivatives.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used DeepSeek and ChatGPT to polish specific sentences to better align with English writing standards. Following the use of these tools, the authors carefully reviewed and edited the content as necessary and assume full responsibility for the content of the published article.

Author Contributions

X.L.: designed, wrote and revised the paper. Y.Z.: wrote the paper and collected the data. J.L.: wrote the paper and collected the data. H.J.: revised the manuscript.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 32371499), Natural Science Foundation of Wuhan (2025040601020158), Science and Technology Major Project of Guangxi (Guike AA24206048, Guike AA24206050-3, Guike JF2504850012), Hubei University of Technology High-Level Talent Research Startup Fund Program (4301/00960), Hubei Provincial Key R&D Major Projects (2025BCA006), Youth Science and Technology Talent Cultivation Special Program of Hubei Province (2025DJA024).

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.

References

1. Prota AE, Katja B, Didier Z, Field JJ, José Fernando D, Karl-Heinz A, et al. Molecular mechanism of action of microtubule-stabilizing anticancer agents. *Science* **2013**, *339*, 587–590. DOI:10.1126/science.1230582
2. Li C, Wang Y, Yin X, Sun X, Sui S, Liu J, et al. Novel alpha-KG/Fe(II)-Dependent Dioxygenases Catalyzing C1beta-Hydroxylation and Construction of 5/7/6-Skeleton of Highly Oxygenated Taxoids. *Angew. Chem. Int. Ed.* **2025**, *137*, e202517041. DOI:10.1002/ange.202517041
3. Winegar PH, Astolfi MC, Holm SF, Hudson GA, Keasling JD. Advances in the microbial biosynthesis of therapeutic terpenoids. *Curr. Opin. Biotechnol.* **2025**, *96*, 103374. DOI:10.1016/j.copbio.2025.103374
4. Zhu Y, Liang F, Chai P, Kampranis SC, Zhao Y. Recent Advances in the Biosynthesis and Biotechnological Production of Taxol. *Engineering* **2025**, *4*, 65–75. DOI:10.1016/j.eng.2025.12.018
5. Tan CL, Yu X, Feng HC, Gershenson J, Liu Y, Li SH. A synthetic biology roadmap for sustainable production of the plant-originated anti-cancer drug paclitaxel. *Trends Biotechnol.* **2026**. DOI:10.1016/j.tibtech.2025.11.013
6. Xiong X, Gou J, Liao Q, Li Y, Zhou Q, Bi G, et al. The Taxus genome provides insights into paclitaxel biosynthesis. *Nat. Plants* **2021**, *7*, 1026–1036. DOI:10.1038/s41477-021-00963-5
7. Cheng J, Wang X, Liu X, Zhu X, Li Z, Chu H, et al. Chromosome-level genome of Himalayan yew provides insights into the origin and evolution of the paclitaxel biosynthetic pathway. *Mol. Plant* **2021**, *14*, 1199–1209. DOI:10.1016/j.molp.2021.04.015
8. Song C, Fu F, Yang L, Niu Y, Tian Z, He X, et al. Taxus yunnanensis genome offers insights into gymnosperm phylogeny and taxol production. *Commun. Biol.* **2021**, *4*, 1203. DOI:10.1038/s42003-021-02697-8
9. Zhang Y, Scossa F, Fernie AR. The genomes of Taxus species unveil novel candidates in the biosynthesis of taxoids. *Mol. Plant* **2021**, *14*, 1773–1775. DOI:10.1016/j.molp.2021.08.017

10. Wang M, Ma R, Fang Z, Zhang L, Zhang Y, Zheng M, et al. Analysis genome of *Pseudotaxus chienii* reveals insights into the origin and evolution of taxane biosynthesis. *Nat. Commun.* **2025**, *17*, 1088. DOI:10.1038/s41467-025-67849-4
11. McClune CJ, Liu JC, Wick C, De La Pena R, Lange BM, Fordyce PM, et al. Discovery of FoTO1 and Taxol genes enables biosynthesis of baccatin III. *Nature* **2025**, *643*, 582–592. DOI:10.1038/s41586-025-09090-z
12. Jiang B, Gao L, Wang H, Sun Y, Zhang X, Ke H, et al. Characterization and heterologous reconstitution of *Taxus* biosynthetic enzymes leading to baccatin III. *Science* **2024**, *383*, 622–629. DOI:10.1126/science.adj3484
13. Yang C, Wang Y, Su Z, Xiong L, Wang P, Lei W, et al. Biosynthesis of the highly oxygenated tetracyclic core skeleton of Taxol. *Nat. Commun.* **2024**, *15*, 2339. DOI:10.1038/s41467-024-46583-3
14. Li C, Yin X, Wang S, Sui S, Liu J, Sun X, et al. A Cytochrome P450 Enzyme Catalyses Oxetane Ring Formation in Paclitaxel Biosynthesis. *Angewandte Chem. Int. Ed.* **2024**, *63*, e202407070. DOI:10.1002/anie.202407070
15. Zhang Y, Wiese L, Fang H, Alseekh S, Perez de Souza L, Scossa F, et al. Synthetic biology identifies the minimal gene set required for Paclitaxel biosynthesis in a plant chassis. *Mol. Plant* **2023**, *16*, 1951–1961. DOI:10.1016/j.molp.2023.10.016
16. Liang F, Xie Y, Zhang C, Zhao Y, Motawia MS, Kampranis SC. Elucidation of the final steps in Taxol biosynthesis and its biotechnological production. *Nat. Synth.* **2025**, *4*, 1212–1222. DOI:10.1038/s44160-025-00800-z
17. Lai M, Weng Y, Wei J, Lu XY, Huang Z, Li H, et al. Designing an oxidase toolbox for site-directed oxidation of taxanes. *Nat. Commun.* **2025**, *17*, 841. DOI:10.1038/s41467-025-67544-4
18. Tang X, Zhang X, Long LP, Wu S, Fan XY, Liu J, et al. Versatile 2-Oxoglutarate-Dependent Dioxygenases Catalyze Radical-Mediated Multifunctional Skeleton Reconstructions and Oxidation Modifications of Taxoids. *J. Am. Chem. Soc.* **2026**, *148*, 11333–11343. DOI:10.1021/jacs.6c01169
19. Bian G, Yuan Y, Tao H, Shi X, Zhong X, Han Y, et al. Production of taxadiene by engineering of mevalonate pathway in *Escherichia coli* and endophytic fungus *Alternaria alternata* TPF6. *Biotechnol. J.* **2017**, *12*, 1600697. DOI:10.1002/biot.201600697
20. Williams DC, Wildung MR, Jin AQ, Dalal D, Oliver JS, Coates RM, et al. Heterologous expression and characterization of a “Pseudomature” form of taxadiene synthase involved in paclitaxel (Taxol) biosynthesis and evaluation of a potential intermediate and inhibitors of the multistep diterpene cyclization reaction. *Arch. Biochem. Biophys.* **2000**, *379*, 137–146. DOI:10.1006/abbi.2000.1865
21. Edgar S, Zhou K, Qiao K, King JR, Simpson JH, Stephanopoulos G. Mechanistic Insights into Taxadiene Epoxidation by Taxadiene-5 α -Hydroxylase. *ACS Chem. Biol.* **2016**, *11*, 460–469. DOI:10.1021/acscchembio.5b00767
22. Edgar S, Li FS, Qiao K, Weng JK, Stephanopoulos G. Engineering of Taxadiene Synthase for Improved Selectivity and Yield of a Key Taxol Biosynthetic Intermediate. *ACS Synth. Biol.* **2017**, *6*, 201–205. DOI:10.1021/acssynbio.6b00206
23. Zhao Y, Liang F, Xie Y, Duan Y-T, Andeadelli A, Pateraki I, et al. Oxetane Ring Formation in Taxol Biosynthesis Is Catalyzed by a Bifunctional Cytochrome P450 Enzyme. *J. Am. Chem. Soc.* **2023**, *146*, 801–810. DOI:10.1021/jacs.3c10864
24. Li C, Yin X, Dai J. The History of Studies on Oxetane Ring Formation in Paclitaxel Biosynthesis. *ChemBiochem* **2025**, *26*, e202400947. DOI:10.1002/cbic.202400947
25. Tong Y, Luo YF, Gao W. Biosynthesis of paclitaxel using synthetic biology. *Phytochem. Rev.* **2021**, *21*, 863–877. DOI:10.1007/s11101-021-09766-0
26. Liu JCT, De La Pena R, Tocol C, Sattely ES. Reconstitution of early paclitaxel biosynthetic network. *Nat. Commun.* **2024**, *15*, 1419. DOI:10.1038/s41467-024-45574-8
27. Li C, Sun X, Chen R, Xie K, Chen D, Liu J, et al. The discovery of missing taxane C13 α -O-deacetylases redelineates the biosynthetic pathway of paclitaxel. *bioRxiv* **2026**. DOI:10.64898/2026.04.28.721278
28. Chau M, Walker K, Long R, Croteau R. Regioselectivity of taxoid-O-acetyltransferases: Heterologous expression and characterization of a new taxadien-5 α -ol-O-acetyltransferase. *Arch. Biochem. Biophys.* **2004**, *430*, 237–246. DOI:10.1016/j.abb.2004.07.013
29. Jennewein S, Rithner CD, Williams RM, Croteau R. Taxoid metabolism: Taxoid 14 β -hydroxylase is a cytochrome P450-dependent monooxygenase. *Arch. Biochem. Biophys.* **2003**, *413*, 262–270. DOI:10.1016/S0003-9861(03)00090-0
30. Walker KD, Klettke K, Akiyama T, Croteau R. Cloning, heterologous expression, and characterization of a phenylalanine aminomutase involved in Taxol biosynthesis. *J. Biol. Chem.* **2004**, *279*, 53947–53954. DOI:10.1074/jbc.M411215200
31. Ramirez-Estrada K, Altabella T, Onrubia M, Moyano E, Notredame C, Osuna L, et al. Transcript profiling of jasmonate-elicited *Taxus* cells reveals a beta-phenylalanine-CoA ligase. *Plant Biotechnol. J.* **2016**, *14*, 85–96. DOI:10.1111/pbi.12359
32. Walker K, Long R, Croteau R. The final acylation step in taxol biosynthesis: Cloning of the taxoid C13-side-chain N-benzoyltransferase from *Taxus*. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9166–9171. DOI:10.1073/pnas.082115799
33. Hefner J, Ketchum REB, Croteau R. Cloning and functional expression of a cDNA encoding geranylgeranyl diphosphate synthase from *Taxus canadensis* and assessment of the role of this prenyltransferase in cells induced for taxol production. *Arch. Biochem. Biophys.* **1998**, *360*, 62–74. DOI:10.1006/abbi.1998.0926

34. Wildung MR, Croteau R. A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis. *J. Biol. Chem.* **1996**, *271*, 9201–9204. DOI:10.1074/jbc.271.16.9201
35. Jennewein S, Long RM, Williams RM, Croteau R. Cytochrome P450 taxadiene 5 alpha-hydroxylase, a mechanistically unusual monooxygenase catalyzing the first oxygenation step of taxol biosynthesis. *Chem. Biol.* **2004**, *11*, 379–387. DOI:10.1016/j.chembiol.2004.02.022
36. Jennewein S, Rithner CD, Williams RM, Croteau RB. Taxol biosynthesis: Taxane 13 alpha-hydroxylase is a cytochrome P450-dependent monooxygenase. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13595–13600. DOI:10.1073/pnas.251539398
37. Walker K, Schoendorf A, Croteau R. Molecular cloning of a taxa-4(20),11(12)-dien-5alpha-ol-O-acetyl transferase cDNA from *Taxus* and functional expression in *Escherichia coli*. *Arch. Biochem. Biophys.* **2000**, *374*, 371–380. DOI:10.1006/abbi.1999.1609
38. Chau M, Jennewein S, Walker K, Croteau R. Taxol biosynthesis: Molecular cloning and of a cytochrome p450 characterization taxoid 7 beta-hydroxylase. *Chem. Biol.* **2004**, *11*, 663–672. DOI:10.1016/S1074-5521(04)00111-5
39. Chau M, Croteau R. Molecular cloning and characterization of a cytochrome P450 taxoid 2 alpha-hydroxylase involved in Taxol biosynthesis. *Arch. Biochem. Biophys.* **2004**, *427*, 48–57. DOI:10.1016/j.abb.2004.04.016
40. Walker K, Croteau R. Taxol biosynthesis: Molecular cloning of a benzoyl-CoA. Taxane 2 alpha-O-benzoyltransferase cDNA from *Taxus* and functional expression in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13591–13596. DOI:10.1073/pnas.250491997
41. Walker K, Croteau R. Molecular cloning of a 10-deacetylbaocatin III-10-O-acetyl transferase cDNA from *Taxus* and functional expression in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 583–587. DOI:10.1073/pnas.97.2.583
42. Sanchez-Munoz R, Perez-Mata E, Almagro L, Cusido RM, Bonfill M, Palazon J, et al. A Novel Hydroxylation Step in the Taxane Biosynthetic Pathway: A New Approach to Paclitaxel Production by Synthetic Biology. *Front. Bioeng. Biotechnol.* **2020**, *8*, 410. DOI:10.3389/fbioe.2020.00410
43. Li T, Li B, Liao C, Zhang H, Wang L, Fu T, et al. Transcriptome analysis provides insights into light condition effect on paclitaxel biosynthesis in yew saplings. *BMC Plant Biol.* **2022**, *22*, 577. DOI:10.1186/s12870-022-03958-2
44. Mubeen S, Li ZL, Huang QM, He CT, Yang ZY. Comparative Transcriptome Analysis Revealed the Tissue-Specific Accumulations of Taxanes among Three Experimental Lines of *Taxus yunnanensis*. *J. Agric. Food Chem.* **2018**, *66*, 10410–10420. DOI:10.1021/acs.jafc.8b03502
45. Wang T, Chen Y, Zhuang W, Zhang F, Shu X, Wang Z, et al. Transcriptome Sequencing Reveals Regulatory Mechanisms of Taxol Synthesis in *Taxus wallichiana* var. *Mairei*. *Int. J. Genom.* **2019**, *2019*, 1596895. DOI:10.1155/2019/1596895
46. Yu C, Luo X, Zhang C, Xu X, Huang J, Chen Y, et al. Tissue-specific study across the stem of *Taxus media* identifies a phloem-specific TmMYB3 involved in the transcriptional regulation of paclitaxel biosynthesis. *Plant J.* **2020**, *103*, 95–110. DOI:10.1111/tbj.14710
47. Yu C, Hou K, Zhang H, Liang X, Chen C, Wang Z, et al. Integrated mass spectrometry imaging and single-cell transcriptome atlas strategies provide novel insights into taxoid biosynthesis and transport in *Taxus mairei* stems. *Plant J.* **2023**, *115*, 1243–1260. DOI:10.1111/tbj.16315
48. Huang Q, Roessner CA, Croteau R, Scott AI. Engineering *Escherichia coli* for the Synthesis of Taxadiene, a Key Intermediate in the Biosynthesis of Taxol. *Bioorganic Med. Chem.* **2001**, *9*, 2237–2242. DOI:10.1016/S0968-0896(01)00072-4
49. Zhou K, Qiao K, Edgar S, Stephanopoulos G. Distributing a metabolic pathway among a microbial consortium enhances production of natural products. *Nat. Biotechnol.* **2015**, *33*, 377–383. DOI:10.1038/nbt.3095
50. Boghigian BA, Armando J, Salas D, Pfeifer BA. Computational identification of gene over-expression targets for metabolic engineering of taxadiene production. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 2063–2073. DOI:10.1007/s00253-011-3725-1
51. Abdallah II, Pramastya H, van Merkerk R, Sukrasno, Quax WJ. Metabolic Engineering of *Bacillus subtilis* Toward Taxadiene Biosynthesis as the First Committed Step for Taxol Production. *Front. Microbiol.* **2019**, *10*, 218. DOI:10.3389/fmicb.2019.00218
52. Biggs BW, Lim CG, Sagliani K, Shankar S, Stephanopoulos G, De Mey M, et al. Overcoming heterologous protein interdependency to optimize P450-mediated Taxol precursor synthesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3209–3214. DOI:10.1073/pnas.1515826113
53. Mutanda I, Li J, Xu F, Wang Y. Recent Advances in Metabolic Engineering, Protein Engineering, and Transcriptome-Guided Insights Toward Synthetic Production of Taxol. *Front. Bioeng. Biotechnol.* **2021**, *9*, 632269. DOI:10.3389/fbioe.2021.632269
54. Ding MZ, Yan HF, Li LF, Zhai F, Shang LQ, Yin Z, et al. Biosynthesis of Taxadiene in *Saccharomyces cerevisiae*: Selection of geranylgeranyl diphosphate synthase directed by a computer-aided docking strategy. *PLoS ONE* **2014**, *9*, e109348. DOI:10.1371/journal.pone.0109348

55. Nowrouzi B, Li RA, Walls LE, d’Espaux L, Malci K, Liang L, et al. Enhanced production of taxadiene in *Saccharomyces cerevisiae*. *Microb. Cell Factories* **2020**, *19*, 200. DOI:10.1186/s12934-020-01458-2
56. Walls LE, Malci K, Nowrouzi B, Li RA, d’Espaux L, Wong J, et al. Optimizing the biosynthesis of oxygenated and acetylated Taxol precursors in *Saccharomyces cerevisiae* using advanced bioprocessing strategies. *Biotechnol. Bioeng.* **2021**, *118*, 279–293. DOI:10.1002/bit.27569
57. Nowrouzi B, Lungang L, Rios-Solis L. Exploring optimal Taxol(R) CYP725A4 activity in *Saccharomyces cerevisiae*. *Microb. Cell Factories* **2022**, *21*, 197. DOI:10.1186/s12934-022-01922-1
58. Jiang Z, Kempinski C, Bush CJ, Nybo SE, Chappell J. Engineering Triterpene and Methylated Triterpene Production in Plants Provides Biochemical and Physiological Insights into Terpene Metabolism. *Plant Physiol.* **2016**, *170*, 702–716. DOI:10.1104/pp.15.01548
59. Besumbes O, Sauret-Gueto S, Phillips MA, Imperial S, Rodriguez-Concepcion M, Boronat A. Metabolic engineering of isoprenoid biosynthesis in *Arabidopsis* for the production of taxadiene, the first committed precursor of Taxol. *Biotechnol. Bioeng.* **2004**, *88*, 168–175. DOI:10.1002/bit.20237
60. Rontein D, Onillon S, Herbette G, Lesot A, Werck-Reichhart D, Sallaud C, et al. CYP725A4 from yew catalyzes complex structural rearrangement of taxa-4(5),11(12)-diene into the cyclic ether 5(12)-oxa-3(11)-cyclotaxane. *J. Biol. Chem.* **2008**, *283*, 6067–6075. DOI:10.1074/jbc.M708950200
61. Li J, Mutanda I, Wang K, Yang L, Wang J, Wang Y. Chloroplastic metabolic engineering coupled with isoprenoid pool enhancement for committed taxanes biosynthesis in *Nicotiana benthamiana*. *Nat. Commun.* **2019**, *10*, 4850. DOI:10.1038/s41467-019-12879-y
62. Fu J, Xu W, Huang W, Wang B, Li S, Zhang J, et al. Importation of taxadiene synthase into chloroplast improves taxadiene production in tobacco. *Planta* **2021**, *253*, 107. DOI:10.1007/s00425-021-03626-z
63. He J, Plácido JPA, Pateraki I, Kampranis S, Favero BT, Lütken H. Hairy root induction of *Taxus baccata* L. by natural transformation with *Rhizobium rhizogenes*. *Horticulturae* **2023**, *9*, 4. DOI:10.3390/horticulturae9010004
64. Ajikumar PK, Xiao WH, Tyo KE, Wang Y, Simeon F, Leonard E, et al. Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. *Science* **2010**, *330*, 70–74. DOI:10.1126/science.1191652
65. Boghigian BA, Salas D, Ajikumar PK, Stephanopoulos G, Pfeifer BA. Analysis of heterologous taxadiene production in K- and B-derived *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1651–1661. DOI:10.1007/s00253-011-3528-4
66. Wu Q-Y, Huang Z-Y, Wang J-Y, Yu H-L, Xu J-H. Construction of an *Escherichia coli* cell factory to synthesize taxadien-5 α -ol, the key precursor of anti-cancer drug paclitaxel. *Bioresour. Bioprocess.* **2022**, *9*, 82. DOI:10.1186/s40643-022-00569-5
67. Wang J-Y, Huang Z-Y, Wu Q-Y, Pan J, Li C-X, Xu J-H. Facile biosynthesis of taxadiene by a newly constructed *Escherichia coli* strain fusing enzymes taxadiene synthase and geranylgeranyl pyrophosphate synthase. *Process Biochem.* **2022**, *122*, 129–136. DOI:10.1016/j.procbio.2022.09.005
68. Engels B, Dahm P, Jennewein S. Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production. *Metab. Eng.* **2008**, *10*, 201–206. DOI:10.1016/j.ymben.2008.03.001
69. Walls LE, Martinez JL, Rios-Solis L. Enhancing *Saccharomyces cerevisiae* Taxane Biosynthesis and Overcoming Nutritional Stress-Induced Pseudohyphal Growth. *Microorganisms* **2022**, *10*, 163. DOI:10.3390/microorganisms10010163
70. Xu M, Xie W, Luo Z, Li CX, Hua Q, Xu J. Improving solubility and copy number of taxadiene synthase to enhance the titer of taxadiene in *Yarrowia lipolytica*. *Synth. Syst. Biotechnol.* **2023**, *8*, 331–338. DOI:10.1016/j.synbio.2023.04.002
71. Min L, Han JC, Zhang W, Gu CC, Zou YP, Li CC. Strategies and Lessons Learned from Total Synthesis of Taxol. *Chem. Rev.* **2023**, *123*, 4934–4971. DOI:10.1021/acs.chemrev.2c00763
72. Hasan MM, Kim HS, Jeon JH, Kim SH, Moon B, Song JY, et al. Metabolic engineering of *Nicotiana benthamiana* for the increased production of taxadiene. *Plant Cell Rep.* **2014**, *33*, 895–904. DOI:10.1007/s00299-014-1568-9
73. Wick C, Somani A, Liu JC, Karunadasa SS, Xu SL, Fordyce PM, et al. FoTO1 orchestrates Taxol biosynthesis through catalytic and non-catalytic mechanisms. *BioRxiv* **2026**. DOI:10.64898/2026.03.21.713420
74. Wang Y, Guo B, Miao Z, Tang K. Transformation of taxol-producing endophytic fungi by restriction enzyme-mediated integration (REMI). *FEMS Microbiol. Lett.* **2007**, *273*, 253–259. DOI:10.1111/j.1574-6968.2007.00801.x
75. El-Sayed ASA, Abdel-Ghany SE, Ali GS. Genome editing approaches: Manipulating of lovastatin and taxol synthesis of filamentous fungi by CRISPR/Cas9 system. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 3953–3976. DOI:10.1007/s00253-017-8263-z
76. Zhong J, Wang Y, Chen Z, Yalikul Y, He L, Liu T, et al. Engineering cyanobacteria as a new platform for producing taxol precursors directly from carbon dioxide. *Biotechnol. Biofuels Bioprod.* **2024**, *17*, 99. DOI:10.1186/s13068-024-02555-9
77. Ansbacher T, Freud Y, Major DT. Slow-Starter Enzymes: Role of Active-Site Architecture in the Catalytic Control of the Biosynthesis of Taxadiene by Taxadiene Synthase. *Biochemistry* **2018**, *57*, 3773–3779. DOI:10.1021/acs.biochem.8b00452

78. Liu H, Chen Q. Computational protein design for given backbone: Recent progresses in general method-related aspects. *Curr. Opin. Struct. Biol.* **2016**, *39*, 89–95. DOI:10.1016/j.sbi.2016.06.013
79. Koksai M, Jin Y, Coates RM, Croteau R, Christianson DW. Taxadiene synthase structure and evolution of modular architecture in terpene biosynthesis. *Nature* **2011**, *469*, 116–120. DOI:10.1038/nature09628
80. Soliman S, Tang Y. Natural and engineered production of taxadiene with taxadiene synthase. *Biotechnol. Bioeng.* **2015**, *112*, 229–235. DOI:10.1002/bit.25468
81. Van Rijn JPM, Escorcía AM, Thiel W. QM/MM study of the taxadiene synthase mechanism. *J. Comput. Chem.* **2019**, *40*, 1902–1910. DOI:10.1002/jcc.25846
82. Kanda Y, Ishihara Y, Wilde NC, Baran PS. Two-Phase Total Synthesis of Taxanes: Tactics and Strategies. *J. Org. Chem.* **2020**, *85*, 10293–10320. DOI:10.1021/acs.joc.0c01287
83. Barton NA, Marsh BJ, Lewis W, Narraidoo N, Seymour GB, Fray R, et al. Accessing low-oxidation state taxanes: Is taxadiene-4(5)-epoxide on the taxol biosynthetic pathway? *Chem. Sci.* **2016**, *7*, 3102–3107. DOI:10.1039/C5SC03463A
84. Schrepfer P, Buettner A, Goerner C, Hertel M, van Rijn J, Wallrapp F, et al. Identification of amino acid networks governing catalysis in the closed complex of class I terpene synthases. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 958–967. DOI:10.1073/pnas.1519680113
85. Yadav VG. Unraveling the multispecificity and catalytic promiscuity of taxadiene monooxygenase. *J. Mol. Catal. B Enzym.* **2014**, *110*, 154–164. DOI:10.1016/j.molcatb.2014.10.004
86. Song X, Wang Q, Zhu X, Fang W, Liu X, Shi C, et al. Unraveling the Catalytic Mechanism of Taxadiene-5 α -hydroxylase from Crystallography and Computational Analyses. *ACS Catal.* **2024**, *14*, 3912–3925. DOI:10.1021/acscatal.3c05807
87. Zhang MF, Xie WL, Chen C, Li CX, Xu JH. Computational redesign of taxane-10 β -hydroxylase for *de novo* biosynthesis of a key paclitaxel intermediate. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 7105–7117. DOI:10.1007/s00253-023-12784-x
88. Li BJ, Wang H, Gong T, Chen JJ, Chen TJ, Yang JL, et al. Improving 10-deacetylbaccatin III-10- β -O-acetyltransferase catalytic fitness for Taxol production. *Nat. Commun.* **2017**, *8*, 15544. DOI:10.1038/ncomms15544
89. Lin SL, Wei T, Lin JF, Guo LQ, Wu GP, Wei JB, et al. Bio-production of Baccatin III, an Important Precursor of Paclitaxel by a Cost-Effective Approach. *Mol. Biotechnol.* **2018**, *60*, 492–505. DOI:10.1007/s12033-018-0090-7
90. Chen JJ, Liang X, Wang F, Wen YH, Chen TJ, Liu WC, et al. Combinatorial mutation on the β -glycosidase specific to 7- β -xylosyltaxanes and increasing the mutated enzyme production by engineering the recombinant yeast. *Acta Pharm. Sin. B* **2019**, *9*, 626–638. DOI:10.1016/j.apsb.2018.11.003
91. Bai J, Li J, Zhang Y, Chang H, Zhang N, Liu Y, et al. FoTO1 is an epoxide isomerase in paclitaxel biosynthesis. *BioRxiv* **2026**. DOI:10.64898/2026.03.30.715439
92. Zhu X, Liu X, Liu T, Wang Y, Ahmed N, Li Z, et al. Synthetic biology of plant natural products: From pathway elucidation to engineered biosynthesis in plant cells. *Plant Commun.* **2021**, *2*, 100229. DOI:10.1016/j.xplc.2021.100229
93. Li J, Liu X, Zhu X, Liu J, Zhang L, Ahmed N, et al. Biochemical synthesis of taxanes from mevalonate. *Synth. Syst. Biotechnol.* **2024**, *9*, 694–700. DOI:10.1016/j.synbio.2024.05.002