Potential of Tree Endophytes as Sources for New Drug Compounds

Mysore V. Tejesvi and Anna Maria Pirttilä

Abstract The novel or designer metabolites produced by fungal endophytes are increasingly recognized by natural chemists due to their diverse structures and as candidates for drug discovery and development. Many of the metabolites belong to different classes i.e. alkaloids, benzopyranones, coumarins, chromones, cytochalasines, enniatines, isocoumarin derivatives, quinones, peptides, phenols, phenolic acids, semiquinones, steroids, terpenoids, xanthones and lactones. One of the most widely studied endophytic genera is *Pestalotiopsis,* from which more than 140 metabolites are reported with antimicrobial, antioxidant and antitumor activities. Besides reviewing the advances made in identifying bioactive metabolites with drug development potential from endophytic fungi, this chapter discusses possibilities and bottlenecks involved in employment of endophytic fungi and their products by the pharmaceutical industry. Furthermore, issues involved in anti-infective discovery and timeline of drug development are discussed in the view of developing new drug compounds from endophytic products.

Abbreviations

ACE	angiotensin I-converting enzyme
AIDS	acquired immune deficiency syndrome
DGGE	denaturing gradient gel electrophoresis
EMEA	European agency for the evaluation of medicinal products
FDA	food and drug administration
HI	human immunodeficiency
IC_{50}	the half maximal inhibitory concentration

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MIC	minimum inhibitory concentration
NDM-1	New Delhi metallo-beta-lactamase
RFLP	restriction fragment length polymorphism
SARS	severe acute respiratory syndrome
TB	tuberculosis

1 Introduction

The drastic rise in the number of publications on compounds from fungal endophytes within the past two decades is due to the creative ability of these fungi to produce secondary metabolites. There is also a rise in the need for new antibiotics, anti-malarial drugs, chemotherapeutic or pharmaceutical agents that are highly effective, possess low toxicity and have a minor environmental impact. The development of resistance in infectious microorganisms like *Staphylococcus*, *Mycobacterium* and *Streptococcus* to existing drugs and the presence of naturally resistant organisms are causing threat to mankind (Mwangi et al. 2007; Hugonnet et al. 2009; Richter et al. 2009). Emerging diseases such as AIDS, SARS and NDM-1 necessitate the discovery and development of new drugs (Kumarasamy et al. 2010). The weakened immune system due to AIDS not only requires specific drugs for treatment but also needs new therapies to combat the secondary problems arisen from it, and, furthermore, the HI virus is developing resistance towards the existing drugs (Richman et al. 2004). Opportunistic pathogens such as *Aspergillus*, *Cryptococcus* and *Candida* are also virulent in immunocompromised patients, and in patients, who need an organ transplant. In addition, parasitic protozoan and nematodal infections such as malaria, leishmaniasis, trypanomiasis and filariasis are causing major problems in many countries and effective drugs against them are needed. Malaria is claiming more lives each year than diseases caused by any other infectious agent, with the exception of AIDS and TB (NIAID Global Health Research Plan for HIV/AIDS 2001), and enteric infections claim more lives of children each year than any other disease (Strobel et al. 2004).

For all these reasons, there is a continuous search for novel natural products. The compounds produced by microorganisms have a history of offering opportunities for innovation in drug discovery and development, and therefore many scientists and researchers have turned their looks back to the microbial world. Exciting possibilities exist for those who are willing to take a risk and venture into the unexplored territories of the world to experience the excitement and thrill of engaging in the discovery of endophytes, their biology and potential usefulness (Strobel 2003). In the past decade, endophytic microbes have attracted considerable attention as completely new sources of novel pharmaceuticals (Strobel 2002).

A number of microbial metabolites have been available in quantities of up to hundreds of kilograms by fermentation technology (Grabley and Thiericke 1999). From the screening of a huge number of microbial extracts, an unexpected diversity of natural compounds with a broad variety of biological activities has

been found (Grabley and Sattler 2003). Therefore, microorganisms associated with plants, rather than the plants themselves, can be raw material with promising therapeutic potential (Strobel 2002). Until now, endophytic microbial metabolites have been studied for a wide range of activities like antioomycete, antibacterial, antifungal, anticancerous and immunosuppressive activities. Endophytes were given considerable credibility as sources of novel compounds through the discovery of Taxol® biosynthesis, and a variety of other antibacterial, antifungal and anticancer metabolites from the endophytic fungi *Taxomyces andreae* and *Pestalotiopsis* spp. (Stierle et al. 1993; Strobel 2003). Endophytes can be used as sources of novel metabolites for medicine, agriculture and industrial uses. The best strategy for finding new bioactive compounds is to survey endophytes from plants restricted to special areas, as a means to isolate fungi that likely were never studied in earlier screening programs (Pelaez et al. 1998).

2 Current Status of Endophyte Research with Respect to Drug Discovery

The research on endophytes is growing enormously, as >650 research articles covering both bacteria and fungi were published during the period between 1991 and 2010 (www.sciencedirect.com) (Fig. 1). When the bibliographic search was restricted to endophyte and metabolite there were 253 published research articles, which shows that roughly 40% of the endophyte researchers were looking for sec-

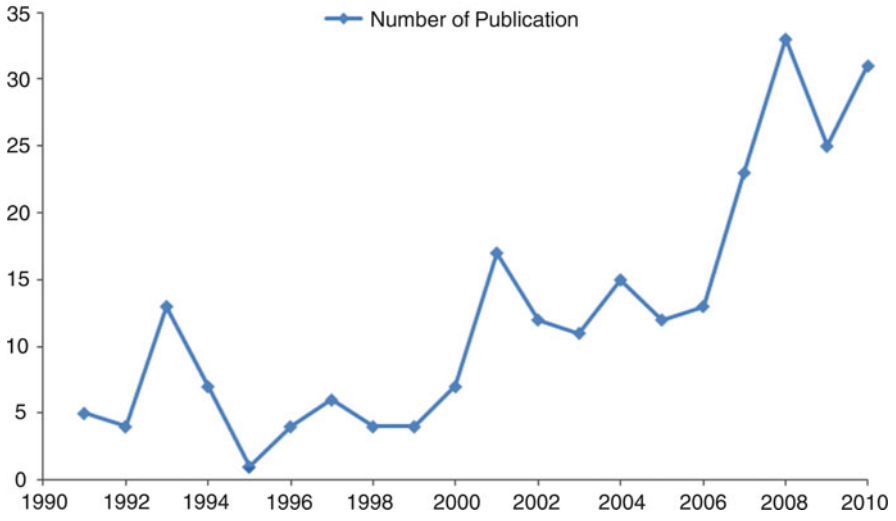


Fig. 1 Number of publication on endophytes from 1990 to 2010 (data used from science direct with a keyword Endophyte + Metabolite). Two hundred and fifty three published articles were found from two decades of research on endophytic metabolites

ondary metabolites (Fig. 1). The potential of endophytic fungi as a source of novel drugs can be seen in terms of number of patents filed and granted on endophytes. When searched with the keyword endophyte (http://www.freepatentsonline.com) >650 patents were filed and granted for using an endophyte as a source for new processes or industrial applications on bioactive metabolites.

3 Medicinal Plants

The plant kingdom is a rich source of structural biodiversity offering a variety of natural products. Plants have been utilized to produce various types of medicines for thousands of years (Samuelsson 2004). These medicines were initially used in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations (Balick and Cox 1997; Samuelsson 2004). More than 50,000 medicinal plants (Schippmann et al. 2002) out of the total of 4, 22,000 flowering plants reported worldwide have been used for various medicinal purposes (Govaerts 2001). The information on the plants usable for these purposes, and the methods of applying them for a particular ailment, were passed down orally through successive generations. Eventually the information on medicinal plants was recorded in herbals. More recently, the use of plants as medicines has focused on the isolation of active compounds, for example the isolation of morphine from opium poppy in the early nineteenth century (Kinghorn 2001; Samuelsson 2004). According to the World Health Organization (WHO 1991), 80% of the world's population is dependent on health-care provided by medicinal plants.

A wide range of medicinal plant parts is used as extracts that can be considered raw drugs that possess specific medicinal properties. The different plant products used to cure various infectious diseases include root, stem, flower, fruit, root, twigs, exudates, and modified plant organs. Whereas some of these raw drugs are collected in small quantities for local use by the native communities and folk healers, many other raw drugs are collected in large quantities and traded in the market as the raw material for herbal industries (Uniyal et al. 2006). The same medicinal plants provide a good source for isolation of endophytic fungi and screening for bioactive metabolites. In this way, the need to sacrifice plants that in some cases are rare or endangered can be avoided.

4 Endophytic Fungi

Endophytic fungi are found practically in every plant species including terrestrial plants such as grasses (Bacon and White 1994; Groppe et al. 1999; Saikkonen et al. 2000), palms (Taylor et al. 1999; Frohlich et al. 2000), banana (Brown et al. 1998; Photita et al. 2004), mangroves (Suryanarayanan et al. 1998; Kumaresan and Suryanarayanan 2002; Ananda and Sridhar 2002) and halophytes (Suryanarayanan

and Kumaresan 2000), and in every subclass including mosses, liverworts, pteridophytes, gymnosperms and angiosperms (Provorov et al. 2002). Endophytes are found in all plant tissues including seeds and ovules (Siegel et al. 1987), fruits (Baayen et al. 2002), stems (Gutierrez-Zamora and Martinez-Romero 2001), roots (Germida et al. 1998), leaves (Cannon and Simmons 2002), inner bark of trees (Tejesvi et al. 2005, 2006), tubers (Sturz et al. 1998), buds (Pirttilä et al. 2000, 2003; Ragazzi et al. 2001), xylem (Hoff et al. 2004) and rachis (Rodrigues and Samuels 1999). Numerous publications are available on their biology (Jennings and Lysek 1996; Clay 1998; Brem and Leuchtmann 2001; Arnold et al. 2003), evolution (Carroll 1998; Saikkonen et al. 2004), occurrence (Kumar et al. 2004; Tejesvi et al. 2006), taxonomy (Petrini 1986; Guo et al. 2000, 2003) and biotechnological applications (Tomita 2003; Strobel 2007).

The impact of endophytic fungi on host plants is largely unknown compared with that of fungal pathogens or mycorrhizal symbionts. Endophytic fungi may influence other fungi present in the same host, existing between the tropical niches of pathogen and mutualist (Hoff et al. 2004). This influence can be expressed directly by inhibition or stimulation of fungal growth, or indirectly via effects on host physiology and morphology. Thus, the genetic variability and unpredictability of pathogen interactions with the host plants can be attributed to endophytes (Saikkonen et al. 1998; Hoff et al. 2004).

Schulz et al. (1993, 1995, 1998) obtained >6,500 endophytic isolates from different organs of more than 500 plants of diverse temperate habitats. The majority of the isolates belonged to ubiquitous genera (e.g. *Acremonium*, *Alternaria*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, *Fusarium*, *Geniculosporium*, *Pestalotiopsis*, *Phoma*, *Pleospora*), concurring with previous results, reviewed by Petrini (1986), that many endophytes are from ubiquitous taxa. The assemblages of endophytes vary with habitat, as different ubiquitous genera are isolated from tropical than from temperate climates (e.g. see the chapters by M. Unterseher and T.S. Suryanarayanan, this volume). Some genera like *Fusarium, Phomopsis* and *Phoma* are common in both tropical and temperate climates, whereas members of Xylariaceae, *Colletotrichum*, *Guignardia*, *Phyllosticta* and *Pestalotiopsis* predominate in the tropics (Frohlich and Hyde 1999; Cannon and Simmons 2002; Suryanarayanan et al. 2003; Arnold 2008). An interesting aspect to investigate is how the occupation of an inter- or intracellular niche within a plant by one fungal group affects the subsequent establishment and evolution of other fungal partnerships (Schulz and Boyle 2005).

5 Antimicrobials from Endophytic *Pestalotiopsis* Species

Pestalotiopsis species have gained much attention in recent years as they produce many important secondary metabolites (Strobel 2002; Tejesvi et al. 2007; Xu et al. 2010). At present, more than 30 *Pestalotiopsis* species have been reported as endophytes, there are 235 species listed in *Index Fungorum* (http://www.indexfungorum.org/Names/Names.asp) and they are usually found in tropical and subtropical plants

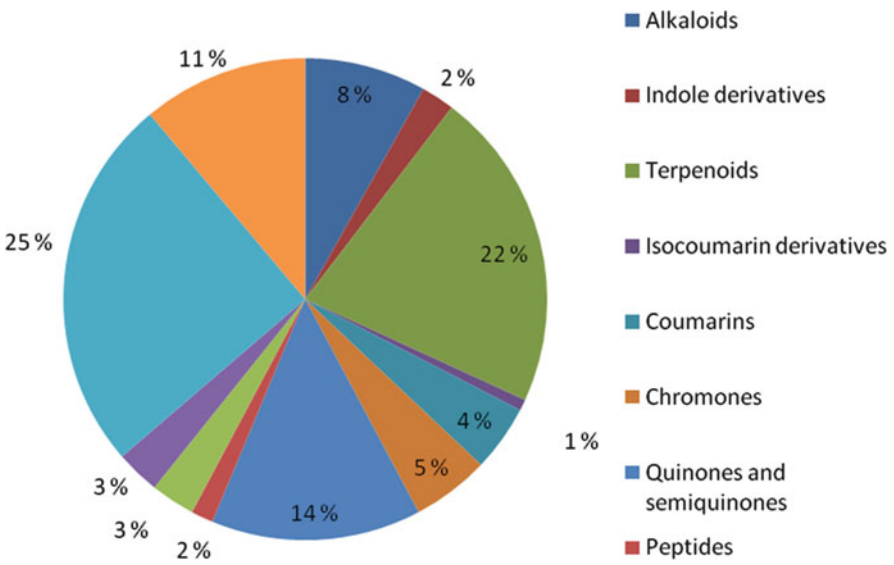
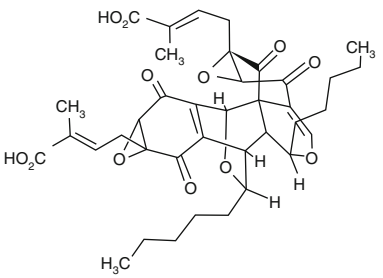


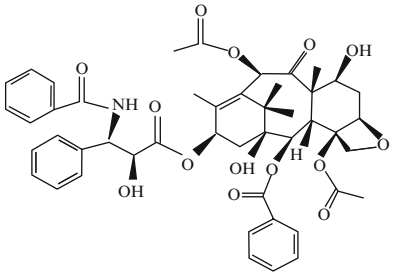
Fig. 2 Different class of secondary metabolite isolated from the genus *Pestalotiopsis*

throughout the world (Tejesvi et al. 2006, 2007; Ding et al. 2009; Liu et al. 2009a). A group of *Pestalotiopsis* species produces secondary metabolites, which have great potential as antioxidants, antimicrobials and anti-tumor compounds (Tan and Zou 2001; Zhang et al. 2006; Xu et al. 2010). However, many endophytic *Pestalotiopsis* species have been unidentified due to the limitation and difficulty in applying classification based on existing morphological characters (Okane et al. 1998; Suryanarayanan and Kumaresan 2000; Suryanarayanan et al. 1998; Toofanee and Dulymamode 2002; Tejesvi et al. 2009). There are >140 metabolites that have been identified and characterized from *Pestalotiopsis* spp., belonging to different classes of compounds such as alkaloids, terpenoids, isocoumarin derivatives, coumarins, chromones, quinones, semiquinones, peptides, xanthones, xanthone derivatives, phenols, phenolic acids, and lactones (Fig. 2), of which some examples with antimicrobial activity are given in the following paragraph.

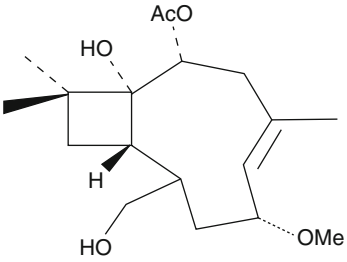
The crude extract of the endophytic *Pestalotiopsis* sp. from the lichen *Clavarioides* sp. yielded six ambuic acid derivatives and a torreyanic acid (Fig. 3), which showed antibacterial activity against *Staphylococcus aureus* with IC_{50} values of 43.9 and 27.8 μM, respectively (Ding et al. 2009). Ambuic acid has also been identified from different *P. microspora* strains isolated from *Taxus baccata*, *Torreya taxifolia*, *Taxodium disticum*, *Wollemia nobelis* and *Dendrobium speciosum* showing potential antifungal activity against plant pathogens (Li et al. 2001). Three new caryophyllene-type sesquiterpene alcohols, 6-hydroxypunctaporonin E, 6-hydroxypunctaporonin B and 6-hydroxypunctaporonin A were isolated from culture filtrate of *P. disseminata*. The compounds 6-hydroxypunctaporonin E and



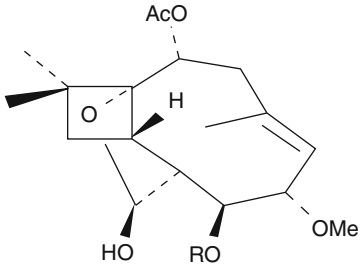
Torreyanic acid



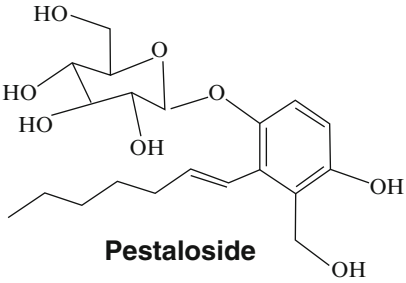
Taxol



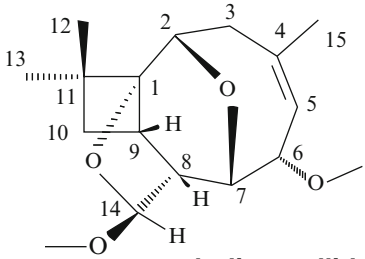
Pestalotiopsins B



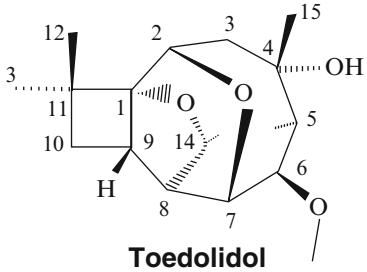
R = H: Pestalotiopsin A
R = CH3: Pestalotiopsin C



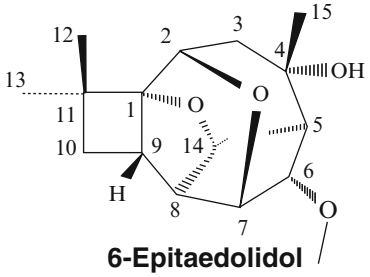
Pestaloside



pestalodiopsodlide A



Toedolidol



6-Epitaedolidol

Fig. 3 Structures of bioactive metabolites produced by endophytic *Pestalotiopsis* spp.

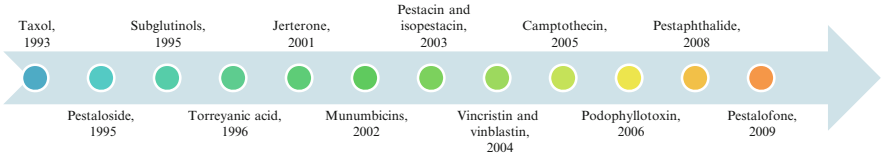


Fig. 4 Timeline of discovery of important secondary metabolites produced by endophytic fungi

6-hydroxypunctaporonin B exhibited antibacterial activities in agar diffusion plate assays at 100 μg/disk against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 29213) (Deyrup et al. 2006). Pestalachloride A, an alkaloid isolated from an endophytic *Pestalotiopsis adusta*, displayed potent antifungal activity against *Fusarium culmorum* with an IC_{50} value of 0.89 μM (Li et al. 2008). *Pestalotiopsis foedan*, isolated from the branches of an unidentified tree, yielded a novel spiroazaphilone derivative, pestafolide A, which exhibited antifungal activity against *Aspergillus fumigatus* (ATCC10894) (Ding et al. 2008).

Jesterone and hydroxy-jesterone are novel cyclohexenone epoxides isolated from a newly described endophytic fungal species *P. jesteri*, which was isolated from the bark of *Fragraea bodenii* (oak tree, family Loganiaceae) (Li and Strobel 2001). Jesterone displayed selective antimycotic activity against the oomycetous fungi such as *Pythium ultimum*, *Aphanomyces* sp., *Phytophthora citrophthora*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, and *Pyricularia oryzae* with MIC values of 94.7, 24.6, 94.7, 24.6, 94.7 and 94.7 μM, respectively (Li and Strobel 2001). Bioassay-guided separation of the culture of *P. fici* yielded five new compounds, pestalofones A–E. Pestalofones C and D exhibited inhibitory effects against *Aspergillus fumigatus* with IC_{50}/MIC values of 1.10/35.3, 0.90/31.2 μM, respectively (Liu et al. 2009b).

Two novel phenols, pestacin and isopestacin (Fig. 4) were isolated from *P. microspora* associated with the combretaceaous plant *Terminalia morobensis* (Strobel et al. 2002; Harper et al. 2003). Pestacin showed a moderate antifungal activity against *Pythium ultimum*, and isopestacin displayed moderate antimycotic activities against plant pathogenic oomycete *Pythium ultimum*, ascomycete *Sclerotinia sclerotiorum* and basidiomycete *Rhizoctonia solani* (Strobel et al. 2002; Harper et al. 2003). Pestaphthalides A and B having moderate antifungal activity were isolated from *P. foedan* of an unidentified tree near Dongzai, Hainan Province, China (Ding et al. 2008). Pestalachlorides B and C are two chlorinated benzophenone derivatives isolated from endophytic *P. adusta*. Pestalachloride B exhibited antifungal activity against the fungal plant pathogen *Gibberella zeae* with an IC_{50} value of 1.1 μM (Li et al. 2008). *Pestalotiopsis microspora* was isolated from *Torreya taxifolia* and it produced pestalopyrone, hydroxypestalopyrone and pestaloside phytotoxins in axenic cultures. Pestaloside exhibited broad-spectrum antifungal activity against the fungi *Cladosporium* sp., *Rhizoctonia solani*, *Geotrichum candidum* and *Agricus campestris* (Lee et al. 1995).

Pestalotiopsis microspora isolated from the inner bark of a small limb of Himalayan yew, *Taxus wallachiana* produces Taxol® in mycelial culture. Taxol® was identified by spectroscopic and chromatographic comparisons similar to authentic Taxol® (Fig. 3). Optimal Taxol® production occurred after 2–3 weeks in still culture at 23°C. ^{14}C Acetate and ^{14}C phenylalanine served as precursors for the fungal ^{14}C Taxol® (Strobel et al. 1996a). A number of unrelated fungal endophytes including *Pestalotia*, *Pestalotiopsis*, *Fusarium*, *Alternaria*, *Pithomyces*, *Monochaetia* also produce taxol in vitro (Strobel et al. 1996b).

6 Methods for Gaining Industry-Level Production Rates of Endophytic Metabolites

Industrial production of bioactive substances (e.g. pharmaceuticals, drugs) requires reproducible, dependable productivity. Microbial fermentation as a means of producing bioactive substances has several advantages: (1) If a microbe is the source organism, in an optimal case it can be grown in tank fermentors, producing an inexhaustible supply of material, (2) microorganisms typically respond favorably to routine culture techniques, whereas tissue culture or growing of plants requires either specialized techniques, or months of growth before harvesting is profitable, (3) product escalation is relatively easy in microorganisms. Various biosynthetic pathways can be optimized by changing the culture conditions for effective development and discovery of the lead compounds. For example, aplasmomycins were produced by *Streptomyces griseus* in the medium only after addition of NaCl (Nakamura et al. 1977; Stierle and Stierle 2005; Imada et al. 2007).

Developing a productive microbial source for anti-infectives and immunosuppressants not only would lower the production cost of the compound but would also make it widely available. The sources of new drugs during the period from 1981 to 2006 indicate that over 60% of the drugs are natural products, and close to 70% of anti-infectives and 63% of anticancerous drugs are derived from natural products (Cragg and Newman 2009). However, even though endophytic fungi show great potential as sources for industry-scale production of Taxol®, so far they have not been applied to industrial use (Ji et al. 2006). Problems such as low fungal biomass produced during fermentation, low yield of Taxol® in culture and lack of knowledge of regulation of the biosynthesis pathway limit the industrial use of these fungi (Ji et al. 2006). A yield of 1 mg/l for Taxol® production would be profitable at industrial scale, but the highest yields reported so far are 15 to 20 times lower (Deng et al. 2009). Taxol® has become a successful natural compound that is widely used as anti-tumor agent, with a higher demand than production rates. Therefore, knowledge of biotechnological tools for large-scale production of Taxol® are needed to engineer endophytic strains, or to produce Taxol® in heterologous hosts.

An endophytic organism can produce secondary metabolites in relatively high yield in culture, particularly when subjected to strain improvement programs

(Penalva et al. 1998). It is feasible to produce and isolate mutants that could readily be cultivated or could generate either additional products or modified products with a higher therapeutic index (Piepersberg 1994). Moreover, the metabolites they produce are largely generated by enzymatic pathways that have the potential to biosynthetically link existing structures to chemical adjuncts in a reproducible manner, at yields that are acceptable for industrial scale (Verdine 1996). To improve Taxol® production, one strain of *Nodulisporium sylviform*, has been subjected to mutagenesis and production of 314–393 μg/L was obtained (Zhao et al. 2005; Zhou et al. 2005). Another way to modify the fungal secondary metabolome is to alter their epigenetic status (Williams et al. 2008). By treating bioactive fungi with enzymes such as DNA methyltransferases or histone deacetylase inhibitors, enhanced chemical diversity can be obtained (Williams et al. 2008). In this sense, natural products produced from microbes exhibit a number of properties that make them excellent candidates for industrial processes.

A typical problem encountered with endophytes is that they produce the bioactive metabolite only for a short while *in vitro* and then die during subculturing, or become impaired in production of the secondary metabolite, or do not grow at all *in vitro*. Similar problems encountered earlier with other microbes lead to the development of metagenomics and metatranscriptomics tools, to access the vast microbial wealth without restrictions of culturability or growth (Handelsman 2004; Green and Keller 2006; Bailly et al. 2007). The culture independent analysis techniques such as terminal restriction fragment length polymorphism (T-RFLP) (Nikolcheva and Bärlocher 2005), denaturing gradient gel electrophoresis (DGGE) (Duong et al. 2006), or direct sequencing of ribosomal sequences (Seena et al. 2008; Tejesvi et al. 2010) are generally used for analyzing the diversity of unculturable fungal communities in plants. However, these methods are not suitable for functional studies of unculturable endophytes, and metagenomic and metatranscriptomic tools are still waiting to be applied to endophytes.

Another reasonably new and highly innovative approach is the biosynthesis of bioactive compounds in heterologous hosts. Heterologous expression of biosynthesis pathway of a compound through the transfer of the pathway genes from the producer organism to another, foreign host, can enable the production of the compound in higher quantities (Wenzel and Muller 2005). *Escherichia coli* is a widely used host for expression of even complex metabolic pathways, such as that of erythromycin (Pfeifer et al. 2001), echinomycin and triostin A (Watanabe 2008). Other hosts used recently are e.g. *Myxococcus xanthus* and *Pseudomonas putida* for the production of epothilone or myxochromide S (Fu et al. 2008), the thermopilic isolate *Corallococcus macrosporus* GT-2 for the production of myxochromide (Perlova et al. 2009) and *Streptomyces lividans* for the production of meridamycin (Liu et al. 2009c). An engineered *Streptomyces avermitilis* mutant was developed very recently and used for the heterologous expression of three different biosynthesis pathways, streptomycin, cephamycin C and pladienolide. Another *Streptomyces* mutant was optimized for terpenoid production by introduction of a synthetic gene optimized for *Streptomyces* codon usage, and this mutant was capable

of producing the plant terpenoid intermediate, amorpha-4,11-diene (Komatsua et al. 2010). With respect to Taxol, already in 2001 Huang et al. accomplished in vivo production of the intermediate taxadiene in *E. coli*, and taxadiene and taxadien-5a-ol were produced in yeast by Engels et al. in 2008. As for now, the production of Taxol itself in a heterologous host remains to be fulfilled. Yeast might be a promising host for such attempts, as the plant-derived artemisin has been successfully produced in yeast (Ro et al. 2006). However, there is an on-going debate on the host selection between *E. coli* and yeast for the heterologous production of plant- and fungi-derived compounds. Although yeast might appear more suitable, there is a conflict of the yeast metabolism interacting with, and/or contaminating the expression of the heterologously introduced pathway (Zhang et al. 2011). Another host that could become useful for the heterologous expression of endophytic products is *Aspergillus nidulans*. This host has been tested and used, e.g. for the heterologous production of Monacolin J (a lovastatin intermediate) (Zirkle et al. 2004). As these methodologies develop further, and access to the genome data of endophytic fungi by pyrosequencing becomes available, further and most promising developments within this arena can be expected.

7 Drug Development Life Cycle

The development of anti-infectives is known to take longer than that of the agro-chemicals or industrial enzymes, because these compounds have to undergo three to four clinical studies. The drug development cycle from laboratory to market is long-lasting with the different phases involved, starting from drug discovery (for example, 10,000 compounds), preclinical trials (about 1–2% of the molecules), human clinical trials (2–5% of the molecules), Food and Drug Administration (FDA) or European Agency for the Evaluation of Medicinal Products (EMEA) review, FDA or EMEA approval and post-market clinical trials (Fig. 5). At the moment, the success rate for anti-infective molecules is about 16% for those approved between 1993 and 2004 (DiMasi et al. 2010) and the time to reach the markets varies between 10 and 12 years, depending on the drug. Because antimicrobials have a short-lived nature as drugs, as they normally are taken only for short periods of time and easily develop resistance, the resulting low profit expectancy and, subsequently, a low interest by pharmaceutical companies complicate the efforts of developing completely new drug compounds (Bradley et al. 2007). The pharmaceutical industry is therefore preferably investing in anti-inflammatory, ACE inhibitors, diabetes and anti-cancerous drugs, which are known to generate long-term revenue. Even though pharmaceutical and biotechnology companies are testing increasing numbers of compounds against various targets by high-throughput screening technologies, the question regarding drug development is whether the products can reach the markets in time for procurement of the disease. Regardless, or due to these problems, the efforts to look for safe, novel compounds from the nature should be persistent and continuous.

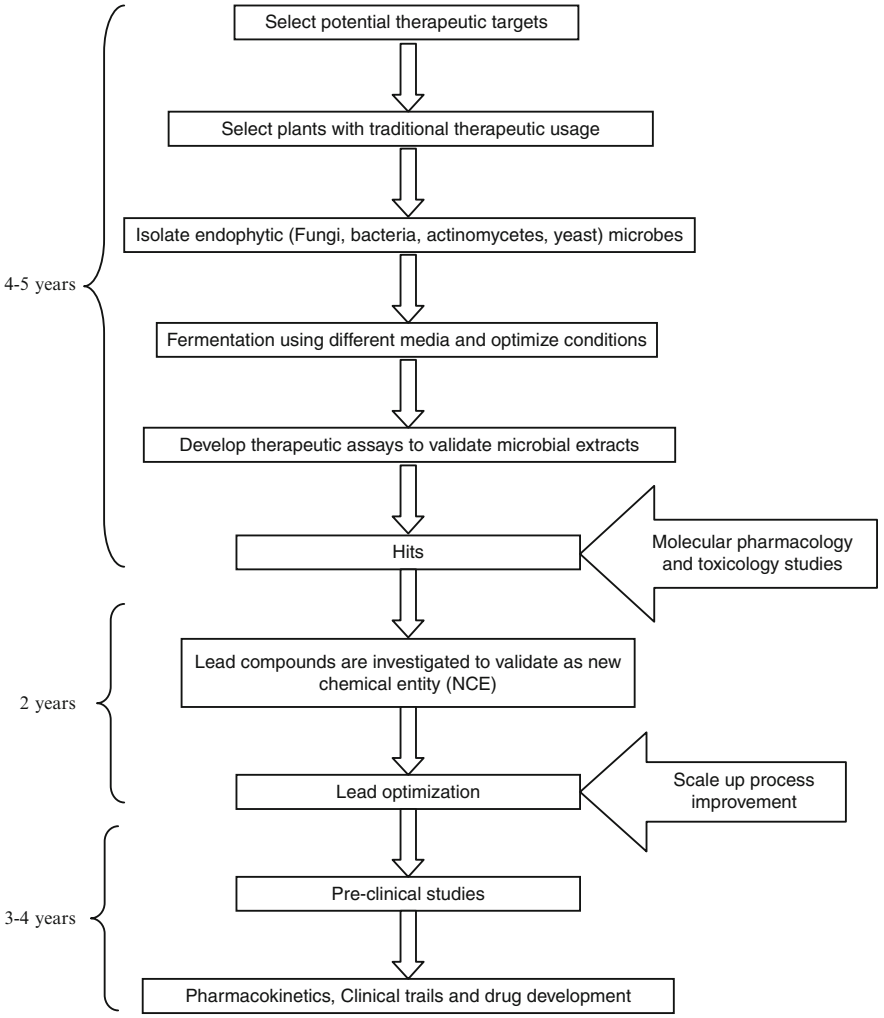


Fig. 5 The endophytes from ethnophamaceutically used plants as a source for new therapeutic leads

8 Conclusion

Endophytic fungi are prolific producers of secondary metabolites and *Pestalotiopsis* species, in particular, are of considerable interest to researchers and pharmacists due to their ability to synthesize a wide range of economically important bioactive molecules. The fermentation and high-throughput screening of a wide array of secondary metabolites with bioassay-guided fraction can yield various new metabolites for various therapeutic targets in the future. The new biotechnological tools have

great promise in enabling the industrial use of endophytic fungi and their products. There is a continuous need for international co-operation to identify and develop antimicrobial drugs to combat various infectious diseases.

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References

Ananda K, Sridhar KR (2002) Diversity of endophytic fungi in the roots of mangrove species on west coast of India. Can J Microbiol 48:871–878

Arnold AE (2008) Endophytic fungi: hidden components of tropical community ecology. In: Schnitzer S, Carson W (eds) Tropical forest community ecology. Blackwell Scientific, London

Arnold AE, Meija LC, Kyllo D et al (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci 100:15649–15654

Baayen RP, Bonants PJM, Verkley G et al (2002) Nonpathogenic isolates of the citrus black spot fungus, *Guignardia citricar*pa, identified as a cosmopolitan endophyte of woody plants, *G. mangiferae* (*Phyllosticta capitalensis*). Phytopathology 92:464–477

Bacon CW, White JF (1994) Stains, media, and procedures for analyzing endophytes. In: Bacon CW, White JF (eds) Biotechnology of endophytic fungi of grasses. CRC, Boca Raton

Bailly J, Fraissinet-Tachet L, Verner M-C et al (2007) Soil eukaryotic functional diversity, a metatranscriptomic approach. ISME J 1:632–642

Balick MJ, Cox PA (1997) Plants, people and culture: the science of ethnobotany. Scientific American Library, New York

Bradley JS, Guidos R, Baragona S et al (2007) Anti-infective research and development – problems, challenges, and solutions. Lancet Infect Dis 7:68–78

Brem D, Leuchtmann A (2001) Epichloe grass endophytes increase herbivore resistance in the woodland grasses *Brachypodium sylvaticum*. Oecologia 126:522–530

Brown KB, Hyde KD, Guest DI (1998) Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Divers 1:27–51

Cannon PF, Simmons CM (2002) Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. Mycologia 94:210–220

Carroll G (1998) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69(1):2–9

Clay K (1998) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10–16

Cragg GM, Newman DJ (2009) Nature: a vital source of leads for anticancer drug development. Phytochem Rev 8:313–331

Deng BW, Liu KH, Chen WQ et al (2009) *Fusarium solani*, Tax-3, a new endophytic taxol-producing fungus from *Taxus chinensis*. World J Microbiol Biot 25:139–143

Deyrup ST, Swenson DC, Gloer JB et al (2006) Caryophyllene sesquiterpenoids from a fungicolous isolate of *Pestalotiopsis disseminate*. J Nat Prod 69:608–611

DiMasi JA, Feldman L, Seckler A et al (2010) Trends in risks associated with new drug development: success rates for investigational drugs. Nature 87:272–277

Ding G, Liu SC, Guo LD et al (2008) Antifungal metabolites from the plant endophytic fungus *Pestalotiopsis foedan*. J Nat Prod 71:615–618

Ding G, Li Y, Fu SB et al (2009) Ambuic acid and torreyanic acid derivatives from the endolichenic fungus *Pestalotiopsis* sp. J Nat Prod 72:182–186

Duong LM, Jeewon R, Lumyong S et al (2006) DGGE coupled with ribosomal DNA phylogenies reveal uncharacterized fungal phylotypes on living leaves of *Magnolia liliifera*. Fungal Divers 23:121–138

Engels B, Dahm P, Jennewein S (2008) Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production. Metab Eng 10:201–206

Frohlich J, Hyde KD (1999) Biodiversity of palm fungi in the tropics: are global fungal diversity estimates realistic? Biodivers Conserv 8:977–1004

Frohlich J, Hyde KD, Petrini O (2000) Endophytic fungi associated with palms. Mycol Res 104:1202–1212

Fu J, Wenzel SC, Perlova O et al (2008) Efficient transfer of two large secondary metabolite pathway gene clusters into heterologous hosts by transposition. Nucleic Acids Res 36:e113

Germida JJ, Siciliano SD, de Freitas R et al (1998) Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus*) and wheat (*Triticum aestivum*). FEMS Microbiol Ecol 26:43–50

Govaerts R (2001) How many species of seed plants are there? Taxon 50:1085–1090

Grabley S, Sattler I (2003) Natural products for lead identification: nature is a valuable resource for providing tools. In: Hillisch A, Hilgenfeld R (eds) Modern method of drug discovery. Birkhauser Verlag, Switzerland

Grabley S, Thiericke R (1999) Bioactive agents from natural sources: trends in discovery and application. In: Scheper T (ed) Advances in biochemical engineering/biotechnology. Springer-Verlag, Berlin-Heidelberg

Green D, Keller M (2006) Capturing the uncultivated majority. Curr Opin Biotechnol 17:236–240

Groppe K, Steinger T, Sanders I (1999) Interaction between the endophytic fungus *Epichloë bromicola* and the grass *Bromus erectus*: effects of endophyte infection, fungal concentration and environment on grass growth and flowering. Mol Ecol 8(11):1827–1835

Guo LD, Hyde KD, Liew ECY (2000) Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. Mol Phylogenet Evol 20:1–13

Guo LD, Huang GR, Wang Y et al (2003) Molecular identification of endophytic fungi from *Pinus tabulaeformis*. Mycol Res 107:680–688

Gutierrez-Zamora ML, Martinez-Romero E (2001) Natural endophytic association between *Rhizobium* and maize (*Zea mays* L). J Biotechnol 91(2):117–126

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685

Harper JK, Arif AM, Ford EJ et al (2003) Pestacin: a 1, 3-dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. Tetrahedron 59:2471–2476

Hoff JA, Klopfenstein NB, McDonald GI (2004) Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). For Pathol 34:255–271

Huang QL, Roessner CA, Croteau R, Scott AI (2001) Engineering *Escherichia coli* for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol. Bioorg Med Chem 9:2237–2242

Hugonnet JE, Tremblay LW, Boshoff HI et al (2009) Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis*. Science 323:1215–1218

Imada C, Koseki N, Kamata M et al (2007) Isolation and characterization of antibacterial substances produced by marine actinomycetes in the presence of seawater. Actinomycetologica 21:27–31

Jennings DH, Lysek G (1996) Endophytic fungi: hidden components of tropical community ecology. In: Schnitzer S, Carson W (eds) Fungal biology: understanding the fungal lifestyle. BIOS Scientific Publishers Ltd, Guildford, UK, 146 p

Ji Y, Bi J-N, Yan B et al (2006) Taxol-producing fungi: a new approach to industrial production of taxol Chinesenese. J Biotechnol 22:1–6

Kinghorn AD (2001) Pharmacognosy in the 21st century. J Pharm Pharmacol 53:135–148

Komatsua M, Uchiyama T, Omura S et al (2010) Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism. Proc Natl Acad Sci USA 107:2646–2651

Kumar SS, Cheung HY, Lau CS et al (2004) In vitro studies of endophytic fungi from *Tripterygium wilfordii* with anti-proliferative activity on human peripheral blood mononuclear cells. J Ethnopharmacol 94:295–300

Kumarasamy KK, Toleman MA, Walsh TR et al (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10:597–602

Kumaresan V, Suryanarayanan TS (2002) Endophyte assemblages in young, mature and senescent leaves of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove litter degradation. Fungal Divers 9:81–91

Lee JC, Yang XS, Schwartz M et al (1995) The relationship between the rarest tree in North America and an endophytic fungus. Chem Biol 2:721–727

Li JY, Strobel GA (2001) Jesterone and hydroxyjesterone antioomycete cyclohexenone epoxides from the endophytic fungus *Pestalotiopsis jester*. Phytochemistry 57:261–265

Li JY, Harper JK, Grant DM et al (2001) Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* sp. and *Monochaetia* sp. Phytochemistry 56:463–468

Li EW, Jiang LH, Guo LD et al (2008) Pestalachlorides A–C, antifungal metabolites from the plant endophytic fungus *Pestalotiopsis adusta*. Bioorg Med Chem 16:7894–7899

Liu H, Jiang H, Haltli B (2009a) Rapid cloning and heterologous expression of the meridamycin biosynthetic gene cluster using a versatile *Escherichia coli*-streptomyces artificial chromosome vector, pSBAC. J Nat Prod 72:389–95

Liu L, Liu S, Niu S et al (2009b) Isoprenylated chromone derivatives from the plant endophytic fungus *Pestalotiopsis fici*. J Nat Prod 72:1482–1486

Liu L, Tian RR, Liu SC et al (2009c) Pestalofones A–E, bioactive cyclohexanone derivatives from the plant endophytic fungus *Pestalotiopsis fici*. Bioorg Med Chem 17:606–613

Mwangi MM, Wu SW, Zhou Y et al (2007) Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by wholegenome sequencing. Proc Natl Acad Sci USA 104:9451–6

Nakamura H, Iitaka Y, Kitahara T et al (1977) Structure of aplasmomycin. J Antibiot 30:714–719

Nikolcheva LG, Bärlocher F (2005) Molecular approaches to estimate fungal diversity. I. Terminal Restriction Fragment Length Polymorphism (T-RFLP). In: Graça MAS, Bärlocher F, Gessner MO (eds), Methods to study litter decomposition. Springer, Berlin/New York, pp 169–175

Okane I, Nagagiri A, Ito T (1998) Endophytic fungi in leaves of ericaceous plants. Can J Bot 76:657–663

Pelaez F, Collado J, Arenal F et al (1998) Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. Mycol Res 102:755–761

Penalva MA, Rowlands RT, Turner G (1998) The optimization of penicillin biosynthesis in fungi. Trends Biotechnol 16:483–489

Perlova O, Gerth K, Kuhlmann S et al (2009) Novel expression hosts for complex secondary metabolite megasynthetases: Production of myxochromide in the thermopilic isolate *Corallococcus macrosporus* GT-2. Microb Cell Fact 8:1

Petrini O (1986) Taxonomy of endophytic fungi in aerial plant tissues. In: Fokkoema NJ, Van den Huevel J (eds) Microbiology of the phyllosphere. Cambridge University Press, Cambridge, pp 175–187

Pfeifer BA, Admiraal SJ, Gramajo H et al (2001) Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli*. Science 291:1790–1792

Photita W, Lumyong S, Lumyong P et al (2004) Are some endophytes of *Musa acuminata* latent pathogens? Fungal Divers 16:131–140

Piepersberg W (1994) Pathway engineering in secondary metabolites-producing actinomycetes. Crit Rev Biotechnol 14:251–285

Pirttilä AM, Laukkanen H, Pospiech H et al (2000) Detection of intracellular bacteria in the buds of Scotch pine (*Pinus sylvestris* L) by in situ hybridization. Appl Environ Microbiol 66:3073–3077

Pirttilä AM, Pospiech H, Laukkanen H et al (2003) Two endophytic fungi in different tissues of Scots pine buds (*Pinus sylvestris* L). Microb Ecol 45:53–62

Provorov NA, Yu A, Borisov et al (2002) Developmental genetics and evolution of symbiotic structures in nitrogen-fixing nodules and arbuscular mycorrhiza. J Theor Biol 214:215–232

Ragazzi A, Moricca S, Capretti P (2001) Endophytic fungi in *Quercus cerris*: isolation frequency in relation to phenological phase, tree health and the organ affected. Phytopathol Mediterr 40:165–171

Richman DD, Morton SC, Wrin T et al (2004) The prevalence of antiretroviral drug resistance in the United States. AIDS 18:1393–1401

Richter SS, Heilmann KP, Dohrn CL et al (2009) Changing epidemiology of antimicrobial-resistant *Streptococcus pneumoniae* in the United States, 2004–2005. Clin Infect Dis 48:e23–33

Ro DK, Paradise EM, Ouellet M et al (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440:940–943

Rodrigues KF, Samuels GJ (1999) Fungal endophytes of *Spondias mombin* leaves in Brazil. J Basic Microbiol 39:131–135

Saikkonen K, Faeth SH, Helander M et al (1998) Fungal endophytes: a continuum of interaction with host plants. Annu Rev Ecol Syst 29:319–343

Saikkonen K, Ahlholm J, Helander M et al (2000) Endophytic fungi in wild and cultivated grasses in Finland. Ecography 23:346–352

Saikkonen K, Wäli P, Helander M (2004) Evolution of endophyte-plant symbioses. Trends Plant Sci 9:275–280

Samuelsson G (2004) Drugs of natural origin: a textbook of pharmacognosy, 5th edn. Sweedish Pharmaceutical Press, Stockholm, 620 p

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues. Inter departmental working group on biological diversity for food and agriculture (FAO), Rome

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686

Schulz BU, Wanke U, Drager S et al (1993) Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycol Res 97:1447–1450

Schulz B, Sucker J, Aust HJ (1995) Biologically active secondary metabolites of endophytic Pezicula species. Mycol Res 99:1007–1015

Schulz B, Guske S, Dammann U et al (1998) Endophyte host interactions II. Defining symbiosis of the endophyte-host interaction. Symbiosis 25:213–227

Seena S, Wynberg N, Bärlocher F (2008) Fungal diversity during leaf decomposition in a stream assessed through clone libraries. Fungal Divers 30:1–14

Siegel MR, Latch GCM, Johnson MC (1987) Fungal endophytes of grasses. Annu Rev Phytopathol 25:293–315

Stierle AA, Stierle DB (2005) Bioprospecting in the Berkeley pit: bio active metabolites from acid mine waste extremophiles. Stud Nat Prod Chem 32:1123–1175

Stierle A, Strobel GA, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*. Science 260:214–216

Strobel GA (2002) Rainforest endophytes and bioactive products. Crit Rev Biotechnol 22:315–333

Strobel G (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544

Strobel G (2007) Plant associated microorganisms (endophytes) as a new source of bioactive natural products. In: Kayser O, Quax WJ (eds) Medical plant biotechnology. Wiley–VCH Verlag, Weinheim

Strobel GA, Hess WM, Ford E (1996a) Taxol from fungal endophytes and the issue of biodiversity. J Ind Microbiol Biot 17(5/6):417–423

Strobel GA, Yang X, Sears J (1996b) Taxol from *Pestalotiopsis microspora* of *Taxus wallachiana*. Microbiology 142:435–440

Strobel GA, Ford E, Worapong J et al (2002) Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. Phytochemistry 60:179–183

Strobel G, Daisy B, Castillo U et al (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Sturz A, Cristic BR, Matheson BG (1998) Associations of bacterial endophytes population from red clover and potato crops with potential for beneficial allelopathy. Can J Microbiol 44:162–167

Suryanarayanan TS, Kumaresan V (2000) Endophytic fungi of some halophytes from an estuarine mangrove forest. Mycol Res 104:1465–1467
Suryanarayanan TS, Kumaresan V, Johnson JA (1998) Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Can J Microbiol 44:1003–1006
Suryanarayanan TS, Venkatesan G, Murali TS (2003) Endophytic fungal communities in leaves of tropical forest trees: diversity and distribution patterns. Curr Sci India 85:489–493
Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459
Taylor JE, Hyde KD, Jones EBG (1999) Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei*, within and outside its geographical range. New Phytol 142:335–346
Tejesvi MV, Mahesh B, Nalini MS et al (2005) Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W and A (Combretaceae). World J Microbiol Biotechnol 21:1535–1540
Tejesvi MV, Mahesh B, Nalini MS et al (2006) Fungal endophyte assemblages from ethnopharmaceutically important medicinal trees. Can J Microbiol 52:427–435
Tejesvi MV, Kini KR, Prakash HS et al (2007) Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. Fungal Divers 24:37–54
Tejesvi MV, Tamhankar SA, Kini KR et al (2009) Phylogenetic analysis of endophytic *Pestalotiopsis* species from ethnopharmaceutically important medicinal trees. Fungal Divers 38:167–183
Tejesvi MV, Ruotsalainen AL, Markkola AM et al (2010) Root fungal endophytes along a primary succession gradient in northern Finland. Fungal Divers 41:125–134
Tomita F (2003) Endophytes in Southeast Asia and Japan: their taxonomic diversity and potential applications. Fungal Divers 14:187–204
Toofanee SB, Dulymamode R (2002) Fungal endophytes associated with *Cordemoya integrefolia*. Fungal Divers 11:169–175
Uniyal SK, Singh KN, Jamwal P et al (2006) Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J Ethnobiol Ethnomed 2:1–14
Verdine GL (1996) The combinatorial chemistry of nature. Nature 384:11–13
Watanabe K (2008) Exploring the biosynthesis of natural products and their inherent suitability for the rational design of desirable compounds through genetic engineering. Biosci Biotechnol Biochem 72:2491–2506
Wenzel SC, Muller R (2005) Recent developments towards the heterologous expression of complex bacterial natural product biosynthetic pathways. Curr Opin Biotechnol 16:594–606
Williams RB, Henrikson JC, Hoover AR et al (2008) Epigenetic remodeling of the fungal secondary metabolome. Org Biomol Chem 6:1895–1897
World Health Organisation (WHO) (1991) Traditional Medicine and Modern Health Care, Progress Report by the Director General, Forty Fourth World Health assembly, A44/19
Xu J, Ebada SS, Proksch P (2010) *Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites. Fungal Divers 44:15–31
Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771
Zhang HR, Boghigian BA, Armando J et al (2011) Methods and options for the heterologous production of complex natural products. Nat Prod Rep 28:125–151
Zhao K, Ping WX, Ma X et al (2005) Breeding of high yield strain of taxol by mutagenesis of protoplast and primary discussion of genetic differences between mutants and their parent strain. Acta Microbiol Sin 45:355–358
Zhou DP, Zhao K, Ping WX et al (2005) Study on the mutagensis of protoplasts from taxol-producing fungus *Nodulisporium sylviforme*. J Am Sci 1:55–62
Zirkle R, Ligon JM, Molnar I (2004) Heterologous production of the antifungal polyketide antibiotic soraphen A of *Sorangium cellulosum* So ce26 in *Streptomyces lividans*. Microbiology 150:2761–2774