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Fungal endophytes – secret producers of bioactive plant metabolites

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Dedicated to Professor Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

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The potential of endophytic fungi as promising sources of bioactive natural products continues to attract broad attention. Endophytic fungi are defined as fungi that live asymptomatically within the tissues of higher plants. This overview will highlight the uniqueness of endophytic fungi as alternative sources of pharmaceutically valuable compounds originally isolated from higher plants, e.g. paclitaxel, camptothecin and podophyllotoxin. In addition, it will shed light on the fungal biosynthesis of plant associated metabolites as well as new approaches developed to improve the production of commercially important plant derived compounds with the involvement of endophytic fungi.

1. Introduction

Fungal endophytes were first defined by Anton de Bary in 1886 as microorganisms that colonize internal tissues of stems and leaves (Wilson 1995). More recent definitions denoted that they are ubiquitous microorganisms present in virtually all plants on earth from the arctic to the tropics (Strobel and Daisy 2003; Huang et al. 2007). They reside asymptomatically in the internal tissues of living plants (Bacon and White 2000; Porras-Alfaro and Bayman 2011), and are thus subjected to constant metabolic interactions with their host (Chandra 2012). The endophyte-host plant relationship was described as a balanced symbiotic continuum ranging from mutualism through commensalism to parasitism (Kogel et al. 2006). This complex relationship continues to present an exciting frontier yet to be explored as such host organisms represent unique ecological niches for diverse communities of symbiotic microbes giving rise to complex interactions of plant microbiomes (Barrow et al. 2008; Porras-Alfaro and Bayman 2011).

Furthermore, co-evolution of endophytes and their host plants is believed to shape natural product patterns of endophytic fungi, which often contribute in multiple ways to endophyte-host communication as well as host fitness and adaptation to environmental challenges (Gunatilaka 2006; Aly et al. 2011). In this context, colonization of host plants by endophytic fungi has been reported to contribute to host plant adaptation to biotic (e.g. pathogens, herbivores) and abiotic (e.g. drought tolerance) stress factors (Redman et al. 2002; Arnold et al. 2003; Waller et al. 2005; Akello et al. 2007; Bae et al. 2009; Giordano et al. 2009; Rodriguez et al. 2009).

Even though endophytes were first described from the Darnel (*Lolium temulentum*) back in 1904 (Freeman 1904), they started to attract substantial attention after the discovery of the paclitaxel (Taxol®) (**1**) producing endophytic fungus *Taxomyces andreanae*, which was isolated from the original source of this important anti-cancer drug *Taxus brevifolia* (Stierle et al. 1993, 1995). This discovery evoked the interest in endophytes as potential new sources for therapeutic agents (Aly et al. 2010, 2011; Debbab et al. 2011, 2012) and set the stage for a more comprehensive examination of the ability of other plants to yield endophytes producing pharmacologically important natural products hitherto only known from plants.

In this review pharmaceutically valuable plant secondary metabolites which were found to be produced by fungal endophytes are reported with special emphasis on source organisms. Furthermore, studies on the fungal biosynthesis of plant associated metabolites are highlighted and new approaches to improve the production of commercially important plant derived compounds involving endophytic fungi are described.

2. Endophytic fungi as producers of plant secondary metabolites

Several studies report on the biosynthesis of important plant secondary metabolites by endophytic fungi residing in the plants that were originally known as the source of such metabolites. These spectacular discoveries raised questions with regard to horizontal gene transfer between host and endophyte (or *vice versa*) (Stierle et al. 1995) and emphasized the potential of using such microorganisms as alternative sources of these metabolites (Priti et al. 2009).

The discovery of the paclitaxel (Taxol®) (**1**) producing endophytic fungus *Taxomyces andreanae* from the Pacific yew tree *Taxus brevifolia* (Taxaceae) (Stierle et al. 1993, 1995) highlighted the ability of endophytes to biosynthesize associated plant metabolites for the first time. This instigated a thorough investigation of other *Taxus* species and other plants for the presence of paclitaxel producing endophytes, aiming at utilizing such strains for the sustained industrial production of this pharmacologically important drug. The multi-billion dollar anti-cancer compound paclitaxel (**1**), produced by the yew plant, has activity against a broad band of tumor types, including breast, ovarian, lung, head and neck cancers, as well as advanced

forms of Kaposi's sarcoma. The compound binds to polymerized tubulin, promoting microtubule formation and microtubule stabilization. Thus, it precludes tubulin molecules from depolymerizing during the processes of cell division and consequently inhibits mitosis and cancer growth (Schiff and Horowitz 1980). Meanwhile, many other endophytic fungal strains, such as *Seimatoantlerium tepuiense*, *S. nepalense* (Bashyal et al. 1999), and *Tubercularia* sp. strain TF5 (Wang et al. 2000), have been reported to produce paclitaxel. A study investigating the endophytic fungal diversity of *Taxus chinensis* verified thirteen fungal species belonging to different genera for producing paclitaxel *in vitro*. One of the isolated fungal strains was *Metarhizium anisopliae*, which yielded 846.1 μg/L of the drug, thus exceeding the amounts so far reported for other paclitaxel-producing fungi (Liu et al. 2009). Of special interest are reports on fungi residing in plants other than *Taxus* species, which were also found to produce paclitaxel. Examples include *Pestalotiopsis microspora* (Strobel et al. 1996), *Periconia* sp. (Li et al. 1998), *Bartalinia robillardoides* and *Colletotrichum gloeosporioides* (Gangadevi and Muthumary 2008a,b). The latter two fungi produced 187.6 and 163.4 μg/L of paclitaxel, respectively, as determined by HPLC quantification (Gangadevi and Muthumary 2008a,b). However, paclitaxel yields from fungal isolates are still too low to be used for sustained industrial production, which relies predominantly on alternative processes including semi-synthesis of the anti-cancer drug starting from the naturally occurring precursor desacetylbaccatin III, which can be isolated in sufficient amounts from needles of other *Taxus* species such as *T. baccata*.

The dimeric indole alkaloid vincristine (**2**) is another mitotic inhibitor clinically used in chemotherapeutic treatment of certain cancer types including leukemia, lymphoma, breast and lung cancer. Vincristine (**2**), obtained from *Catharanthus roseus* (Apocynaceae), exerts its action by binding to tubulin dimers and inhibiting their assembly to microtubule structures thus arresting mitosis. A preliminary evidence for the production of vincristine by *Fusarium oxysporum*, endophytic in *C. roseus*, was reported (Lingqi et al. 2000).

Naturally occurring topoisomerase I inhibitors include the pentacyclic quinoline alkaloid camptothecin (**3**), the parent compound of the semi-synthetic derivatives irinotecan (**3a**) and topotecan (**3b**) that are clinically used against ovarian, small lung and refractory ovarian cancers (Srivastava et al. 2005). Topoisomerases are a class of enzymes that catalyze and guide the twisting and unknotting of DNA during replication and transcription (Ling-Hua et al. 2003). In addition, **3** was reported to inhibit RNA synthesis (Bendixen et al. 1990). Camptothecin was originally obtained from *Camptotheca acuminata* (Nyssaceae) (Wall et al. 1966), but it occurs also in systematically unrelated plant families such as Icacinaceae (*Nothapodytes nimmoniana*, *Pyrenacantha klaineana*, *Merrilliodendron megacrapum*, *Apodytes dimidiata*), Apocynaceae (*Ervatamia heyneana*), Rubiaceae (*Ophiorrhiza pumila*, *O. mungos*), and Gelsemiaceae (*Mostuea brunonis*) (Ramesha et al. 2008; Wink 2008; Shweta et al. 2010). The patchy distribution of this alkaloid in various plant families was thought to be originally caused by endophytes through infection of the respective plants or gene transfer (Wink 2008). Recently, the potent antineoplastic camptothecin (**3**) and two of its analogues, 9-methoxycamptothecin (**4**) and 10-hydroxycamptothecin (**5**), showing similar potency to **3** but improved aqueous solubility, were identified in the mycelia of the endophyte *Fusarium solani* isolated from *Camptotheca acuminata*. The latter compounds are reported mainly from *C. acuminata* and *N. nimmoniana* in rather low concentrations. Interestingly, differences in the genetic makeup of the fungus investigated in this study and *F. solani* isolated from other sources were observed, which may arise from a horizontal gene transfer from the host into the fungal genome (Kusari et al. 2009a). Other studies reported **3** from cultures of endophytic *Entrophospora infrequens*, isolated from *N. nimmoniana*, with a maximum yield of 0.575 ± 0.031 and 4.96 ± 0.73 mg/100 g dry cell mass in shake flasks and in a bioreactor, respectively (Puri et al. 2005; Amna et al. 2006). Such findings inspired further studies to explore other endophytic fungi from various plant sources for the production of **3** and its analogues. For instance, two endophytic fungal strains of *Fusarium solani*, obtained from *A. dimidiata*, were reported to produce **3–5** with yields in the range of 8.2–53.6 μg/100 g of dry cell mass (Shweta et al. 2010).

Podophyllum species (Berberidaceae) are known to produce the important lignan podophyllotoxin (**6**), which is the precursor to clinically used anti-cancer drugs such as etoposide (**6a**) and teniposide (**6b**). Podophyllotoxin exerts its antineoplastic action by preventing the polymerization and assembly of tubulin into the mitotic-spindle microtubules, thus arresting the cell cycle at mitosis (Guerram et al. 2012). The semi-synthetic derivatives, however, show a different mechanism of action as potent inhibitors of topoisomerase II, thereby blocking the ligation step of the cell cycle (covalent sealing of single-strand breaks in DNA during replication), harming genome integrity, and subsequently leading to apoptosis and cell death (Loike et al. 1978; Wigley 1995). This difference in the mode of action was related to the presence of the bulky glucoside moiety which is absent in **6** (Wigley 1995). The endophytic fungal strains *Trametes hirsuta* and *Phialocephala fortinii*, isolated from *Podophyllum hexandrum* and *P. peltatum*, respectively, were reported to produce podophyllotoxin at a yield ranging from 0.5 to 189 μg/L (Eyberger et al. 2006; Puri et al. 2006). Podophyllotoxin accumulation by *Fusarium oxysporum*, an endophyte of the medicinal plant *Juniperus recurva* (Cupressaceae) producing up to 28 μg/g dry weight of mycelia of podophyllotoxin, was also described (Kour et al. 2008). A recent study reported on production of podophyllotoxin by endophytic *F. solani*, from the roots of *P. hexandrum*, which was able to produce 29.0 μg/g dry weight (Nadeem et al. 2012). Similarly, endophytic *Aspergillus fumigatus*, isolated from *Juniperus communis*, was found to produce the anticancer pro-drug deoxypodophyllotoxin (**7**) with a maximum yield of 4 ± 2 μg/100 g dry weight of mycelia and 3 ± 2 μg/L of spent broth. Deoxypodophyllotoxin is not only a possible precursor of podophyllotoxin, but it shows broad therapeutic efficacy against a variety of malignancies as well (Kusari et al. 2009b).

The naphthodianthrone, hypericin (**8**), first identified from *Hypericum perforatum* (St. John's wort), is one of the main constituents of *Hypericum* species (Clusiaceae), which have been used for centuries against mild forms of depression and anxiety (Nahrstedt and Butterweck 1997). The compound may contribute to the antidepressant action by its monoamine oxidase (MAO) inhibiting activity and its significant affinity for σ-receptors (Nahrstedt and Butterweck 1997; Raffa 1998). Further studies reported wound healing, anti-inflammatory, antimicrobial and antioxidant activities for **8** (Kusari et al. 2008), as well as a significant antiviral activity against diverse enveloped viruses (Kubin et al. 2005). Production of hypericin, as well as its proposed main precursor in the endophytic biochemical pathway, emodin (**9**), was detected in the fungal biomass of *Thielavia subthermophila*, inhabiting *H. perforatum*, with yields of 35 ± 2 and 113 μg/100 g dry weight of fungal mycelia, respectively (Kusari et al. 2008, 2009c).

A recent report describes the production of azadirachtin A and B (**10** and **11**) from endophytic *Eupenicillium parvum*, isolated from the neem tree, *Azadirachta indica* (Meliaceae) (Kusari et al. 2012). Azadirachtins are a class of natural phagorepellents and antifeedants exclusively known from *A. indica*. They

exert their action by negatively affecting feeding and mating behavior, as well as postembryonic development and growth of insects (Hummel et al. 2012), thus contributing to the remarkable insect-repellent property of the neem tree (Lay et al. 1993). They are of great potential as lead compounds for the development of natural insecticides with less hazards on health and environment (Biswas et al. 2002). The parent compound azadirachtin A (**10**) was found to exert its antifeedent effect by stimulating specific deterrent chemoreceptors on the insect mouthpart and impeding the perception of phagostimulants by other chemoreceptors (Mordue et al. 1998). When taken up into the cells, the compound was found to inhibit cell division and protein synthesis by modifying or preventing transcription or translation of proteins, and it also caused significant inhibition of reproductive processes of male and female insects (Mordue and Nisbet 2000).

Lolium species (Poaceae), including common pasture and forage grasses as fescues and ryegrasses, are often colonized by endophytic *Neotyphodium* species. Loline alkaloids (**12**), first exclusively detected in endophyte infected grasses, were found to exhibit deterrent and toxic effects towards invertebrate and vertebrate herbivores, thus contributing to grass protection (Schardl et al. 2007). Further investigations on this class of compounds, which resemble simplified pyrrolizidine alkaloids with potent broad-spectrum insecticidal activity, demonstrated that a common endophyte of *Lolium pratense*, *Neotyphodium uncinatum*, was fully able to synthesize some of the most common loline alkaloids (Blankenship et al. 2001). Interestingly, a study showed that endophytic *N. uncinatum* inhabiting *L. pratense* provided defensive loline alkaloids not only to its host, but also to a root hemiparasitic plant of the host grass, *Rhinanthus serotinus* (Orobanchaceae), thus enhancing the resistance of the hemiparasitic plant to its generalist aphid herbivore *Aulacorthum solani* (Lehtonen et al. 2005).

Locoweeds, including the legume genera *Astragalus* and *Oxytropis* (Fabaceae), are known for the production of toxic indolizidine alkaloids, such as swainsonine (**13**), which cause poisoning of livestock. An endophytic fungus of the genus *Embellisia* was isolated from *Astragalus* and *Oxytropis* species and shown to produce swainsonine (Ralphs et al. 2008).

A similar example was reported for the mangrove plant *Hibiscus tiliaceus* (Malvaceae). Chemical examination of the plant as well as fermentation broth of its endophyte *Phomopsis* sp. revealed the presence of oleanane-type triterpenes, suggesting a possible transfer of the biosynthetic machinery of the oleanane skeleton during evolution (Li et al. 2006, 2008).

3. Fungal biosynthesis of plant associated metabolites

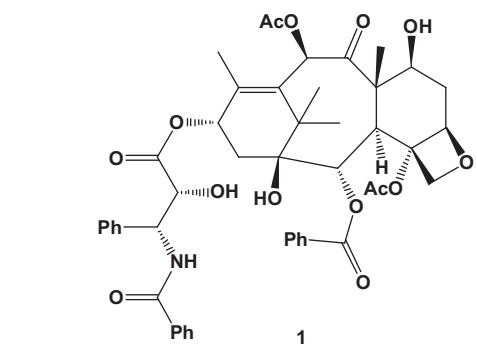
As shown above many endophytes are apparently able to synthesize the same natural products that also occur in plants. Whether a horizontal gene transfer occurred at some time during co-evolution of plants and endophytes that enabled the receiving partner to perform the same biosynthetic reactions as present in the donor remains to be clarified. Determination of fungal contribution to the secondary metabolite profiles of plants is of great interest as it would offer an explanation for the patchy distribution of natural products, including certain alkaloids, cardiac glycosides and anthraquinones in the plant kingdom (Wink 2008). It has been hypothesized that the best suited ecological settings for plant-endophyte interactions, are mostly found in the tropical environment (Rodriguez et al. 2009), as this habitat apparently favours horizontal gene transfers or genetic recombinations between host plants and their endophytic counterparts resulting in the production of the same secondary metabolites (Kusari et al. 2009c). An answer to this intriguing question, however, can only be given after analysis of biogenetic gene clusters from host plants and endophytes. Furthermore, regulatory mechanisms determining the site of genomic detachment and/or integration in the host and its endophyte, as well as the expression of gene clusters in the recipient system still need to be addressed. It is nevertheless assumed that production of these respective compounds *in planta* does not proceed exclusively by endophytes but is rather the consequence of concomitant plant and fungal biosynthesis (Kusari et al. 2008).

The earliest reports on detection of plant metabolites in fungi date back to the 1930 s where the phytohormones gibberellins (**14**) were detected in *Fusarium fujikuroi* (Yabuta and Sumiki 1938). In spite of the production of structurally identical gibberellins by higher plants and *F. fujikuroi*, biosynthetic studies disclosed significant differences at the chemical, biochemical and genetic levels indicating independent development of the corresponding complex biosynthetic pathways and the absence of horizontal gene transfer (Hedden et al. 2002). Only few gibberellin-producing fungi are known to date, including *F. konzum*, *Phaeosphaeria* sp. and some *Sphaceloma* species, mainly *S. manihoticola*. Interestingly, despite of distant relatedness, the latter fungus showed close gene clusters similarity, identical gene functions, and conserved intron positions to *F. fujikuroi* suggesting a common evolutionary origin of the two fungi (Bömke et al. 2008).

In many cases, studies on biosynthetic pathways of plant associated metabolites in plants and endophytic fungi revealed similar but still distinct metabolic pathways for their production (Chandra 2012). For instance, the gene 10-deacetylbaccatin-III-10-*O*-acetyl transferase, which is involved in the biosynthesis of paclitaxel (**1**), was isolated from *Clasdosporium cladosporiodes* endophytic in *Taxus media*. The fungal gene showed 99% and 97% identity to the corresponding genes of the host *T. media* and *T. wallichiana*, respectively, indicating independent production of taxol by the endophytic fungus (Jennewein et al. 2001).

In another study, a significant decrease of camptothecin (**3**) production by endophytic *Fusarium solani*, isolated from *Camptotheca acuminata*, was observed upon repeated subculturing of the fungus. Investigations on fungal camptothecin pathway, using a homology-based approach and high-precision isotoperatio mass spectrometry, revealed that the endophyte employs indigenous enzymes, including geraniol 10-hydroxylase, secologanin synthase and tryptophan decarboxylase for the synthesis of camptothecin precursors. However, it uses host strictosidine synthase, probably carried over into the fungal biomass during isolation, to complete the final biosynthesis steps. Based on these findings, a cross-species biosynthetic pathway was suggested. In an attempt to improve camptothecin production, the endophyte was artificially inoculated into its living host plant and then re-isolated. Interestingly, in spite of reconstituted host-endophyte interaction camptothecin biosynthesis could not be re-established. Biosynthetic gene analysis showed diverse mutations in the seventh subculture generation of the endophyte, whereas no damage was observed for control genes of primary metabolic processes (Kusari et al. 2011).

It was suggested that the complex enzymatic conversion of emodin (**9**) to hypericin (**8**) in *Hypericum perforatum* is regulated by the Hyp-1 phenolic coupling protein (Bais et al. 2003). Efforts made to isolate a homologous sequence to host plant *hyp-1* gene from its endophyte *Thielavia subthermophila* by reverse transcription-polymerase chain reaction (RT-PCR) resulted in isolation of a gene transcript of different size than the genomic fragment amplified on DNA template from *H. perforatum*, attributable to introns present within the gene sequence. However, DNA sequencing did not show any nucleotide similarity to the published *hyp-1* gene. The absence of a homologous



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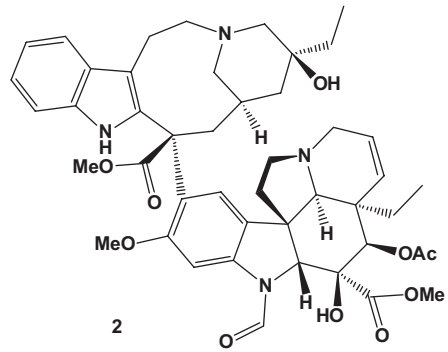

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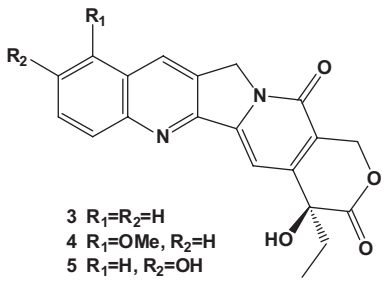
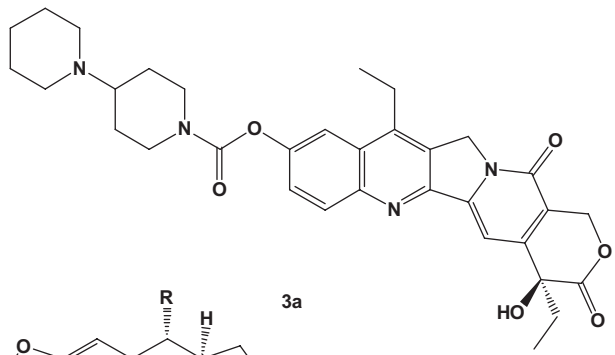


3 R_1=R_2=H
4 R_1=OMe, R_2=H
5 R_1=H, R_2=OH



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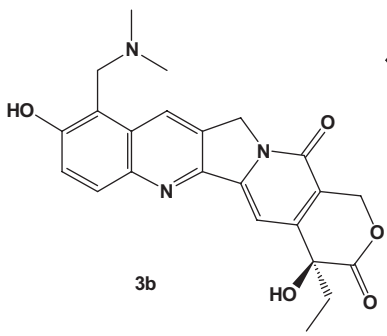
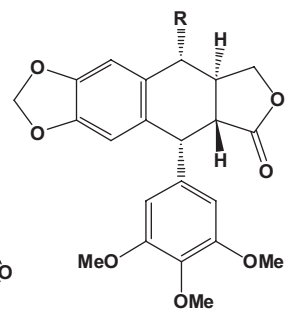
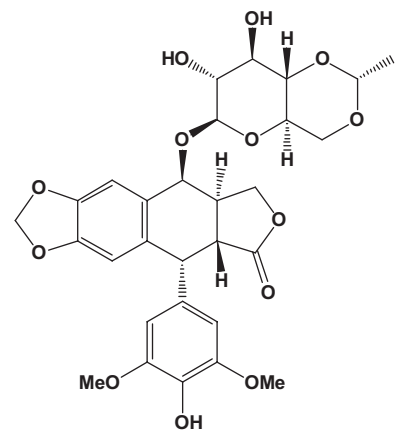


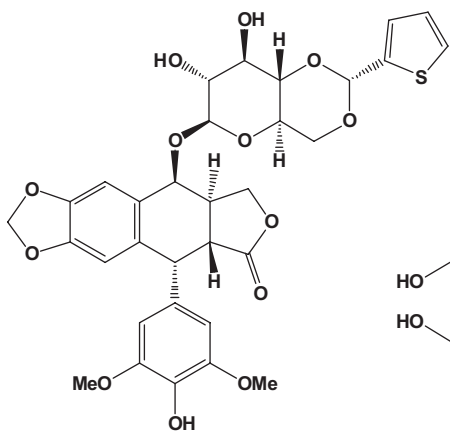
3b



6 R=OH
7 R=H



6a



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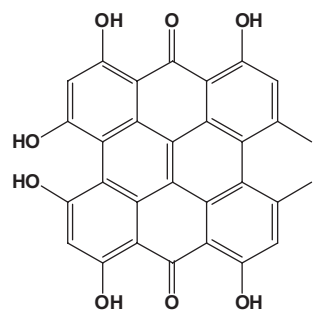

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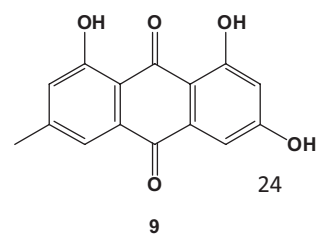

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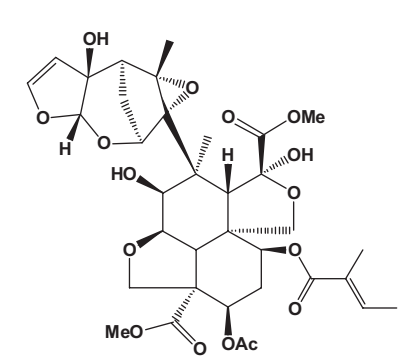
sequence to *hyp-1* gene in *T. subthermophila* suggested that hypericin biosynthesis in the endophytic fungus occurs through a different pathway and/or is regulated by a different molecular mechanism than in the host plant or in host cell suspension cultures (Kusari et al. 2009c).

Thus, the hypothesis of "traitspecific endophytic infallibility" was proposed, which suggests that being subject to the same selection pressure as their host plants, endophytes experience independent adaptive processes during co-evolution with their hosts resulting in the development of similar molecular machineries to produce the same host-specific metabolites. This is governed by host and endophyte specific genotypes as well as suitable environmental factors (Kusari et al. 2009c).

4. Endophytic fungal involvement for improving the production of commercially important plant derived compounds

The development of modern biotechnological techniques offers great opportunities to significantly improve the production



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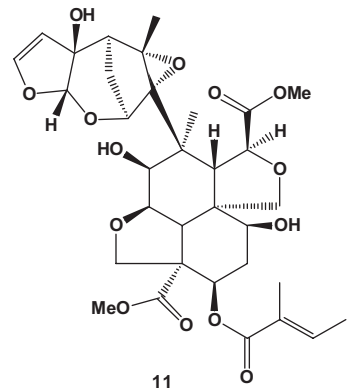

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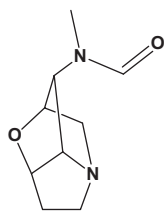

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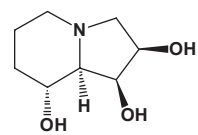

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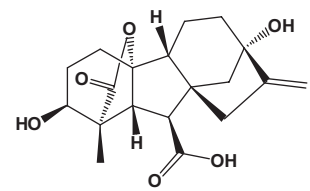

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of valuable bioactive secondary metabolites. Using co-culture methods, as well as genetic engineering by transferring, over-expressing or modifying genes encoding the rate-limiting enzymes of secondary metabolites biosynthetic pathways may be of great potential for the industrial mass production of such valuable metabolites by fungal large scale fermentation.

A co-culture method, by which suspension cells of *Taxus chinensis* var. *mairei* and its endophytic fungi *Fusarium mairei* were cultured in a co-bioreactor, was described to improve paclitaxel (**1**) production in a shorter culture time. Optimization of the co-culture conditions resulted in a significant increase in the yield of paclitaxel from *Taxus* cell cultures to reach 25.63 mg/L of paclitaxel within 15 days (equivalent to 1.71 mg/L per day), which is 38-fold higher than the yield of single culture (0.68 mg/L within 15 days) (Li et al. 2009).

Methods for genetic engineering of the paclitaxel biosynthetic pathway to improve paclitaxel production by endophytic fungi are on the rise. The restriction enzyme-mediated integration (REMI) transformation was applied to an endophytic paclitaxel-producing fungus, *Ozonium* sp. BT2, isolated from *Taxus chinensis* var. *mairei*. This method is commonly used to transfer nonhomologous linearized DNA into host chromosome by the *in vivo* action of restriction enzymes. The transformation of endophytic *Ozonium* sp. BT2 resulted in stable hygromycin B resistant fungal transformants, thus representing the first report of successful transformation of paclitaxel-producing endophytic fungi by a relatively simple and efficient procedure, which may be applied to improve paclitaxel production by these fungi (Wang et al. 2007). In a more recent study a protocol for *Agrobacterium tumefaciens*-mediated transformation (ATMT) of the paclitaxel-producing endophytic fungus, *Cladosporium cladosporioides* MD2 isolated from *Taxus x media*, was successfully developed. In this protocol fungal spores were used as host cells and important parameters of the transformation system were optimized to obtain stable fungal transformants with hygromycin B resistance (Zhang et al. 2011).

Very recently, the construction of a fungus expression vector (pV2$^+$-TS-pAN7-1) containing taxadiene synthase gene (*ts*), a rate-limiting enzyme gene for paclitaxel biosynthesis, was described. This vector was used for the transformation of the paclitaxel-producing endophytic fungus *Ozonium* sp. EFY-21, obtained from *T. chinensis* var. *mairei*, leading to successful integration of the *ts* gene into the genome of independent fungal transformants. LC-MS analysis indicated an increased paclitaxel production for one of the transgenic strains obtained, which exhibited an over-expression of the *ts* gene, as proven by reverse transcription – polymerase chain reaction (RT-PCR) (Wei et al. 2012).

Similar approaches were developed to improve the production of podophyllotoxin (**6**) and its derivatives by mimicking plant responses to elicitation of endophytic fungi. In this context, cell suspension cultures of *Linum album* (Linaceae), developed from internode portions of an *in vitro* germinated plant, were co-cultured with the axenically cultivable arbuscular mycorrhiza-like fungi, *Piriformospora indica* and *Sebacina vermifera*, by inoculating different levels of the fungi in *L. album* growing culture. Both fungi showed phytopromotional effect increasing plant cell growth by 21% and 17.6%, respectively, in comparison to control cultures, which may be explained by an increased absorption of mineral nutrients by plant cells. In addition, both fungi enhanced podophyllotoxin and 6-methoxypodophyllotoxin production by about four- and eight-fold, respectively, probably by elicitation through induction of hypersensitive response in the plant cells resulting in activation of plant defense pathways. Furthermore, a significant increase of phenylalanine ammonia lyase (PAL) enzyme activity, responsible for the rate limiting step of lignan biosynthesis, was observed during the co-culture studies. Interestingly, addition of dead fungal cells to *L. album* cell suspension cultures increased the production of podophyllotoxins and PAL activity to a significantly lower level than addition of live fungal cells (Baldi et al. 2008). In a similar study, autoclaved and filter-sterilized culture filtrate of the root endophytic fungus *P. indica* was added to growing *L. album* hairy root cultures to evaluate its influence on growth and lignan production. In agreement with the previous study, podophyllotoxin and 6-methoxypodophyllotoxin yields were improved by 3.8 and 4.4 times, respectively, with a corresponding increase in PAL activity in comparison to control cultures (Kumar et al. 2012).

5. Conclusion

In the continuous search for novel drug sources, endophytic fungi have proven to be a promising, largely untapped reservoir of natural products, which have been optimized by evolutionary, ecological and environmental factors to represent effec-

tive chemically diverse bioactive agents. Furthermore, some endophytes are capable to fully synthesize medicinally important natural products originally known exclusively from plants, thus raising the prospect of using such organisms as alternative and sustainable sources to meet the clinical needs of these substances. This would offer an inexhaustible, reproducible and cost-effective supply of these compounds, and prevent extinction of producing plants. However, in practice commercial application of endophytic fungi for the industrial production of such substances has not been achieved so far and its feasibility has still to be proven. Efforts have to be invested to prevent reduced production of secondary metabolites upon repeated sub-culturing, to identify potential elicitors for compound production in cultures, and to improve our understanding of fungal biosynthetic processes.

Recent advancements in molecular biology of fungal secondary metabolism may improve our knowledge on the regulation and mode of expression of fungal biogenetic gene clusters *in planta*, which may in turn help to understand the biochemical and molecular basis of plant secondary metabolite production by endophytes. However, factors affecting endophytic fungi in axenic cultures are significantly different from those arising in the host plant. Thus, a deeper understanding of the host-endophyte relationship at the molecular and genetic levels, as well as interactions of endophytes with other microorganisms within the complex plant microbiomes, and the impact of complex environmental conditions may help to induce and optimize secondary metabolite production under laboratory conditions and on industrial scale to improve the production yield of desired bioactive natural products.

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