综 述

内生真菌紫杉醇生物合成的研究现状与展望

赵凯[1]，宇璐[1]，金昱言[1]，马学玲[2]，刘丹[1]，王晓华[1]，王歆[1]

1 黑龙江大学 生命科学学院微生物重点实验室，黑龙江 哈尔滨 150080
2 哈尔滨医科大学附属第四医院 神经内科，黑龙江 哈尔滨 150001

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摘 要：紫杉醇是重要的抗癌药物之一，已经证明其对多种癌症具有显著疗效。目前，人们主要是从红豆杉的树皮中提取、分离和纯化紫杉醇，但由于红豆杉为生长缓慢、散生、濒危的珍稀植物，且随着紫杉醇临床用途的不断拓宽，市场需求的稳定增长，单纯依靠从红豆杉树皮中提取紫杉醇已经无法满足日益增长的市场需求。为了解决紫杉醇的药源不足，科学家已把目光从红豆杉树分离提取紫杉醇转向了其他替代方法，如化学全合成、半合成、组织培养与细胞培养、微生物发酵法生产紫杉醇等。因此，了解内生真菌紫杉醇生物合成的分子基础和遗传调控机制，对解析内生真菌紫杉醇生物合成机制、构建高产紫杉醇基因工程菌株和早日实现内生真菌紫杉醇工业化生产具有重要的科学意义和现实意义。结合本课题组多年来的科研工作，概述了红豆杉细胞紫杉醇生物合成途径、内生真菌发酵生产紫杉醇的优势、产紫杉醇内生菌的分离研究现状和生物多样性及紫杉醇生物合成相关基因的研究现状。内生真菌生物发酵合成紫杉醇是可以无限生产、大量获取紫杉醇、解决紫杉醇药源短缺问题的很有前景的方法之一。

关键词：内生真菌，紫杉醇，生物合成，相关基因

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Corresponding author: Xin Wang. Tel: +86-451-86608586; Fax: +86-451-86609016; E-mail: tianronghaise@126.com
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Advances and prospects of taxol biosynthesis by endophytic fungi

Kai Zhao[1], Lu Yu[1], Yuyan Jin[1], Xueling Ma[2], Dan Liu[1], Xiaohua Wang[1], and Xin Wang[1]

1 *Key Laboratory of Microbiology*, *School of Life Science*, *Heilongjiang University*, *Harbin* 150080, *Heilongjiang*, *China*
2 *Department of Neurosurgery*, *the Fourth Affiliated Hospital of Harbin Medical University*, *Harbin* 150001, *Heilongjiang*, *China*

Abstract: Taxol is one of the most important chemotherapeutic drugs against cancer. Taxol has been mainly extracted from the bark of yews for a long time. However, methods for the extraction of taxol from the bark of Taxus species were inefficient and environmentally costly. As a result of the high ecological toll exacted on trees with the potential for *Pacific yew* extinction, investigators began to look for other methods of taxol production. Recently, increasing efforts have been made to develop alternative means of taxol production, such as using complete chemical synthesis, semi-synthesis, *Taxus* spp. plant cell culture and microbe fermentation. Using microbe fermentation in the production of taxol would be a very prospective method for obtaining a large amount of taxol. Therefore, it is necessary to understand the molecular basis and genetic regulation mechanisms of taxol biosynthesis by endophytic fungi, which may be helpful to construct the genetic engineering strain with high taxol output. In this paper, the taxol biosynthesis pathway from *Taxus* cells and the advantages of taxol biosynthesis by endophytic fungi were discussed. The study on the isolation and biodiversity of taxol-producing endophytic fungi and the taxol biosynthesis related genes are also discussed.

Keywords: endophytic fungi, taxol, biosynthesis, related genes

Taxol is a diterpenoid with anticancer activities, and was first isolated from the bark of *Taxus brevifolia* Nutt by Wani et al [1]. It is a natural anti-cancer drug with high efficiency, low toxicity, and broad-spectrum, and has been widely used for the treatment of many malignant cancers, such as metastatic breast cancer, advanced ovarian cancer, nonsmall-cell lung cancer and Kaposi's sarcoma [2-3]. Up to now, taxol is extracted, isolated and purified mostly from the bark of *Taxus* species. *Taxus* grows very slow, sparse, and thus becomes the rare tree species; in addition, studies have showed that the taxol content in the *Taxus* species is fairly low. Therefore, it is very difficult to solve the taxol source if depending solely on extraction from yew. Chemical synthesis of taxol has been also attempted, which has many disadvantages, such as complex synthesis route, uncontrollable reaction conditions, higher costs, and these limited the method to be used only in laboratory. In semi-synthesis pathway, the precursors such as Baccatin Ⅲ and 10-Deacetylbaccatin Ⅲ have also to be extracted from *Taxus*. Plant cell culture or plant callus induction can only produce taxol at low output with high cost. The discovery of taxol producing endophytic fungi is a step stone towards the exploration of taxol sources. Taxol production by endophytic fungi fermentation has many advantages, including high growth rate, short growth period, simple medium composition, the controllable culture conditions and low costs, which therefore become the preference of the researchers. This method has now become a very effective method for exploring the taxol sources.

The use of endophytic fungi for biosynthesis of taxol by fermentation technique has yet on the lab

level, and there is still a gap towards industry-based level. The taxol yield from the taxol-producing stain is very low, and little has been known on the molecular biological basis of taxol synthesis, which contributed to its low output. Up to now, few reports on the taxol biosynthesis-related genes by endophytic fungi have been published. This promotes great difficulties and challenges for the use of modern biotechnology to modify the low-output strain genetically, therefore obtaining the high-output strain is necessary. In this view, the understanding of the molecular basis and genetically-regulating mechanisms of taxol biosynthesis by endophytic strain will make it possible to modulate the taxol biosynthesis by endophytic fungi at molecular level, elucidate the mechanisms of the taxol biosynthesis by endophytic fungi and construct high-output strain genetically. This may solve the sources of taxol and realize the production at industrial levels. This review stated the biosynthesis pathway of taxol by *Taxus* cells, the advantages of taxol production by endophytic fungi, the present advance of the isolation and biodiversities of taxol-producing endophytic fungi, and the taxol biosynthesis-related genes.

1 The biosynthetic pathway of taxol

The biosynthetic pathway of taxol from *Taxus* cells has been elucidated nowadays, while little is known about the taxol biosynthesis by endophytic fungi. The biosynthetic pathway of taxol from *Taxus* cells is generally divided into three stages, that is, the synthesis of isopentenylpyrophosphate (IPP), a kind of terpene precursor, taxol carbocycle skeleton Baccatin III synthesis and taxol side chain synthesis.

1.1 Synthesis of isopentenyl pyrophosphate (IPP)

Rohmer indicated that the biosynthesis of terpenoid involved both the mevalonate (MVA) and non-MVA (MEP) pathways [4]. Eisenreich et al proved that the taxane was synthesized by MEP pathway [5]. Both the MVA and MEP pathways were related, although the former existed in cytoplasm, and the latter existed in plasmids. IPP synthesized from these pathways is the precursor of the tricyclic diterpene in taxol biosynthesis.

1.2 Biosynthesis of Baccatin III

IPP and its isomer dimethyl-propene-pyrophosphoric acid (DMAPP) can form geranyl-pyrophosphoric acid (GPP) through condensation reaction. GPP may transform into FPP by adding one IPP. The FPP may condense with the third IPP to form geranylgeranyl diphosphate (GGPP). GGPP can be catalyzed by taxol-diene-cyclase for cyclization into taxa-4 (5) (12)-diene, which is the backbone of taxol-tricyclic-diterpene. This is the speed-limiting step in taxol synthesis. Baccatin III, which is the last diterpene intermediate in taxol biosynthesis pathway and also the direct precursor of taxol biosynthesis was obtained after hydroxylation at C_1, C_2, C_5, C_7, C_9,C_{10} and C_{13}, the formation of epoxypropane circle at C_4 and C_5, acylation at C_2, C_5, C_{10}, ketone at C_9 [6-9].

1.3 Synthesis of taxol side chain

The C_{13} side chain of taxol is the key factor for ensuring the anticancer activities of taxol. The side chain structures have greater effects on the taxol synthesis speed than that of the backbone. Therefore, the study on the biosynthetic steps of side chains may be important for increasing taxol output. Phenylalanine is a key precursor for side chain synthesis. Under the catalysis of aminomutase, α-phenylalanine can transform into β-phenylalanine, which was then transformed into phenylisoserine after hydroxylation at C_2 site. The phenylisoserine is the precursor of taxol C_{13} side chain. Phenylisoserine would then interact with taxol backbone to produce taxol [10].

2 Isolation of taxol-producing strains

At present, great progress has been obtained in the synthesis of taxol and taxane-like compounds by

fermentation using taxol and taxane-like compounds producing strain screened from yew endofungi. Stierle et al isolated *Taxomyces andreanae*, a taxol-producing endophytic fungus from *T. brevifolia*. From then on, the isolation and identification of taxol-producing endophytic fungi were carried out by many researchers. Up to now, more than 20 endophytic fungi genera have been found, which existed in many hosts, including yew, and non yew plants such as hazelnut, *Wollemi* and *Torreya grandifolia* indicating the biodiversity of taxol-producing fungi and their hosts (Table 1).

Table 1 Taxol-producing endophytic fungi discovered

Hosts	Strains	The content of taxol in fermentation liquid (μg/L)	References
Taxus baccata	Tbp-2 (*Monochaetia* sp.)	0.10	11
	Tbp-9 (*Fusarium lateritium*)	0.13	11
	Tbx-2 (*Pestalotia bicilia*)	1.08	11
	BT115 (*Botryodiplodia theobromae*)	280.50	12
	SBU-16 (*Stemphylium sedicola*)	6.90	13
	Ja-69 (*Alternaria* sp.)	0.16	11
	Ja-73 (*Pestalotiopsis microspora*)	0.27	11
Taxus cuspidata	HQD_{33} (*Nodulisporium sylviforme*)	51.06–125.70	14
	HQD_{48} (*N. sylviforme*)	51.06–125.70	14
	TPF-1 (*N. sylviforme*)	448.52	14
	HD1353 (*Alternaria taxi*)	–	15
	HD104 (*Botrytis taxi*)	–	16
	HDF-68 (*N. sylviforme)*	468.62	17
	$HDFS_{4\text{-}26}$ (*N. sylviforme*)	516.37	18
	HDl81-23 (*Botrytis* sp.)	206.34	18
	HD86-9 (*Aspergillus niger*)	273.46	19
	BKH 27(*Phomopsis*)	418.00	20
Taxus yunnanensis	Tax-1 (*Rhizoctonia* sp.)	1.43	21
	Tax-X (*Phoma*)	32.93	21
	Tax-26 (*Penicillium*)	8.24	21
	Tax-X (*Botrytig*)	4.09	21
	Tax-23 (*Trichoderma*)	19.59	21
	Tax-56 (*Mucor)*	1.08	21
	Tax-60 (*Chaetomium*)	21.10	21
Taxus chinensis	IFBC-Z38 (*Aspergillus niger*)	1 000.00	22
	YN6 (*Pestalotiopsis* sp.)	120.00–140.00	23
	XC1-07	1 124.34	24
	LB-10 (*Metarhizium anisopliae*)	846.10	25
	BJ-11 (*Aspergillus carbonarius*)	127. 20	26
	H-27 (*Cladosporium tenuissiumu*)	846.10	27
Taxus chinensis var. *mairei*	TF5 (*Tubercularia* sp.)	185.40	28
	12.3.2 (*Penicillium*)	–	29

续表 1

	XH004 (*Bionectria*)	33.90–430.46	30
	D62 (*Fusarium* sp.)	148.95	31
	LNUF014 (*Fusarium*)	53.68	32
	XT5 (*Ectostroma* sp.)	276.75	33
	XT2 (*Botrytis* sp.)	161.24	33
	XT17 (*Papulaspora* sp.)	10.25	33
	TPF6 (*Alternaria alternate*)	84.50	34
	Y1117 (*Fusarium*)	2.70	35
	F1 (*Pestalotiopsis*)	8.50	36
Torreya grandifolia	F2 (*Fusarium*)	31.50	36
	F3 (*Pestalotiopsis*)	31.10	36
	MD3 (*Aspergillus candidus*)	112.00	37
	MD2 (*Cladosporium cladosporioides*)	80.00	38
Taxusx medica	M57 (*Rhizopus*)	45.00–50.00	39
	Z58 (*Hypocrea* sp.)	2.50–3.00	40
	060B1 (*Mucor* sp.)	2.50–3.00	41
Taxodium distichum	UH23 (*Fusarium mairei*)	20.00	42
	Cp-4 (*Pestalotiopsis microspora*)	0.01–1.49	43
Taxus wallachiana	Ne-32 (*Pestalotiopsis microspora*)	50.00	11
Taxus sumatrana	P-96 (*Pithomyces* sp.)	0.09	11
Podocarpus	EPTP-1 (*Aspergillus fumigatus*)	560.00	44
Cardiospermum helicacabum	CHP-11 (*Pestalotiopsis pauciseta*)	113.30	45
Citrus medica	CHP-11 (*Phyllosticta citricarpa*)	265.00	46
Taxus brevifolia	*Taxomyces andreanae*	0.02–0.05	47
Terminalia arjuna	TAP-15 (*Pestalotiopsis terminaliae*)	211.10	48
Wrightia tinctoria	*Phyllosticta tabernaemontanae*	461.00	49
Ginkgo biloba	SBU-16 (*Phoma betae*)	795.00	50
Taxus baccata L. subsp. *wallichiana* (Zucc.) Pilger	TBPJ-B (*Fusarium redolens*)	66.00	51
Corylus avellana	NRRL 62431 (*Penicillium aurantiogriseum)*	–	52

3 Advances on the taxol-biosynthesis related genes

The study on the taxol biosynthesis pathway using *Taxus* cells has achieved great advances in recent years, and some of the genes encoding key enzymes have been isolated, identified and cloned (Table 2). However, there exist great differences in gene sequences encoding taxol biosynthesis between *Taxus* cells and endophytic fungi, which can be supported by the finding that candidate taxol biosynthetic genes from the taxol synthesizing in endophytic fungi were significantly different and had evolved independently from the host plants [53]. Up to now, few reports have been published on the isolation of taxol biosynthesis related genes from taxol-producing fungi.

3.1 Clone of taxol-biosynthesis related genes from *Taxus* cells

3.1.1 Taxane 14-β hydroxylase gene

The expression inhibition of Taxane 14-β hydroxylase gene may block the taxane pathway of

Table 2 Related enzymes of taxol biosynthesis from *Taxus* cells

Hosts	Enzymes	References
Taxus canadensis	Taxadiene 2-α hydroxylase	54
Taxus cuspidata	Taxadiene 5-α hydroxylase	55
Taxus cuspidata	Taxane 13-α hydroxylase	56
Taxus cuspidata	Taxane 7-β hydroxylase	57
Taxus cuspidata	Taxane 10-β hydroxylase	58
Taxus cuspidata	Taxane 14-β hydroxylase	59
Taxus cuspidata	Taxa-4(20),11(12)-dien-5-alpha-ol-O-acetyltransferase, *TAT*	60
Taxus canadensis	PAM	61
Taxus cuspidata	10-deacetylbaccatin III-10-O-acetyltransferase, *DBAT*	62
Taxus cuspidata	C-13 phenylpropanoid side chain-CoAacyltransferase, *BAPT*	63
Taxus canadensis	3'-N-debenzoyltaxol N-benzoyltransferase	64
Taxus canadensis	GGPPS	65
Taxus cuspidata	Taxane2α-O-benzoyltransferase, *TBT*	66
Taxus cuspidata	transcription factor AP2	67
Taxus x media	3-hydroxy-3-methylglutaryl enzyme A reductase, *HMGR*	68

the intermediate product to C_{14} oxygen substitution [69]. Jennewein et al [59] cloned genes expressing Taxane 14-β hydroxylase from *Taxus*, and pointed out that as no substitution at C_{14} site exists in taxol, 14-β hydroxylase can not remain in the target drug pathway, and may be related to the transduction pathway of *Taxus* cells. Li et al [70] inhibited Taxane 14-β hydroxylase gene expression in *Taxus media* effectively by using the RNAi technique, which provides theoretical basis for improving the yield of taxol.

3.1.2 Deacetyl Baccatin III-10β-O-acetyltransferase gene

In the taxol biosynthesis pathway, Baccatin III was formed by catalyzing with deacetyl Baccatin III-10β-O-acetyl-transferase (DBAT). This gene was first cloned by Walker et al [62]. Cheng et al also cloned the DBAT gene from *Taxus chinensis* var. *mairei* [71].

3.1.3 Geranylgeranyl diphosphate synthase gene

Geranylgeranyl diphosphate synthase (GGPPS) can catalyze to form Geranylgeranyl diphosphate (GGPP), which is the common precursor of diterpenes. GGPPS is the key enzyme for taxol biosynthesis. Hefner et al discovered the gene from *T. canadensis*, which contains 393 amino acid residues. GGPPS can provide necessary jasmonic methyl ester, inducing *T. canadensis* to produce the precursors for taxol synthesis [65]. Yu et al cloned 6 full-length cDNA encoding the important taxol genes including *GGPPS* gene [72]. Lan et al cloned GGPPS gene from *T. wallichiana* [73]. Wang et al cloned full-length sequence of GGPPS gene, and proved the high homogeneity of the protein with other plant-derived GGPPS [74]. Our group has obtained *GGPPS* gene fragments from *T. cuspidate*, which is 371 bp, and the gene has 99 % homogeneity with that of *GGPPS* gene recorded in GenBank[72].

3.1.4 Taxadiene synthase gene

Taxadiene synthase (TS) catalyze GGPP cyclization to form taxol-4(5),11(12)-diene, which is the backbone of taxol Tricyclic-diterpene. TS is the most important enzyme catalyzing taxol biosynthesis and the first oriental step of taxol biosynthesis, which aroused the investigator`s interest [76]. Wildung et al cloned the TS gene for the first time

from *T. brevifolia*, which is 98 303 Da, containing 2 586 nucleotides-encoding ORF, and 862 amino acid residues[77]. Liang et al cloned cDNA segments of *TS* gene from *T. yunnanensis*, with 98.42% identity to that reported by Wildung [78]. Xiao et al obtained the full length cDNA of *TS* gene from *T. chinensis* var. *mairei* [79].

3.1.5 Taxane 13α-hydroxylase gene

Taxane 13α-hydroxylase, with typical features of P450, is the key enzyme in the downstream of taxol biosynthesis by *Taxus* cells, which catalyzes the hydroxylation of C_{13} side chain from taxol diene-5α-itol to form taxol diene-5α,13α-diol [80]. This gene is 1 458 bp in length and has high identity with Taxane 10β-hydroxylase gene. The gene was firstly cloned and sequenced from *Taxus* cells by Jennewein et al [76]. Teng et al cloned Taxane 13α hydroxylase gene from *T. cuspidate* and constructed plant expression vector and transformed into tobacco [81]. Li et al [82] and Huang et al [80] cloned the gene from *T. cuspidate*. These studies provided molecular basis for production of taxol and its precursor using metabolic engineering.

3.1.6 Taxol diene-5α-itol-acety transferase gene

Taxol diene-5α-itol-acetyl transferase (*TAT*) is composed of 439 amino acid residues, with molecular weight of 50 kDa. At pH 9.0, TAT has good affinity to taxol dienetol and acetyl CoA. *TAT* is acetic taxol-4(20),11(12)-diene-5α-ester with a very low output in *Taxus* cells. Therefore, the taxol synthesis efficiency can be greatly affected by this enzyme. Walker et al firstly cloned the gene, and determined its important role in taxol biosynthesis, which can be used as the goal of improving taxol output [63].

3.1.7 4C-13 phenylpropanoidoyl CoA transferease gene

4C-13 phenylpropanoidoyl CoA transferease (BAPT) can catalyze the formation of 3′-N-debenzoyl-taxol, which is the direct precursor of taxol biosynthesis from β-phenylalanoyl-CoA and Baccatin III. Further study should be performed to prove whether this is the limiting step of taxol biosynthesis from *yew*. However, it is apparent that the enzyme used in this step is very important in taxol biosynthesis pathway. *BAPT* cDNA gene is 1 335 bp, encoding 445 amino acid residues, which was first found and cloned from *T. cuspidata* by Walker et al [63], who also pointed out that the gene could increase taxol output, activities and water-solubility when transferred into suitable host. Han et al cloned the *BAPT* full-length cDNA gene of 1 456 bp from three different *Taxus*, and there was 97.4 % identity among these sequences [83].

3.1.8 Taxadiene 5α-hydroxylase gene

Taxadiene 5α-hydroxylase is a multifunctional monooxygenase from microsome cytochrome P450. Jennewein et al clone the gene for the first time by screening cDNA library of *Taxus* [55].

3.1.9 Taxadiene 2α-hydroxylase gene

Taxadiene 2α-hydroxylase gene is 1 488 bp length, encoding 495 amino acids. As Taxadiene 2α-hydroxylase and Taxadiene 7β-hydroxylase can catalyze products from the other side to form the same products, it can be speculated that the taxol-biosynthesis may involve complicated net. Chau et al discovered and cloned the gene from *Taxus* [54].

3.2 Genes related to taxol-biosynthesis by endophytic fungi

Although the study on taxol synthesis by endophytic fungi has acquired great progress, the work is still limited in laboratory. One reason is the low taxol output from the isolated taxol-producing endophytic fungi, making it difficult for industrial production. Therefore, the focus should be on how to increase the taxol output through endophytic fungi biosynthesis. Based on the documents reported, three ways may be used to increase the taxol output through endophytic fungi biosynthesis: firstly, the gene encoding rate-limiting enzyme in taxol biosynthesis isolated from *Taxus* can be transferred into taxol-producing fungi to increase the expression

level of the key enzyme, and improve the taxol synthesis ability; secondly, optimization of the fermentation culture using the metabolic engineering by filling several substances including carbon sources, nitrogen sources, precursors, inducer and the metabolic bypass inhibitors [84-85]. There are many reports using these measures on the taxol study from *Taxus* cells, while very few reports have been concerned with taxol biosynthesis by endophytic fungi fermentation. Our group has studied the factors that affect the biosynthesis of taxol from taxol-producing fungi, such as culture temperature, the initial pH of culture, the rotation speed, and dissolved oxygen, and then determined the optimal fermentation conditions. Our group has also studied the effects of adding different concentrations of carbon sources, nitrogen sources, precursors, inducers, the metabolic bypass inhibitors and their synergism on the metabolic regulation during the biosynthesis of taxol from taxol-producing fungi, and we have obtained the optimal medium composition [86]. Thirdly, the genes encoding key enzymes isolated from taxol-producing fungi may be induced into the microbes, and construct new taxol-high output engineered strain to produce taxol using other fungi, bacteria or even yeast. However, there is no report on the taxol biosynthesis-related genes from *N. sylviforme*. Our group has also constructed the differential expression cDNA subtracting library of taxol-high output engineered strain and starting strain with a low output of taxol, cDNA library of taxol synthesis period subsidizing non-synthesis period of taxol-producing strain, high ratio full-length cDNA library and its genetic transformed system with high efficiency, screened the mutants contributing taxol-output changes, isolated seven taxol synthesis related genes by *N. sylviforme*, which were genes encoding diterpene synthetase, diterpene-5α-hydroxylase, GGPP synthetase, taxane-10β-hydroxylase, diterpene-5α-itol-acetyl transferase, taxane-2α-hydroxylase, taxane-13α-hydroxylase, respectively. These works may provide new insight into constructing high-output taxol genetic-engineering fungi by inducing the genes encoding the enzyme used for taxol biosynthesis by taxol-producing fungi through gene over-expression into microbes. And the study also provides a way for elucidating the taxol biosynthesis pathway by endophytic fungi and its biosynthesis mechanisms.

4 Prospective

It is possible to construct genetic-engineering strain with high yield of taxol, with the deep understanding of the screening of the taxol-synthesis related genes from endophytic fungi and the analysis of their functions. Simultaneously, using the constructed genetic-engineering strain with high yield of taxol as starting strain, the metabolic pathway and its mechanisms of biosynthesis of the genetic-engineered strain with high yield of taxol would be elucidated through classic methods for metabolic study such as inducer addition, resting cell, isotope tracer, blocked mutant, and mRNA differential display, transcription sequencing and protein expression differential analysis technique. It is believed that taxol production in large scale by taxol-producing endophytic fungi fermentation must be a leading direction, which would solve the taxol shortage and reduce the cost. This technique would become the important pathway for taxol resources.

On the other hand, with the developing of technology, many new research methods and ideas have been applied in the study of taxol production, such as high-throughput amplicon sequencing and ecological method [53, 87-89]. These results reveal that taxol biosynthetic pathway may differ between these microbes and *Taxus*, indicating that taxol biosynthesis in *Taxus* root endophytes may have evolved independently. These results also suggest that diversity of endophytes in *Taxus* is rich and the resident fungi within a host plant interact with one another to stimulate taxol biosynthesis, either

directly or through their metabolites and the endophyte secondary metabolism should be studied in the context of its native ecosystem.

REFERENCES

[1] Wani MC, Taylor HL, Wall ME, et al. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc, 1971, 93(9): 2325–2327.

[2] Sun L, Simmerling C, Ojima I. Recent advances in the study of the bioactive conformation of taxol. ChemMedChem, 2009, 4(5): 719–731.

[3] Mekhail TM, Markman M. Paclitaxel in cancer therapy. Expert Opin Pharmacother, 2002, 3(6): 755–766.

[4] Rohmer M, Knani M, Simonin P, et al. Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem J, 1993, 295(pt 2): 517–524.

[5] Eisenreich W, Menhard B, Hylands PJ, et al. Studies on the biosynthesis of taxol: the taxane carbon skeleton is not of mevalonoid origin. Proc Natl Acad Sci USA, 1996, 93(13): 6431–6436.

[6] Kong JQ, Wang W, Zhu P, et al. Recent advances in the biosynthesis of taxol. Acta Pharm Sin, 2007, 42(4): 358–365 (in Chinese).
孔建强, 王伟, 朱平, 等. 紫杉醇生物合成的研究进展. 药学学报, 2007, 42(4): 358–365.

[7] Xiong ZQ, Yang YY, Zhao N, et al. Diversity of endophytic fungi and screening of fungal paclitaxel producer from *Anglojap yew*, *Taxus x media*. BMC Microbiol, 2013, 13: 71.

[8] Lee YH, Lee YR, Kim KH, et al. Baccatin III, a synthetic precursor of taxol, enhances MHC-restricted antigen presentation in dendritic cells. Int Immunopharmacol, 2011, 11(8): 985–991.

[9] Liu WH, Yao B, Zhu SQ, et al. Advances in studies on biosynthetic pathway of taxol precursor and its correlative biotechnology. Chin Tradit Herb Drugs, 2009, 40(8): 1327–1331 (in Chinese).
刘万宏, 姚波, 祝顺琴, 等. 紫杉醇前体生物合成途径及生物技术研究进展. 中草药, 2009, 40(8): 1327–1331.

[10] Jiang M, Stephanopoulos G, Pfeifer BA. Downstream reactions and engineering in the microbially reconstituted pathway for taxol. Appl Microbiol Biotechnol, 2012, 94(4): 841–849.

[11] Strobel G, Yang XS, Sears J, et al. Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*. Microbiology, 1996, 142(2): 435–440.

[12] Venkatachalam R, Subban K, Paul MJ. Taxol from *Botryodiplodia theobromae* (BT 115)-an endophytic fungus of *Taxus baccata*. J Biotechnol, 2008, 136(S): S189–S190.

[13] Mirjalili MH, Farzaneh M, Bonfill M, et al. Isolation and characterization of *Stemphylium sedicola* SBU-16 as a new endophytic taxol-producing fungus from *Taxus baccata* grown in Iran. FEMS Microbiol Lett, 2012, 328(2): 122–129.

[14] Zhou DP, Sun JQ, Yu HY, et al. *Nodulisporium*, a genus new to China. Mycosystema, 2001, 20(2): 148–149.

[15] Ge QP, Ping WX, Ma X, et al. Identification of taxol-producing strain HU1353. J Microbiol, 2004, 24(3): 19–21 (in Chinese).
葛菁萍, 平文祥, 马玺, 等. 紫杉醇产生菌 HU1353 的鉴定. 微生物学杂志, 2004, 24(5): 19–21.

[16] Wang Y, Ma X, Ping WX, et al. A new species of *Botrytis*. J Fungal Res, 2006, 4(4): 62–64 (in Chinese).
王颖, 马玺, 平文祥, 等. 葡萄抱属一新种菌物研究. 菌物研究, 2006, 4 (4): 62–64.

[17] Zhao K, Sun LX, Ma X, et al. Improved taxol production in *Nodulisporium sylviforme* derived from inactivated protoplast fusion. Afr J Biotechnol, 2011, 10(20): 4175–4182.

[18] Zhao K, Zhao LF, Jin YY, et al. Isolation of a taxol-producing endophytic fungus and inhibiting

effect of the fungus metabolites on HeLa cell. Mycosystema, 2008, 27(5): 735–744 (in Chinese).
赵凯, 赵立斐, 金媛媛, 等. 一株产紫杉醇内生真菌的分离及其代谢产物抑制HeLa细胞生长作用. 菌物学报, 2008, 27(5): 735–744.

[19] Zhao K, Ping WX, Li Q, et al. *Aspergillus niger* var. *taxi*, a new species variant of taxol-producing fungus isolated from *Taxus cuspidata* in China. J Appl Microbiol, 2009, 107(4): 1202–1207.

[20] Kumaran RS, Hur BK. Screening of species of the endophytic fungus *Phomopsis* for the production of the anticancer drug taxol. Biotechnol Appl Biochem, 2009, 54(1): 21–30.

[21] Chen YJ, Zhang Z, Wang Y, et al. Screening endophytic fungus to produce taxol from *Taxus Yunnanensis*. Biotechnology, 2003, 13(2): 10–11 (in Chinese).
陈毅坚, 张灼, 王艳. 云南红豆杉 (*Taxus yunnanensis*) 内生真菌中产紫杉醇真菌的筛选. 生物技术, 2003, 13(2): 10–11.

[22] Chen JH, Liu JJ, Zang GG, et al. Screening of taxol producing endophytic fungi and regulation of fermentation conditions. J Cent South Univ: Nat Sci, 2004, 35(1): 65–69 (in Chinese).
陈建华, 刘佳佳, 臧巩固, 等. 紫杉醇产生菌的筛选与发酵条件的控制. 中南大学学报: 自然科学版, 2004, 35(1): 65–69.

[23] Zhang P, Liu B, Zhou PP, et al. Isolation and identification of a taxol-producing endophytic fungus YN6. Chin J Biochem Mol Biol, 2011, 27(10): 961–967 (in Chinese).
张鹏, 刘博, 周蓬蓬, 等. 一株产紫杉醇内生真菌 YN6 的分离及鉴定. 中国生物化学与分子生物学报, 2011, 27(10): 961–967.

[24] Jin R, Kang JC, Wen TC, et al. A study on optimal fermentation of an endophytic fungus producing taxol. Mycosystema, 2011, 30(2): 235–241 (in Chinese).
靳瑞, 康冀川, 文庭池, 等. 一株产紫杉醇内生真菌液体发酵工艺的优化. 菌物学报, 2011, 30(2): 235–241.

[25] Geng Z, Liu KH, Zhao YX, et al. Isolation, identification of an endophytic taxol-producing fungus obtained from *Taxus Chinensis*. Microbiol China, 2010, 37(2): 199−203 (in Chinese).
耿直, 刘开辉, 赵赟鑫, 等. 一株产紫杉醇中国红豆杉内生真菌的分离和鉴定. 微生物学通报, 2010, 37(2): 199−203.

[26] Li Q, Ji GS, Zhu J, et al. Isolation and identification of a strain BJ-11 which can produce taxol using cellulose. J Jiangsu Univ Sci Tech: Nat Sci Ed, 2012, 26(6): 611–614 (in Chinese).
李强, 季更生, 朱婧, 等. 一株利用纤维素产紫杉醇菌株 BJ-11 的分离鉴定. 江苏科技大学学报: 自然科学版, 2012, 6: 611–614.

[27] Liu KH, Ding XW, Deng BW, et al. Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*. J Ind Microbiol Biotechnol, 2009, 36(9): 1171–1177.

[28] Wang JF, Li GL, Lu HY, et al. Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairei*. FEMS Microbiol Lett, 2000, 193(2): 249–253.

[29] Chen SJ, Liu JJ, Gong HX, et al. Identification and antibacterial activity of secondary metabolites from *Taxus* endophytic fungus. Chin J Biotech, 2009, 25(3): 368–374 (in Chinese).
陈淑娟, 刘佳佳, 龚汉祥, 等. 红豆杉内生真菌次生代谢物的分离鉴定及其抗菌活性分析. 生物工程学报, 2009, 25(3): 368–374.

[30] Yu Y, Hu CH. Separation and identification of a new *Taxus chinensis* var. *mairei* endophytic fungus (*Bionectria* sp.*)* and the activity of its metabolites. J Southwest Univ: Nat Sci Ed, 2007, 29(6): 131–135 (in Chinese).
余艳, 胡昌华. 南方红豆杉内生真菌 *Bionectria* sp.的分离、鉴定及代谢产物活性研究. 西南大学学报: 自然科学版, 2007, 29(6): 131–135.

[31] Lu LY, Qin Z, Xu JK, et al. Production of taxol by an endophytic fungus isolated from *Taxus chinensis var. Mairei*. Chin Med Biotechnol, 2010, 5(3): 202–207 (in Chinese).
卢陆洋, 秦竹, 徐金库, 等. 一株产紫杉醇内生真菌的分离及其代谢产物的研究. 中国医药生

物技术, 2010, 5(3): 202–207.

[32] Ai HX, Feng YK, Zhu CY, et al. Isolation and identification of a taxol-producing endophytic fungus LNUF014. J Microbiol, 2010, 30(4): 58–62 (in Chinese).
艾海新, 冯玉康, 朱春玉, 等.1 株产紫杉醇内生真菌 LNUF014 的鉴定及产物检测. 微生物学杂志, 2010, 30(4): 58–62.

[33] Hu K, Tan F, Tang KX, et al. Isolation and screening of endophytic fungi synthesizing taxol from *Taxus chinensis* var. *mairei*. J Southwest China Normal Univ: Nat Sci, 2006, 31(1): 134–137 (in Chinese).
胡凯, 谈锋, 唐克轩, 等. 南方红豆杉中产紫杉醇内生真菌的分离和筛选西南师范大学学报: 自然科学版, 2006, 31(1): 134–137.

[34] Tian RP, Yang Q, Zhou GL, et al. Taxonomic study on a taxol producing fungus isolated from bark of *Taxus chinensis* var. *mairei*. J Wuhan Bot Res, 2006, 24(6): 541–545 (in Chinese).
田仁鹏, 杨桥, 周国玲, 等. 一株产紫杉醇的南方红豆杉内生真菌的分离及分类研究. 武汉植物学研究, 2006, 24(6): 541–545.

[35] Cheng L, Ma QM, Tao GJ, Tao WY, Wang RM, Yang J, Guo XL. Systematic identification of a paclitaxel-producing endofungus. Ind Microbiol, 2007, 37(4): 23–30 (in Chinese).
程龙, 马奇明, 陶冠军, 等. 一株产紫杉醇的丝状真菌的系统分类鉴定. 工业微生物, 2007, 37(4): 23–30.

[36] Li JL. Studies on the taxol-producing of *Taxus* endophytic fungus. Harbin: Northeast Agricultural University, 2006: 17–20 (in Chinese).
李嘉琳. 产紫杉醇的红豆杉内生真菌研究. 哈尔滨: 东北农业大学, 2006.

[37] Zhang P, Zhou PP, Yu LJ. An endophytic taxol-producing fungus from *Taxus* x *media*, *Aspergillus candidus* MD3. FEMS Microbiol Lett, 2009, 293(2): 155–159.

[38] Zhang P, Zhou PP, Yu LJ. An endophytic taxol-producing fungus from *Taxus media*, *Cladosporium cladosporioides* MD2. Curr Microbiol, 2009, 59(3): 227–232.

[39] Li TQ, Zhang ZJ, Zhang P, et al. Isolation and identification of a taxol-producing endophytic fungus identified from Taxus media. Agric Sci Technol, 2010, 11(5): 38–40, 68.

[40] Miao LY, Zhang P, Liu B, et al. Isolation and identification of a taxol-producing endophytic fungus Z58. Chin J Biochem Mol Biol, 2012, 28(12): 1141–1146 (in Chinese).
苗莉云, 张鹏, 刘博, 等. 产紫杉醇内生真菌 Z58 的分离和鉴定. 中国生物化学与分子生物学报, 2012, 28(12): 1141–1146.

[41] Zhang P, Liu B, Xu M, et al. Isolation and identification of a taxol-producing endophytic fungus O60B1. Hubei Agric Sci, 2012, 51(23): 5315–5317, 5323 (in Chinese).
张鹏, 刘博, 徐曼, 等. 产紫杉醇内生真菌 O60B1 的分离及鉴定. 湖北农业科学, 2012, 51(23): 5315–5312.

[42] Dai WL, Tao WY. Preliminary study on fermentation conditions of taxol-producing endophytic fungus. Chem Ind Eng Prog, 2008, 27(6): 883–886, 891 (in Chinese).
代文亮, 陶文沂. 一株紫杉醇产生菌发酵条件的初步研究. 化工进展, 2008, 27(6): 883–891.

[43] Li JY, Strobel G, Sidhu R, et al. Endophytic taxol-producing fungi from bald cypress, *Taxodium distichum*. Microbiology, 1996, 142(8): 2223–2236.

[44] Sun DF, Ran XQ, Wang JF. Isolation and identification of a taxol-producing endophytic fungus from *Podocarpus*. Acta Microbiol Sin, 2008, 48(5): 589–595 (in Chinese).
孙端方, 冉雪琴, 王嘉福. 一株产紫杉醇罗汉松内生真菌的分离和鉴定. 微生物学报, 2008, 48(5): 589–595.

[45] Gangadevi V, Muthumary J. A novel endophytic taxol-producing fungus *Chaetomella raphigera* isolated from a medicinal plant, *Terminalia arjuna*. Appl Biochem Biotechnol, 2009, 158(3): 675–684.

[46] Kumaran RS, Muthumary J, Hur BK. Taxol from

Phyllosticta citricarpa, a leaf spot fungus of the angiosperm *Citrus medica*. J Biosci Bioeng, 2008, 106(1): 103–106.

[47] Stierle A, Strobel G, Stierle D. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of pacific yew. Science, 1993, 260(5105): 214–216.

[48] Gangadevi V, Muthumary J. Taxol production by *Pestalotiopsis terminaliae*, an endophytic fungus of *Terminalia arjuna* (arjun tree). Biotechnol Appl Biochem, 2009, 52(1): 9–15.

[49] Kumaran RS, Muthumary J, Hur BK. Isolation and identification of an anticancer drug, taxol from *Phyllosticta tabernaemontanae*, a leaf spot fungus of an angiosperm, *Wrightia tinctoria*. J Microbiol, 2009, 47(1): 40–49.

[50] Kumaran RS, Choi YK, Lee S, et al. Isolation of taxol, an anticancer drug produced by the endophytic fungus, *Phoma betae*. Afr J Biotechnol, 2012, 11(4): 950–960.

[51] Garyali S, Kumar A, Reddy MS. Taxol production by an endophytic fungus, *Fusarium redolens*, isolated from *Himalayan yew*. J Microbiol Biotechnol, 2013, 23(10): 1372–1380.

[52] Yang YF, Zhao HN, Barrero RA, et al. Genome sequencing and analysis of the paclitaxel-producing endophytic fungus *Penicillium aurantiogriseum* NRRL 62431. BMC Genomics, 2014, 15(1): 69.

[53] Zhang Q, Liu HW, Sun GL, et al. Baseline survey of root-associated microbes of *Taxus chinensis* (Pilger) Rehd. PLoS ONE, 2015, 10(3): e0123026.

[54] Chau M, Croteau R. Molecular cloning and characterization of a cytochrome P450 taxoid 2α-hydroxylase involved in Taxol biosynthesis. Arch Biochem Biophys, 2004, 427(1): 48–57.

[55] Jennewein S, Long RM, Williams RM, et al. Cytochrome p450 Taxadiene 5α-hydroxylase, a mechanistically unusual monooxygenas catalyzing the first oxygenation step of Taxol biosynthesis. Chem Biol, 2004, 11(3): 379–387.

[56] Jennewein S, Rithner CD, Williams RM, et al. Taxol biosynthesis: taxane 13 alpha-hydroxylase is a cytochrome P450-dependent monooxygenase. Proc Natl Acad Sci USA, 2001, 98(24): 13595–13600.

[57] Chau M, Jennewein S, Walker K, et al. Taxol biosynthesis: molecular cloning and characterization of a cytochrome P450 taxoid 7β-hydroxylase. Chem Biol, 2004, 11(5): 663–672.

[58] Schoendorf A, Rithner CD, Williams RM, et al. Molecular cloning of a cytochrome P450 taxane10β-hydroxylase cDNA from *Taxus* and functional expression in yeast. Proc Natl Acad Sci USA, 2001, 98(4): 1501–1506.

[59] Jennewein S, Rithner CD, Williams RM, et al. Taxoid metabolism: taxoid 14β-hydroxylase is a cytochrome P450-dependent monooxygenase. Arch Biochem Biophys, 2003, 413(2): 262–270.

[60] Walker K, Schoendorf A, Croteau R. Molecular cloning of a taxa-4(20), 11(12)-dien-5α-ol-O-acetyl transferase cDNA from *Taxus* and functionalexpression in *Escherichia coli*. Arch Biochem Biophysics, 2000, 374(2): 371–380.

[61] Jennewein S, Wildung MR, Chau M, et al. Random sequencing of an induced *Taxus* cell cDNA library for identification of clones involved in Taxol biosynthesis. Proc Natl Acad Sci USA, 2004, 101(24): 9149–9154.

[62] Walker K, Croteau R. Molecular cloning of a 10-deacetylbaccatin III-10-O-acetyl transferase cDNA from *Taxus* and functional expression in *Escherichia coli*. Proc Natl Acad Sci USA, 2000, 97(2): 583–587.

[63] Walker K, Fujisaki S, Long R, et al. Molecular cloning and heterologous expression of the C-13 phenylpropanoid side chain-CoA acyltransferase that functions in Taxol biosynthesis. Proc Natl Acad Sci USA, 2002, 99(20): 12715–12720.

[64] 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(14): 9166–9171.

[65] 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(1): 62–74.

[66] Walker K, Croteau R. Taxol biosynthesis: molecular cloning of a benzoyl-CoA: taxane 2α-O-benzoyltransferase cDNA from *Taxus* and functional expression in *Escherichia coli*. Proc Natl Acad Sci USA, 2000, 97(25): 13591–13596.

[67] Dai YL, Qin QL, Dai DL, et al. Isolation and characterization of a novel cDNA encoding methyl jasmonate-responsive transcription factor *TcAP2* from *Taxus cuspidata*. Biotechnol Lett, 2009, 31(11): 1801–1809.

[68] Liao ZH, Tan QM, Chai YR, et al. Cloning and characterisation of the gene encoding HMG-CoA reductase from *Taxus media* and its functional identification in yeast. Funct Plant Biol, 2004, 31(1): 73–81.

[69] Nims E, Dubois CP, Roberts SC, et al. Expression profiling of genes involved in paclitaxel biosynthesis for targeted metabolic engineering. Metab Eng, 2006, 8(5): 385–394.

[70] Li FL. Studies on regulation of the gene expression of taxoid 13α-, 14β-hydroxylase and analysis of protein profile in *Taxus* sp. cells treated with methyl jasmonate [D]. Beijing: Chinese Academy of Medical Science and Peking Uion Medical College, 2009 (in Chinese).
李凤岚. 红豆杉紫杉烷13α-,14β-羟基化酶基因调控及茉莉酸甲酯诱导下的蛋白表达 [D]. 北京: 中国协和医学院, 2009.

[71] Cheng SJ, Huang SJ, Guo LQ, et al. Cloning and sequence analysis of 10-deacetylbaccatin-10-O-acetyl transferase gene from *Taxus chinensis* var. *mairei*. Biotechnol Bull, 2011, (1): 107–112 (in Chinese).
程抒劼, 黄仕杰, 郭丽琼, 等. 南方红豆杉 10-去乙酰巴卡亭Ⅲ -10-乙酰转移酶基因的克隆与生物信息学分析. 生物技术讯, 2011, 22(22): 107–110.

[72] Yu XL. Molecular cloning of genes coding enzymes in the upstream biosynthesis pathway of taxol [D]. Tianjin: Tianjin University, 2004 (in Chinese).
于湘莉. 紫杉醇生物合成上游途径中重要酶基因克隆 [D]. 天津: 天津大学, 2004.

[73] Lan XZ, Sun M. Cloning and characterization of the geranylgeranyl diphosphate synthase gene in *Taxus walliciana*. J Southwest Agri Univ: Nat Sci, 2006, 28(4): 537–543 (in Chinese).
兰小中, 孙敏. 红豆杉香叶基香叶基焦磷酸合成酶基因克隆分析. 西南农业大学学报, 2006, 28(4): 537–43.

[74] Wang YC. Genetic transformation of Taxol-producing endophytic fungi and molecular cloning of genes involved in Taxol biosynthesis pathway[D]. Shanghai: Shanghai Jiao Tong University, 2007 (in Chinese).
汪业春. 产紫杉醇内生真菌的遗传转化及紫杉醇合成途径相关基因的克隆[D]. 上海: 上海交通大学, 2007.

[75] Wang X, Xiao Y, Liu D, et al. Cloning the cDNA fragment of *GGPPS* gene from *Taxus cuspidata*. J Natl Sci Heilongjiang Univ, 2012, 29(4): 536–540 (in Chinese).
王歆, 肖野, 刘丹. 东北红豆杉细胞 GGPPS 基因 cDNA 的克隆. 黑龙江大学学报, 2012, 29(4): 536–540.

[76] Chow SY, Williams HJ, Pennington JD, et al. Studies on taxadiene synthase: interception of the cyclization cascade at the verticillene stage and rearrangement to phomactatriene. Tetrahedron, 2007, 63(27): 6204–6209.

[77] 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(16): 9201–9204.

[78] Liang SY, He LG, Zheng XQ. Cloning,

sequencing and plant vector construction of a taxadiene synthase gene of *Taxus yunnanensis*. Life Sci Res, 2005, 9(1): 24–28.

[79] Xiao Y, Zhao D, Wang G. cDNA cloning of taxadiene synthase functioning in Taxol biosynthesis. Sci Agri Sin, 2006, 39(10): 2138–2146 (in Chinese).
肖颖, 赵冬, 王刚. 紫杉醇合成途径中紫杉烯合成酶 cDNA 的克隆. 中国农业科学, 2006, 39(10): 2138–2146.

[80] Huang SJ, Cheng SJ, Guo XY, Xin YH, et al. Cloning and sequence analysis of taxane 13α-hydroxylase gene from *Taxus chinensis* var. *mairei*. Biotechnology, 2010, 20(6): 10–14 (in Chinese).
黄仕杰, 程抒劼, 郭心悦, 等. 南方红豆杉紫杉烷 13α-羟化酶基因的克隆及序列分析. 生物技术, 2010, 20(6): 10–14.

[81] Teng WJ. Study on cloning, expression and regualtion of taxane 13α-hydroxylase from *Taxus*. Beijing: Chinese Academy of Forestry, 2008 (in Chinese).
滕文静. 红豆杉紫杉烷 13a-羟基化酶基因的克隆及表达调控的研究. 北京: 中国林业科学研究院, 2008.

[82] Li FL, Qiu DY, Ma XJ, et al. Suppression of taxoid 14β-hydroxylase gene expression in *Taxus* x media *via* RNA interference. China Biotechnol, 2009, 29(5): 55–60 (in Chinese).
李凤岚, 邱德有, 马小军. 利用 RNAi 抑制曼地亚红豆杉细胞紫杉烷 14β-羟基化酶基因的表达. 中国生物工程杂志, 2009, 29(5): 55–60.

[83] Han L, Hu YL, Zhu JB, et al. Cloning and analysis of *BAPT* gene from three different *Taxus*. J Shihezi Univ: Nat Sci, 2008, 26(5): 562–565 (in Chinese).
韩丽, 胡鸢雷, 祝建波. 三种红豆杉 *BAPT* 基因的克隆及序列分析. 石河子大学学报: 自然科学版, 2008, 26(5): 562–565.

[84] Zhao K, Ping WX, Zhou DP. Recent advance and prospect on taxol production by endophytic fungus fermentation-a review. Acta Microbiol Sin, 2008, 48(3): 403–407 (in Chinese).
赵凯, 平文祥, 周东坡. 内生真菌发酵生产紫杉醇的研究现状与展望. 微生物学报, 2008, 48(3): 403–407.

[85] Zhao K, Sun LX, Wang X, et al. Screening of high taxol producing fungi by mutagenesis and construction of subtracted cDNA library by suppression subtracted hybridization for differentially expressed genes. Acta Microbiol Sin, 2011, 51(7): 923–933 (in Chinese).
赵凯, 孙立新, 王旋, 等. 高产紫杉醇菌株的诱变选育及其差异表达基因消减 cDNA 文库的构建. 微生物学报, 51(7): 923–933.

[86] Zhao K, Zhou DP, Wang W. Effects of medium components on taxol production of *Nodulisporium sylviforme*. J Fungal Res, 2003, 1(1): 24–27 (in Chinese).
赵凯, 周东坡, 王伟. 培养基组成对树状多节孢 (*Nodulisporium sylviforme*) 紫杉醇产量的影响. 菌物研究, 2003, 1 (1): 24–27.

[87] Soliman SSM, Raizada MN. Interactions between co-habitating fungi elicit synthesis of taxol from an endophytic fungus in host *Taxus* plants. Front Microbiol, 2013, 4: 1–14.

[88] Kusari S, Singh S, Jayabaskaran C. Biotechnological potential of plant-associated endophytic fungi: hope versus hype. Trends Biotechnol, 2014, 32(6): 297–303.

[89] Kusari S, Singh S, Jayabaskaran C. Rethinking production of Taxol® (paclitaxel) using endophyte biotechnology. Trends Biotechnol, 2014, 32(6): 304–311.

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