Endophytic fungi for producing bioactive compounds originally from their host plants

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Plant endophytic fungi are an important and novel resource of natural bioactive compounds with their potential applications in agriculture, medicine and food industry. In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully discovered from the endophytic fungi. During the long period of co-evolution, a friendly relationship was formed between each endophyte and its host plant. Some endophytes have the ability to produce the same or similar bioactive compounds as those originated from their host plants. This chapter mainly reviewed the research progress on the endophytic fungi for producing plant-derived bioactive compounds such as paclitaxel, podophyllotoxin, camptothecine, vinblastine, hypericin and diosgenin etc. The relations between the endophytic fungi and their host plants, some available strategies for efficiently promoting production of these bioactive compounds, as well as their potential applications in the future are also discussed. It is beneficial for us to better understand and take advantage of plant endophytic fungi.

Keywords endophytic fungi; bioactive compounds; host plants; co-evolution relations

1. Introduction

Plant endophytic fungi are defined as the fungi which spend the whole or part of their lifecycle colonizing inter-and/or intra-cellularly inside the healthy tissues of the host plants, typically causing no apparent symptoms of disease. They are important components of plant micro-ecosystems [1-3]. Plant endophytic fungi have been found in each plant species examined, and it is estimated that there are over one million fungal endophytes existed in the nature [4]. Plant endophytic fungi have been recognized as an important and novel resource of natural bioactive products with potential application in agriculture, medicine and food industry [5-7]. Since the "gold" bioactive compound paclitaxel (taxol) discovered from the endophytic fungus *Taxomyces andreanae* in 1993 [8], many scientists have been increasing their interests in studying fungal endophytes as potential producers of novel and biologically active compounds. In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully discovered from the endophytic fungi. These bioactive compounds could be classified as alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones [2, 9]. During the long period of co-evolution, a friendly relationship was gradually set up between each endophytic fungus and its host plant. The host plant can supply plenteous nutriment and easeful habitation for the survival of its endophytes. On the other hand, the endophytes would produce a number of bioactive compounds for helping the host plants to resist external biotic and abiotic stresses, and benefiting for the host growth in return [3, 10]. Some endophytic fungi have developed the ability to produce the same or similar bioactive substances as those originated from the host plants. This is beneficial for us to study the relations between the endophytes and their host plants, and to develop a substitutable approach for efficiently producing these scarce and valuable bioactive compounds [6, 11].

This chapter mainly describes the research progress on the endophytic fungi for producing bioactive compounds such as paclitaxel, podophyllotoxin, camptothecine, vinblastine, hypericin and diosgenin (Fig. 1), which were also produced by their host plants. The potential relationships of the endophytes with their host plants, some available strategies for efficiently promoting production of these bioactive compounds, as well as their potential application in the future are also discussed.

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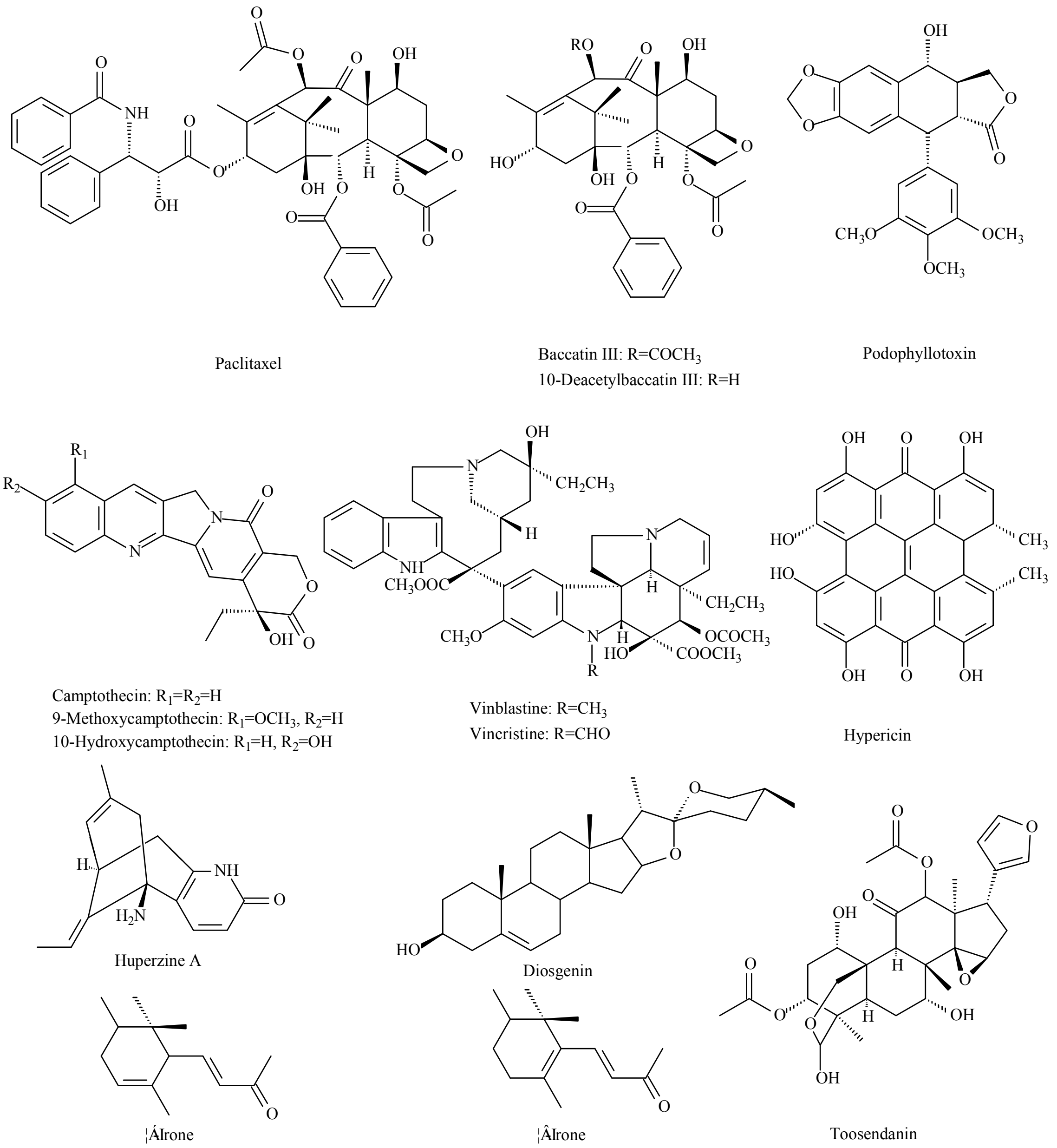

Fig. 1 Structures of the bioactive compounds from the endophytic fungi and their host plants.

2. Endophytic fungi for producing paclitaxel and its analogues

Paclitaxel (taxol), as a well-known and highly functionalized tetracyclic diterpenoid bioactive compound found originally from the bark of *Taxus brevifolia* in 1971 [12], has been proved with an efficient action against prostate, ovarian, breast and lung cancers. Its primary mechanism of action is related to the ability to stabilize the microtubules and to disrupt their dynamic equilibrium [13]. Up to now, the major supply of paclitaxel has been from the wild *Taxus* plants. However, it is found in extremely low amounts in various parts such as the needles, barks and roots of *Taxus* species. In order to satisfy the growing demand of market and make it more widely available, the alternative resource and potential strategy should be developed. In the last 40 years, many efficient approaches such as field cultivation, plant cell and tissue culture, chemical synthesize for paclitaxel production have been developed, and much progress has been achieved [14]. However, it is not realistic for producing paclitaxel with these measures as the problems of time-consuming, lower yield and non economic. Fortunately, a paclitaxel producing endophytic fungus *Taxomyces andreanae* was successfully discovered from the Pacific yew (*Taxus brevifolia*) in 1993 [8]. This tremendous finding firstly showed that the plant endophytic fungi also had the ability to produce paclitaxel, giving us a novel and promising approach to produce this valuable compound. Since then, many scientists have been increasing their interests in studying fungal endophytes as potential candidates for producing paclitaxel. Extensive research such as searching for paclitaxel-producing endophytic fungi from *Taxus* species as well as from other related plant species, microbial fermentation processes and genetic engineering for improving paclitaxel production has been developed, and much progress has been achieved during the past two decades. By now, at least 19 genera of endophytic fungi (i.e. *Alternaria*, *Aspergillus*, *Botryodiplodia*, *Botrytis*, *Cladosporium*, *Ectostroma*, *Fusarium*, *Metarhizium*, *Monochaetia*, *Mucor*, *Ozonium*, *Papulaspora*, *Periconia*, *Pestalotia*, *Pestalotiopsis*, *Phyllosticta*, *Pithomyces*, *Taxomyces*, *Tubercularia*) were screened to have the ability to produce paclitaxel and its analogues (i.e. baccatin III, 10-deacetylbaccatin III) (Table 1). The hosts of paclitaxel-producing fungi mainly include *Taxus* (i.e. *T. baccata*, *T. cuspidata*, *T. media*, and *T. yunnanensis*) and non-*Taxus* species (i.e. *Cardiospermum helicacabum*, *Citrus medica*, *Cupressus* sp., *Ginkgo biloba*, *Hibiscus rosa-sinensis*, *Podocarpus* sp., *Taxodium distichum*, *Terminalia arjuna*, *Torreya grandifolia*, and *Wollemia nobilis*). Such a great number and wide range implies that both paclitaxel-producing fungi and their hosts have biological diversity. These results also showed us a promising way that the endophytic fungi would be an alternative paclitaxel-producing resource.

Table 1 Paclitaxel-producing endophytic fungi and their host plants.

Endophytic fungus	Fungal strain	Host plant	Paclitaxel yield (μg/L)	Reference
Alternaria sp.	Ja-69	*Taxus cuspidata*	0.16	[15]
Alternaria sp.	-	*Ginkgo biloba*	0.12-0.26	[16]
Alternaria alternata	TPF6	*Taxus chinensis* var. *mairei*	84.5	[17]
Aspergillus fumigatus	EPTP-1	*Podocarpus* sp.	557.8	[18]
Aspergillus niger var. *taxi*	HD86-9	*Taxus cuspidata*	273.6	[19]
Botryodiplodia theobromae	BT115	*Taxus baccata*	280.5	[20]
Botrytis sp.	XT2	*Taxus chinensis* var. *mairei*	161.24	[21]
Botrytis sp.	HD181-23	*Taxus cuspidata*	206.34	[22]
Cladosporium cladosporioides	MD2	*Taxus media*	800	[23]
Ectostroma sp.	XT5	*Taxus chinensis* var. *mairei*	276.75	[21]
Fusarium arthrosporioides	F-40	*Taxus cuspidata*	131	[24]
Fusarium lateritium	Tbp-9	*Taxus baccata*	0.13	[15]
Fusarium mairei	Y1117	*Taxus chinensis* var. *mairei*	2.7	[25]
Fusarium mairei	UH23	*Taxus chinensis* var. *mairei*	286.4	[26]
Fusarium solani	-	*Taxus celebica*	1.6	[27]
Fusarium solani	Tax-3	*Taxus chinensis*	163.35	[28]
Metarhizium anisopliae	H-27	*Taxus chinensis*	846.1	[29]
Monochaetia sp.	Tbp-2	*Taxus baccata*	0.10	[15]
Mucor rouxianus	DA10	*Taxus chinensis*	-	[30]
Ozonium sp.	BT2	*Taxus chinensis* var. *mairei*	4-18	[31]
Papulaspora sp.	XT17	*Taxus chinensis* var. *mairei*	10.25	[21]
Periconia sp.	No. 2026	*Torreya grandifolia*	0.03-0.83	[32]
Pestalotia bicilia	Tbx-2	*Taxus baccata*	1.08	[15]
Pestalotiopsis guepinii	W-1f-2	*Wollemia nobilis*	0.49	[33]
Pestalotiopsis microspora	Ja-73	*Taxus cuspidata*	0.27	[15]
Pestalotiopsis microspora	Ne-32	*Taxus wallachiana*	0.5	[15]
Pestalotiopsis microspora	No. 1040	*Taxus wallachiana*	0.06-0.07	[34]
Pestalotiopsis microspora	Cp-4	*Taxodium distichum*	0.05-1.49	[35]
Pestalotiopsis microspora	Ne 32	*Taxus wallachiana*	0.34-1.83	[36]

Table 1 Contd....

Endophytic fungus	Fungal strain	Host plant	Paclitaxel yield (μg/L)	Reference
Pestalotiopsis pauciseta	CHP-11	*Cardiospermum helicacabum*	113.3	[37]
Pestalotiopsis sp.	W-x-3	*Wollemia nobilis*	0.13	[33]
Pestalotiopsis sp.	W-1f-1	*Wollemia nobilis*	0.17	[33]
Pestalotiopsis terminaliae	TAP-15	*Terminalia arjuna*	211.1	[38]
Phyllosticta citricarpa	No.598	*Citrus medica*	265	[39]
Phyllosticta dioscoreae	No.605	*Hibiscus rosa-sinensis*	298	[40]
Phyllosticta spinarum	No.625	*Cupressus* sp.	235	[41]
Pithomyces sp.	P-96	*Taxus sumatrana*	0.095	[15]
Taxomyces andreanae	-	*Taxus brevifolia*	0.024-0.05	[8]
Taxomyces sp.	-	*Taxus yunnanensis*	2.3	[42]
Tubercularia sp.	TF_5	*Taxus chinensis* var. *mairei*	185.4	[43]
Unidentified	YF_1	*Taxus yunnanensis*	-	[44]

3. Endophytic fungi for producing podophyllotoxin

Podophyllotoxin (PDT), a well-known aryltetralin lignan with potent anticancer, antiviral, antioxidant, antibacterial, immunostimulation and anti-rheumatic properties, mainly occurs in genera of *Diphylleia*, *Dysosma*, *Sabina* (also called *Juniperus*), and *Sinopodophyllum* (also called *Podophyllum*) [45-52]. PDT has been used as a precursor for chemical synthesis of the anticancer drugs like etoposide, teniposide and etopophose phosphate [48, 51]. At present, the major supply of podophyllotoxin is from the natural *Sinopodophyllum* plants. As the over-exploitation, the *Sinopodophyllum* plants have been declared to be endangered species. In order to satisfy the increasing demand and make it more available, the alternative resource and strategy for efficiently producing this valuable compound should be developed.

Yang et al. first reported about six endophytic fungi obtained from *Sinopodophyllum hexandrum*, *Diphylleia sinensis* and *Dysosma veitchii* that had the ability to produce podophyllotoxin [45]. Later, Lu et al. also declared that an endophytic *Alternaria* sp. obtained from *Sabina vulgaris* could produce PDT [46]. Eyberger et al. successfully obtained two endophytic *Phialocephala fortinii* strains PPE5 and PPE7 from the rhizomes of *Sinopodophyllum peltatum* that could produce PDT with the yield of 0.5-189 μg/L in liquid suspension culture [51]. Puri et al. reported an endophytic fungus *Trametes hirsuta* isolated from *Sinopodophyllum hexandrum* that could produce PDT and its glycoside in Sabouraud broth culture [52]. Cao et al. examined an endophytic fungus *Alternaria* sp. isolated from *Sinopodophyllum hexandrum* that could produce PDT [47]. Kour et al. also discovered a PDT-producing endophytic fungus *Fusarium oxysporum* obtained from *Sabina recurva* in 2008 [48]. These results give us a promising way of exploring the endophytic fungi as the alternative source to produce podophyllotoxin and its analogues.

Table 2 Podophyllotoxin-producing endophytic fungi and their host plants.

Endophytic fungus	Fungal strain	Host plant	Podophyllotoxin content or yield	Reference
Alternaria sp.	-	*Sinopodophyllum hexandrum* (=*Podophyllum hexandrum*)	-	[45]
Alternaria sp.	SC13	*Sabina vulgaris*	-	[46]
Alternaria neesex	Ty	*Sinopodophyllum hexandrum*	2.4 μg/L	[47]
Fusarium oxysporum	JRE1	*Sabina recurva* (=*Juniperus recurva*)	28 μg/g	[48]
Monilia sp.	-	*Dysosma veitchii*	-	[45]
Penicillium sp.	-	*Sinopodophyllum hexandrum*	-	[45]
Penicillium sp.	-	*Diphylleia sinensis*	-	[45]
Penicillium sp.	-	*Dysosma veitchii*	-	[45]
Penicillium implicatum	SJ21	*Diphylleia sinensis*	-	[49]
Penicillium implication	2BNO1	*Dysosma veitchii*	-	[50]
Phialocephala fortinii	PPE5, PPE7	*Sinopodophyllum peltatum*	0.5-189 μg/L	[51]
Trametes hirsuta	-	*Sinopodophyllum hexandrum*	30 μg/g	[52]

4. Endophytic fungi for producing camptothecine and its analogues

Camptothecin (CPT), a pentacyclic quinoline alkaloid, was firstly isolated from the wood of *Camptotheca acuminata* (Nyssaceae) by Wall et al. in 1966 [53]. CPT and its analogue10-hydroxycamptothecin have been regarded as two of the most effective antineoplastic agents. The primary action mechanism of CPT is by virtue of inhibiting the intra-nuclear enzyme topoisomerase-1, which is required in DNA replication and transcription during molecular events [54]. Hycamtin (topotecan) and Camtostar (irinotecan), two of the famous CPT semi-synthetic drugs, have already been in clinical use against ovarian, small lung and refractory ovarian cancers popularly all over the world [55]. At present, the major supply of this bioactive compound CPT is still from the wild trees *Camptotheca acuminata* and *Nothapodytes nimmoniana* (Icacinaceae). As the growing demand of this compound, it has resulted in extensive cropping of the trees in China and India. It is necessary to further find high yielding candidates and alternative sources to produce this bioactive compound and its analogues [56, 57].

Puri et al. first reported an endophytic fungus *Entrophospora infrequens* obtained from *Nothapodytes foetida* that had the ability to produce camptothecin in 2005 [58]. Later, Amna et al. performed the kinetic studies of the growth and CPT accumulation of the endophyte *E. infrequens* in suspension culture with the either shake flasks or bioreactor, and demonstrated that this endophyte would be a potential alternate microorganism source to produce CPT [56]. Rehman et al. successfully discovered a CPT-producing endophytic fungus *Neurospora* sp. from the seeds of *Nothapodytes foetida* in 2008 [59]. More recently, Kusari et al. reported an endophytic fungus *Fusarium solani* obtained from *Camptotheca acuminata* could produce CPT, 9-methoxycamptothecin and 10-hydroxycamptothecin in Sabouraud dextrose broth [60]. Min and Wang reported an unidentified endophytic fungal strain XK001 could produce 10-hydroxycamptothecin with the yield of 677 μg/L [61]. Shweta et al. successfully found two endophytic *Fusarium solani* strains MTCC9667 and MTCC9668 had the ability to produce CPT and 9-methoxycamptothecin (0.45 μg/g), and the endophyte MTCC9668 could also produce 10-hydroxycamptothecin as much as 0.08 μg/g [57]. These findings showed that the endophytic fungi could be an alterative resource to produce CPT and its analogues.

Table 3 Camptothecin-producing endophytic fungi and their host plants.

Endophytic fungus	Fungal strain	Host plant	Camptothecin content or yield	Reference
Entrophospora infrequens	RJMEF 001	*Nothapodytes foetida*	-	[58]
Entrophospora infrequens	5124	*Nothapodytes foetida*	49.6 μg/g	[56]
Fusarium solani	INFU/Ca/KF/3	*Camptotheca acuminata*	-	[60]
Fusarium solani	MTCC 9667	*Apodytes dimidiata*	0.37 μg/g	[57]
Fusarium solani	MTCC 9668	*Apodytes dimidiata*	0.53 μg/g	[57]
Neurospora sp.	ZP5SE	*Nothapodytes foetida*	-	[59]
Unidentified	XK001	*Camptotheca acuminata*	-	[61]

5. Endophytic fungi for producing vinblastine and its analogues

Vinblastine and vincristine, the terpenoid indole alkaloids derived from the coupling of vindoline and catharanthine monomers, are two of the well-known anticancer agents [62, 63]. The primary action mechanism of vincristine is via interference with microtubule formation and mitotic spindle dynamics, disruption of intracellular transport and decreased tumour blood flow, with the latter probably as a consequence of anti-angiogenesis [62, 64]. Guo et al. first reported an endophytic fungus *Alternaria* sp. isolated from the phloem of *Catharanthus roseus* that had the ability to produce vinblastine in 1998 [65]. Later, Zhang et al. successfully discovered an endophytic *Fusarium oxysporum* from the pholem of *C. roseus* that could produce vincristine [66]. Yang et al. also found an unidentified vincristine-producing endophytic fungus from the leaves of *C. roseus* in 2004 [67]. These results indicate that some endophytic fungi could be a potential source to produce either vinblastine or vincristine.

6. Endophytic fungi for producing other bioactive compounds originally from their host plants

Other pronounced bioactive compounds originated from the host plants could also be biosynthesized by their endophytic fungi mainly include huperzine A, α-irone, β-irone, diosgenin, hypericin and toosendanin (shown in Table 4). Li et al first reported an endophytic fungus *Acremonium* (2F09P03B) obtained from *Huperzia serrata* that could produce huperzine A that was a lycopodium alkaloid. They further optimized its fermentation conditions [68]. Zhou et al. reported an endophytic fungus *Penicillium chrysogenum* obtained from *Lycopodium serratum* could also produce huperzine A as much as 4.761 mg/L in liquid culture [73]. Ju et al. successfully discovered two endophytic fungi *Blastomyces* sp. (HA15) and *Botrytis* sp. (HA23) from *Phlegmariurus cryptomerianus* that had the ability to produce huperzine A [69].

Zhou and his co-workers screened a few diosgenin-producing endophytic fungi from *Paris polyphylla* var. *yunnanensis* [70, 71]. Zhang et al. reported an endophytic fungus *Rhizopus oryzae* (94Y-01) from the rhizomes of *Iris germanica* that could produce α- and β-irones for which the culture conditions were then optimized [74]. Wang et al. discovered three endophytic fungal isolates from *Melia azedarach* that had the ability to produce toosendanin [75]. Kusari et al. reported an endophytic fungus isolated from the stems of *Hypericum perforatum* (St. John's Wort) had the ability to produce hapericin and emodin in rich mycological medium with shake flasks [72]. All the results mentioned above clearly showed that a promising way that the endophytic fungi would be an alternative resource for efficiently producing valuable bioactive compounds in the future.

Table 4 Other bioactive compounds-producing endophytic fungi and their host plants.

Endophytic fungus	Fungal strain	Host plant	Bioactive compounds	Reference
Acremonium sp.	2F09P03B	*Huperzia serrata*	Huperzine A	[68]
Blastomyces sp.	HA15	*Phlegmariurus cryptomerianus*	Huperzine A	[69]
Botrytis sp.	HA23	*Phlegmariurus cryptomerianus*	Huperzine A	[69]
Cephalosporium sp.	84	*Paris polyphylla* var. *yunnanensis*	Diosgenin	[70, 71]
Chaetomium globosum	INFU/Hp/KF/34B	*Hypericum perforatum*	Hypericin, Emodin	[72]
Paecilomyces sp.	80	*Paris polyphylla* var. *yunnanensis*	Diosgenin	[70, 71]
Penicillium chrysogenum	SHB	*Lycopodium serratum*	Huperzine A	[73]
Rhizopus oryzae	94Y-01	*Iris germanica*	α-Irone, β-Irone	[74]
Unidentified	O-L-5, O-SC II-4, O-RC-3	*Melia azedarach*	Toosendanin	[75]

7. Conclusions and future perspectives

Plant endophytic fungi, as a novel and abundant microorganism resource, owning the special ability to produce the same or similar compounds originated from their host plants, as well as other bioactive compounds, have increased many investigators′ interesting in both basic research and applied fields. In the past two decades, scientists mainly focused on the investigation of endophytic fungal diversity, relationships between endophytic fungi and their host plants, seeking for natural bioactive compounds originated from the endophytic fungi, and improving the productivity of some potential candidates by taking advantage of genetic engineering, microbial fermentation projects and other measures [5]. Up to now, hundreds of plants have been investigated for their endophytic fungi, and most of them have been proved to be rich with endophytic fungi. Many novel and valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully obtained from the endophytic fungi [76]. The evidence of plant-associated microbes discovered in the fossilized tissues of stems and leaves indicated that the endophytic associations may have evolved from the time that higher plants first appeared on the earth, hundreds of millions of years ago [77]. Carroll suggested that some phytopathogens in the environment were related to endophytes and had an endophytic origin [78]. A few microorganisms appear actively to penetrate plant tissues through invading openings or wounds, as well as proactively using hydrolytic enzymes such as cellulase and pectinase [2]. During the long period of co-evolution, the endophytic fungi have adapted themselves to their special microenvironments gradually by genetic variation, including uptake of some plant DNA segments into their own genomes, as well as insertion their own DNA segments into the host genomes. This could have led to certain endophytes own the ability to biosynthesize some ″phytochemicals″ originated from their host plants [2, 8]. One typical example was the production of gibberellins from both fungi and plants [79]. The outline of the bioactive compounds from both endophytic fungi and their host plants along with their potential applications is shown in Fig. 2.

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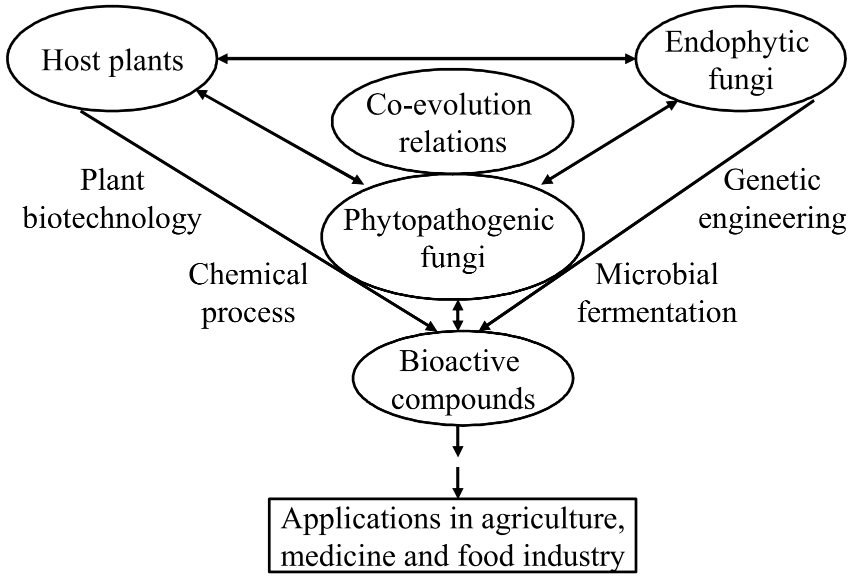

Fig. 2 Outline of the bioactive compounds from both endophytic fungi and their host plants along with their potential applications.

It is believed that the plant endophytic fungi as a novel mine of natural bioactive compounds have their great potential applications in agriculture, medicine and food industry [5, 7, 80]. Taking advantage of modern biotechnology such as genetic engineering, metabolic technology and microbial fermentation process, we can better understand and manipulate this important microorganism resource, and make it more benefit for the mankind. First, the most important step is to search for potential endophytic fungi resources from the nature. And then, through mutations, protoplast fusion, gene manipulation and other DNA recombination techniques, the high productivity candidates suitable for industrial fermentation could be established. Furthermore, colonizing and expression of relevant functional genes in the biosynthetic pathways are also beneficial for improving the productivity of the candidates. It is well known that microorganism fermentation is a sophisticated project, and it has been widely used in many occasions for a long period of time. Penicillin, avermectin, validamycin and other well-known antibiotics have been successfully developed through fermentation process. Compared with plant cell culture, the culture medium for the fungal cells is simple, inexpensive with the abundant supply, and the production cost is relatively low. Moreover, the period of fermentation is short, and the microbial fermentation process can provide the best growth and breeding conditions, and the various culture parameters can be strictly controlled according to our requests. In addition, the microbial fermentation conditions can be easily optimized, and many feasible strategies could be adopted for efficiently enhancing the bioactive compound production during the fermentation process, such as feeding precursors, adding biotic and abiotic elicitors, appending inhibitors, using special enzymes and other substances through metabolic investigation.

In summary, plant endophytic fungi, as a novel and important microbial resource for producing bioactive compounds originally from their hosts, have attracted many researchers' attentions on their theoretical study as well as potential applications. After more than two decades of research, much progress has been achieved though there are still many issues (i.e. increasing compound yield in fermentation culture, elucidating biosynthetic pathway of the compounds in the endophytic fungi, etc.) needed to be further clarified and resolved.

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