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Review

Fungal endophytes and bioprospecting

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ABSTRACT

Horizontally transmitted fungal endophytes are an ecological group of fungi, mostly belonging to the Ascomycota, that reside in the aerial tissues and roots of plants without inducing any visual symptoms of their presence. These fungi appear to have a capacity to produce an array of secondary metabolites exhibiting a variety of biological activity. Although the ability of fungi to produce unique bioactive metabolites is well known, endophytes have not been exploited, perhaps because we are only beginning to understand their distribution and biology. This review emphasizes the need to routinely include endophytic fungi in the screening of organisms for bioactive metabolites and novel drugs; it also underscores the need to use information obtained concerning fungal secondary metabolite production from other groups of fungi for a targeted screening approach.

1. Introduction

For several years, natural products have been used directly as drugs or have provided the basic chemical architecture for deriving such drugs. There are at least 200,000 natural metabolites with bioactive properties (Bérdy, 2005). For instance, about 52 % of the new chemicals introduced into the market worldwide between 1981 and 2002 were natural products or their derivatives (Chin *et al.*, 2006). Besides plants, microorganisms constitute a major source of natural products with desirable bioactive properties. More than 20,000 bioactive metabolites of microbial origin were known by the end of 2002 (Bérdy, 2005). Fungi are among the most important groups of eukaryotic organisms that are being explored for metabolites for clinical applications. Existing drugs of fungal origin include β-lactam antibiotics, griseofulvin, cyclosporine A, taxol, ergot alkaloids, and lovastatin. More new natural products of varied chemical structures are continually being reported from fungi (Grabley and Sattler, 2003; Mitchell *et al.*, 2008; Stadler and Keller, 2008). The versatile synthetic capability of fungi reflects their heterotrophic and absorptive mode of nutrition and the ability to exploit a variety of substrates and habitats (Hyde, 2005; Suryanarayanan and Hawksworth, 2005). We know only about 7 % of the estimated 1.5 million species of fungi (Hawksworth, 2004), and only very few of these have been cultivated and screened for drug production. It is therefore logical to postulate that we have only discovered a small

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percentage of economically important metabolites of fungal origin.

2. Products of pharmaceutical importance from fungi

Bacteria and fungi are well known for producing many novel chemicals that are directly used as drugs or function as lead structures for synthetic modifications (e.g. Kock *et al.*, 2001; Bode *et al.*, 2002; Donadio *et al.*, 2002; Chin *et al.*, 2006; Gunatilaka, 2006; Lam, 2006; Mitchell *et al.*, 2008; Stadler and Keller, 2008). Although mycophenolic acid, the first fungal secondary metabolite was obtained from *Penicillium glaucoma* as early as 1896 (see Bérdy, 2005), it was the tremendous success of penicillin as an antibiotic in the early 1940s that shifted the focus of natural product-based drug sources from plants to microorganisms. In the 5 y from 2000 to 2005, 23 novel drugs obtained from plants and microorganisms for treating various human disorders such as cancer, neurological disorders, infectious and cardiovascular diseases, metabolic and immunological diseases and genetic disorders were brought to the market (Chin *et al.*, 2006). In the 9 y from 1993, about 1500 metabolites have been reported from fungi to have anti-tumor or antibiotic activity (Peláez, 2005). Some of the recently approved drugs of fungal origin are micafungin, an anti-fungal metabolite from *Coleophoma empetri* (Frattarelli *et al.*, 2004), mycophenolate, a product of *Penicillium brevicompactum* used for preventing renal transplant rejection (Curran and Keating, 2005), rosuvastatin from *Penicillium citrinum* and *P. brevicompactum* used for treating dyslipidemias (Scott *et al.*, 2004) and cefditoren pivoxil, a broad spectrum antibiotic derived from *Cephalosporium* sp. (Darkes and Plosker, 2002). Derivatives of fumagillin, an antibiotic produced by *Aspergillus fumigatus* (Chun *et al.*, 2005), and illudin-S, a sesquiterpenoid from *Omphalotus illudens* (McMorris *et al.*, 1996) exhibit anti-cancer activities. Fungal metabolites find important applications in agriculture as well (Anke and Thines, 2007). It is pertinent to mention that, among the microfungi, only certain genera such as *Aspergillus* and *Penicillium* have been rigorously screened for bioactive compounds; of the 6450 bioactive metabolites from microfungi, more than 30 % are obtained from these two genera (Bérdy, 2005).

3. Horizontally transferred fungal endophytes and bioprospecting

Horizontally transmitted fungal endophytes are an ecological group of fungi mostly belonging to the Ascomycota that reside in the aerial tissues and roots of plants without inducing any visual symptoms of their presence (Sánchez-Márquez *et al.*, 2007; Oses *et al.*, 2008; Rungjindamai *et al.*, 2008; Tao *et al.*, 2008). Although the existence of symptomless fungi inside plant tissues has long been recognized [the term endophyte was coined by de Bary (1866)], it is only recently that horizontally spread fungal endophytes have been studied in detail by mycologists, ecologists and plant pathologists (Arnold, 2007; Sieber, 2007; Saikkonen, 2007; Hyde and Soytong, 2008). The term 'endophyte' has been variously defined (see Hyde and Soytong, 2008), all of which are variations of the central theme that the endophytes survive in living tissues of plants for a short or prolonged period without producing any visible symptoms. Endophytes have a cryptic existence and their main role in the ecosystem appears to be that of decomposers, as they are among the primary colonizers of dead plant tissues (Kumaresan and Suryanarayanan, 2002; Hyde and Soytong, 2008; Oses *et al.*, 2008). There is some evidence to suggest that a few endophytes protect plants against diseases (Arnold *et al.*, 2003), ward off insect pests (Akello *et al.*, 2007), and increase the fitness of plants by enhancing their tolerance to abiotic stress (Redman *et al.*, 2002; Bae *et al.*, 2008); endophyte infection can also impose a significant cost on the host plant (Arnold and Engelbrecht, 2007). For a more detailed account of different aspects of fungal endophyte biology, refer to Arnold (2007), Saikkonen (2007), Sieber (2007), and Hyde and Soytong (2008).

There are a few studies on endophytes as sources of natural products of pharmaceutical and agricultural importance (e.g Tan and Zou, 2001; Gunatilaka, 2006). We suggest that horizontally transmitted cryptic fungal symbionts (namely the endophytes of non-grass plants) should to be screened for biologically active compounds with the intensity with which the soil fungi have been previously investigated (Tan and Zou, 2001; Smith *et al.*, 2008). Endophytic fungi appear to be metabolically more innovative than soil fungi (Schulz *et al.*, 2002) or fungi associated with algae (Schulz *et al.*, 2008) with regard to bioactive compounds. That they produce unique bioactive metabolites is well known (Mitchell *et al.*, 2008; Stadler and Keller, 2008). This is perhaps an outcome of their constant need to interact with the host milieu. Endophyte–plant host interactions are different from pathogen–plant host interactions since neither associate really 'wins'; neither disease symptoms develop on the plant host nor is the fungus eliminated by the plant host (Pinto *et al.*, 2000; Schulz *et al.*, 2002; Stone *et al.*, 2004; Sieber, 2007; Saikkonen, 2007). This situation entails sustained and prolonged reactions against the defense mechanisms of the host by the endophyte and this could act as selection pressure for developing novel metabolic pathways – a potentially beneficial situation for bio-prospectors (Calhoun *et al.*, 1992; Schulz *et al.*, 1995; Lu *et al.*, 2000; Wang *et al.*, 2000; Tan and Zou, 2001; Weber *et al.*, 2007).

Although the advent of combinatorial chemistry has shifted the research focus away from natural products, fungal endophytes continue to be a source for novel drugs (Strobel and Daisy, 2003; Wang *et al.*, 2004; Bérdy, 2005; Mitchell *et al.*, 2008; Sappapan *et al.*, 2008; Wang *et al.*, 2008); they produce an array of metabolites of varied structural groups such as terpenoids, steroids, xanthones, chinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasines and enniatines (Schulz *et al.*, 2002). The metabolites of endophytic fungi include antibacterial, anti-viral, anti-fungal and anti-cancer activities (Gunatilaka, 2006). Some of the metabolites are xanthine oxidase inhibitors toxic to brine shrimps, eosinophil inhibitors, acetylcholinesterase inhibitors, β-glucuronidase inhibitors, insecticides, root growth accelerators, anti-inflammatory agents and insulin receptor activators (Gunatilaka, 2006). Fungal endophytes associated with higher plants appear to be a good source of novel anti-oxidants as well (Huang *et al.*, 2007, 2008).

A few endophytes have been screened for novel antibiotics (Li *et al.*, 1996; Zou *et al.*, 2000; Bérdy, 2005; Shiono *et al.*, 2005; Silva *et al.*, 2005; Gu *et al.*, 2007; Phongpaichit *et al.*, 2007; Lösgen *et al.*, 2008). There are reports of anti-cancer chemicals from endophytes (Firáková *et al.*, 2007) such as paclitaxel (Stierle *et al.*, 1993), Hsp 90 inhibitors (Turbyville *et al.*, 2006), sequoiatones A and B (Stierle *et al.*, 1999) and camptothecin (Amna *et al.*, 2006). Novel compounds of endophyte origin include new lactones (Chen *et al.*, 2003), and enalin derivative (Hormazabal *et al.*, 2005), colletotrichic acid (Zou *et al.*, 2000), myrocin A and apiosporic acid (Klemke *et al.*, 2004), phomopsilactone (Silva *et al.*, 2005), cyclopentanoids (Teuscher *et al.*, 2006), and (+)-ascochin and (+)-ascodiketone (Krohn *et al.*, 2007), chaetocyclinones (Lösgen *et al.*, 2007), pestalotheols A–D (Li *et al.*, 2008), isofusidienols (Lösgen *et al.*, 2008) and naphthoquinone spiroketals (Macías-Rubalcava *et al.*, 2008).

The presence of endophytes in plant tissues can influence the volatiles produced by the plant (Mucciarelli *et al.*, 2007). Jallow *et al.* (2008) reported that *Acremonium strictum*, an unspecialized root endophyte, modifies the volatile profile of the host plant thereby influencing host selection and oviposition behaviour of a polyphagous moth. Jimenez-Romero *et al.* (2008) isolated lactones from endophytic *Xylaria* sp. with potential to function as leads for anti-malarial drugs. Apart from elaborating novel bioactive chemicals, endophytes can also bring about stereoselective biotransformations of chemicals, thus aiding in drug modifications (Borges *et al.*, 2007). Endophytes of tropical plants, constitute a species rich ecological assemblage of fungi (Arnold and Lutzoni, 2007; Huang *et al.*, 2008) and considering the synthetic potential of endophytes, they should be included in any program that aims at screening of fungi for novel metabolites.

Our experience with fungal endophytes isolated mostly from dicotyledonous trees of different types of forests of southern Western Ghats also suggests that endophytes are a promising source of bioactive compounds. We screened fungal endophytes isolated from 55 dicotyledonous trees belonging to 29 families and growing in dry deciduous, dry thorn, moist deciduous or evergreen forests of the Nilgiri Biosphere Reserve, Western Ghats for bioactive metabolites. The endophytes were grown as static cultures in Potato Dextrose broth for 20 d at 27 °C. Secondary metabolites from the culture filtrates were obtained by solvent extraction and tested for their effects on seed germination and plant cell division, and for their anti-algal, anti-fungal and anti-insect properties. Of the 107 isolates tested on rice seeds, 40 %–50 % inhibited and promoted root growth, respectively; nearly 98 % of the isolates reduced the plumule growth (Fig 1a, b). About 95 % of the 44 isolates screened reduced the mitotic index in onion root meristem while 7 % increased the mitotic

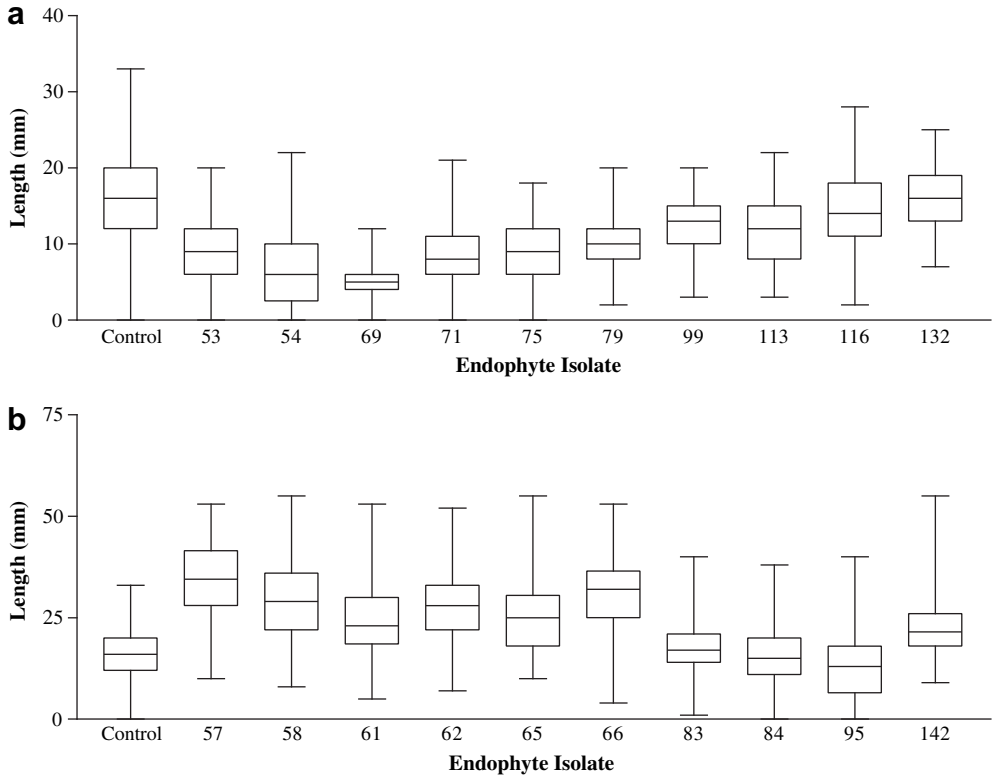

Fig. 1 – (a) Box-Whisker plots comparing the rank medians of the length of radicle of germinating rice grains (ADT 43) treated with culture filtrates of select endophytes. Bars below and above the boxes indicate minimum and maximum values. The bottom and top horizontal lines of a box represent the 25 and 75 percentile values with the median value indicated by a line within a box. (b) Box-Whisker plots comparing the rank medians of the length of radicle of germinating rice grains (ADT 43) treated with culture filtrates of select endophytes. Bars below and above the boxes indicate minimum and maximum values. The bottom and top horizontal lines of a box represent the 25 and 75 percentile values with the median value indicated by a line within a box.

index (Fig 2). Culture filtrate extracts of 56 % of the 145 endophyte isolates showed insecticidal activity. Similarly, 15 %–37 % of the 148 isolates showed anti-fungal and anti-algal activity, respectively.

We screened 110 of these fungal endophyte isolates for their anti-cancer activity by treating cells of mouse fibroblast cell line L-929 with organic solvent extracts of the culture filtrates. Cytotoxic effects of the fractions of the culture filtrates were assessed by MTT colorimetric assay (Mosmann, 1983). Subsequently, the extracts showing activity were fractionated by analytical RP HPLC and all the fractions (each 0.5 min) were tested for cytotoxic effects. Furthermore, the effects of the active fractions on nuclear morphology, cell division, actin microfilaments and endomembrane system of PtK_2 potoroo cells were studied using immunofluorescence techniques. These bioassays revealed that some of the endophytes produced metabolites that impair cell division. Active extracts were further analyzed by HPLC-MS. Biological and analytical information together with information from databases (DNP on CD-ROM) helped to identify known compounds. Notable among the active endophytes was a *Chaetomium* sp. that produced chaetoglobosins, which are cytochalasin analogs that inhibit actin polymerization (Yahara *et al.*, 1982) (Fig 3). *Chaetomium* is a genus known to produce different types of secondary metabolites including chaetomin, chaetoglobosins, chaetoquadrins, oxaspirodion, chaetospiron, orsellides and chaetocyclinones (Lösgen *et al.*, 2007). A *Phomopsis* sp. produced phomopsolide A and B which have been reported to act as boring and feeding deterrents of elm bark beetles (Grove, 1985). An *Alternaria* endophyte elaborated several solanapyrones (Table 1 and Fig 4). These phytotoxic compounds are inhibitors of DNA polymerases (Mizushina *et al.*, 2002). Endophytic *Nigrospora oryzae* produced aphidicolin (3α, 16β, 17, 18-Aphidicolianetetrol – Fig 4) and several of its derivatives, nigrosporolide, phomalactone, bostrycin and epoxyexserohilone. Aphidicolin is a tetracyclic diterpene-tetraol and an inhibitor of nuclear DNA synthesis in eukaryotes (Spadari *et al.*, 1982). It inhibits DNA synthesis by interfering with DNA polymerase α enzyme. Ikegami *et al.* (1979) reported that this metabolite inhibits the S phase of the cell cycle. Nigrosporolide is a 14 membered lactone which inhibits plant growth and was first reported from *Nigrospora sphaerica* (Harwooda *et al.*, 1995). Furthermore, one of our *Fusarium* isolates produced apicidin which is a histone deacetylase inhibitor that inhibits cell division (Han *et al.*, 2000), and enniatins which are known to function as inhibitors of the yeast transporter protein Pdr5p (Hiraga *et al.*, 2005).

Our study with 150 endophyte isolates using different bioassays showed that certain genera such as *Alternaria*, *Chaetomium*, *Colletotrichum*, *Curvularia*, *Nigrospora* and *Xylaria* produce a larger number of bioactive compounds.

4. Plant–endophyte interactions affect metabolite production

Our knowledge of interaction of horizontally transmitted fungal endophytes with their plant host at any level, be it structural, physiological or gene level, is, at best, unclear. The endophytes are likely to adopt the same strategy as the plant pathogenic fungi in order to enter a plant host (Chapela *et al.*, 1993; also see Sieber, 2007 and Stone and Petrini, 1997). Even such a generalization has to be made with some caution: in the case of direct-penetrating biotrophic fungal pathogens, the epidermal surface of the host is the first line of defense explaining to a certain extent host-specificity among them (Viret *et al.*, 1994; Heath, 2002; Valkama *et al.*, 2005). Even though both endophytes and biotrophs derive nutrients from their hosts without killing them, many endophytes such as *Phomopsis*, *Phoma*, *Colletotrichum* and *Phyllosticta* have a wide host range and colonize several taxonomically unrelated plant hosts (Pandey *et al.*, 2003; Jeewon *et al.*, 2004; Murali *et al.*, 2006; Sieber, 2007) suggesting that they have developed adaptations to overcome different types of host defences. Apart from the balanced antagonism hypothesis proposed by Schulz *et al.* (1999) attempting to explain the type of interaction between fungal endophytes and their plant hosts, there is little information on the post-penetrative stages of endophyte–host interaction. Abang *et al.* (2009) showed that the asymptomatic endophytic *Colletotrichum gloeosporioides* is unable to produce a toxic metabolite which the pathogenic strains produced and induced disease symptoms in the plant host. It is not clear if several post penetration defense reactions of the hosts such as programmed cell death, induction of phytoalexins and pathogenesis related proteins observed for pathogen–host interactions (De Lorenzo and Ferrari,

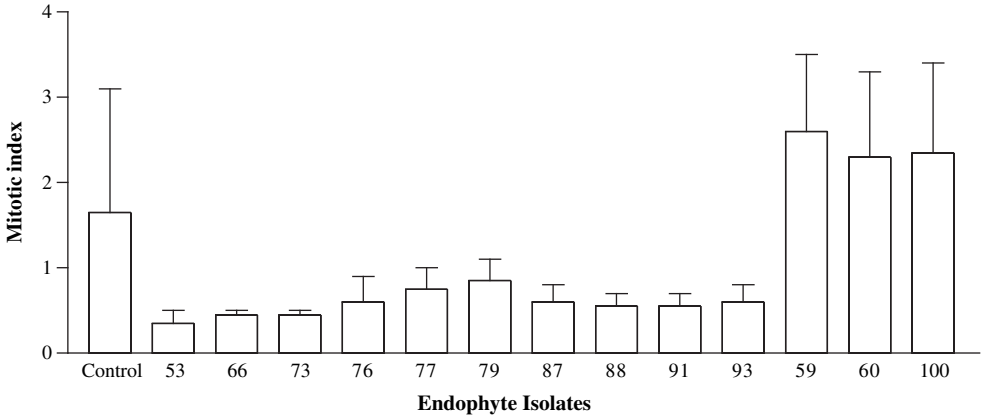

Fig. 2 – Effect of endophyte secondary metabolites on cell division in onion root meristem (bars represent standard deviation).

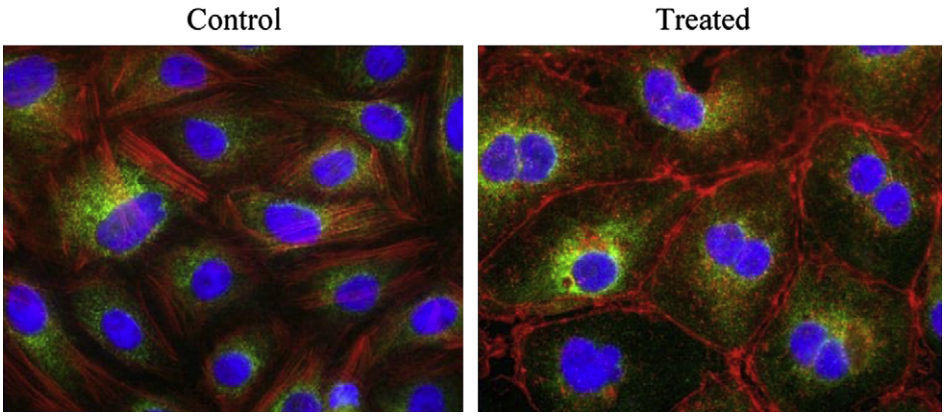

Fig. 3 – Effect of culture extract from endophytic *Chaetomium* sp. on potoroo kidney cell line PtK2. Note cell enlargement, loss of actin fibres and failure of cell division after nuclear division. (Nucleus stained with blue fluorescent dye and actin with red fluorescent dye).

2002) are also operative in endophyte–host interactions (Sieber, 2007). The observation of Moricca and Ragazzi (2008) indicates that the type of interaction between an endophyte and a plant is controlled by the genes of both organisms and modulated by the environment. A benign endophyte residing in the host tissue in a symptomless state or one that may be beneficial to its host may turn into a pathogen in response to some environmental cue (Hendry *et al.*, 2002). It is logical to assume that such a shift in the nature of the endophyte would also result in a change in its metabolite profile. Pathogenic fungi can reside as symptomless endophytes in plant tissues (Kuldau and Yates, 2000; Vettraino *et al.*, 2005; Suryanarayanan and Murali, 2006); futhermore, the species diversity of foliar endophyte assemblage is known to change with the leaf age (Stone, 1987; Okane *et al.*, 1998; Taylor *et al.*, 1999; Photita *et al.*, 2004; Suryanarayanan and Thennarasan, 2004). These facts indicate that sampling endophytes from a plant community for bioprospecting on a single occasion may not capture the entire spectrum of endophytes and their metabolites. The effort has to be sustained to obtain the whole extent of secondary metabolites.

The endophyte may be in a metabolically aggressive environment constantly encountering host defense chemicals (Cabral *et al.*, 1993; Peters *et al.*, 1998; Schulz *et al.*, 1999). Such a hostile environment may account for the evolution of the potentially increased synthetic ability of the endophytes. This perhaps explains the apparent anomaly observed when a species of endophyte isolated from a plant host produces a bioactive compound but fails to do so when isolated from another plant species (Li *et al.*, 1996). We have found that the herbicidal activity of secondary metabolite(s) of an endophytic *Phyllosticta capitalensis* differed with the plant host from which the endophyte was isolated. This probably means that the plant host (and ultimately its metabolism) influences the synthetic ability of an endophyte. This indicates that bioprospecting for endophyte natural products should be host plant based as opposed to fungal taxon based. Strobel and Daisy (2003) emphasize this in their review while stating that 'plants from unique environmental settings' and 'plants with an unconventional biology' as well as plants with established ethnobotanic values would be more promising sources of endophytes producing novel biochemicals. Endophytic fungi associated with traditionally used medicinal plants especially of the tropics could be a rich source of functional metabolites (Weber *et al.*, 2004; Tejesvi *et al.*, 2007; Aly *et al.*, 2008; Boonman *et al.*, 2008; Huang *et al.*, 2008; Sappapan *et al.*, 2008). In this regard, the endophyte–plant host association could also be exploited in enhancing the production of useful metabolites by the plant host (Wang *et al.*, 2004). In addition to plants of the tropical rain forests, those growing in harsh habitats such as hot and cold deserts, saline and acidic soils and marine habitats have to be screened for bioactive metabolite-producing endophytes (Raghukumar, 2008; Schulz *et al.*, 2008).

We observed some tissue-specificity but less host taxon specificity in endophytes associated with mangrove trees (Kumaresan *et al.*, 2002). Pang *et al.* (2008) obtained similar results for endophytes of *Kandelia candel* growing in Hong

Table 1 – Some bioactive secondary metabolites isolated from endophytic fungi in our study

Fungus	Chemical list
Phomopsis sp.	Phomopsolide A
	Phomopsolide B
Chaetomium sp.	Chaetoglobosin A
	Chaetoglobosin C
	Chaetoglobosin D
	Chaetoglobosin G
Alternaria alternata	Prosalanapyrone I
	Prosalanapyrone II
	Prosalanapyrone III
	Solanopyrone A, B, C, D, E, F, G
Nigrospora oryzae	Aphidicolin
	Aphidicolene
	Aphidicolaneodiol
	Aphidicolanatriol
	Aphidicolanepentol
Curvularia sp.	Cytochalasin B
	Cytochalasin F
Fusarium sp.	Apicidin
	Enniatin A1
	Enniatin B
	Enniatin E

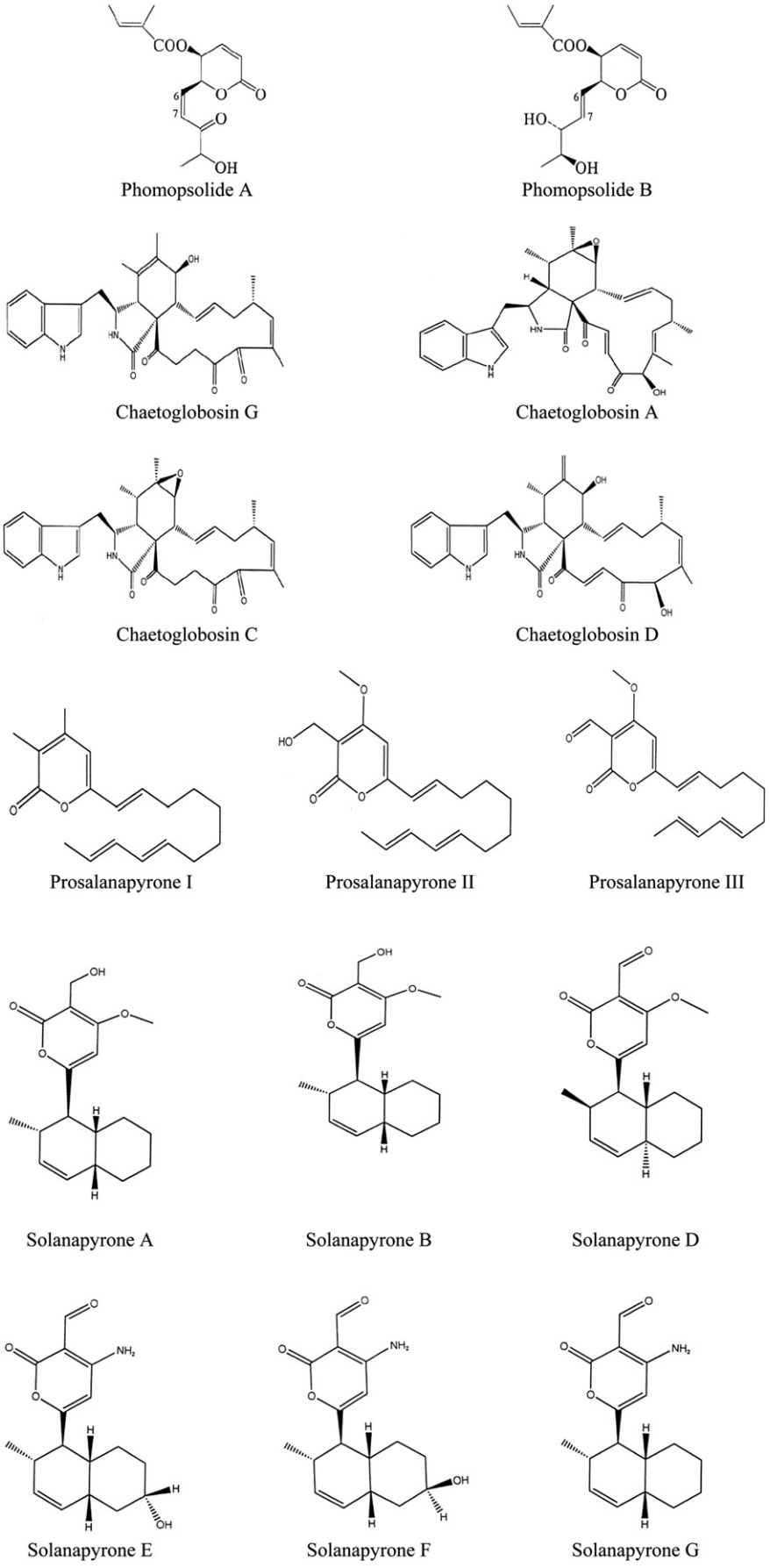

Fig. 4 – Structure of the some bioactive secondary metabolites isolated from endophytic fungi in our study.

Kong mangroves. Endophytes should therefore be screened from different tissues of a host plant rather than targeting endophytes isolated from similar tissue of different plant species (Hyde and Soytong, 2008).

The endophyte–host relationship possibly involves the evolution of strategies and counter strategies by the mutualists. Different species of the horizontally transmitted endophytic fungi cause multiple and discrete infections in a given tissue of a plant such as a leaf (Fisher and Petrini, 1987; Suryanarayanan *et al.*, 1998, 2000, 2002; Arnold *et al.*, 2000; Fröhlich *et al.*, 2000; Gamboa and Bayman, 2001). It is not known if such an interaction of different species of endophytes (or an interaction between endophytes and pathogens) in a given host tissue attenuates or enhances the metabolic capability of one another. The work of Bailey *et al.* (2006) indicates that endophyte infections alter the pattern of gene expression in the host plant. Assays of endophytes in single and multiple infections under *in vitro* conditions (using callus or cell cultures) would provide some insights into this, ultimately leading to devising procedures to obtain better yields of the metabolites of interest (Pirttilä *et al.*, 2004).

Only endophytes associated with angiosperms and gymnosperms have been studied to any extent for novel metabolites. Endophytes of other taxonomic groups of plants such as algae, bryophytes, pteridophytes and even lichens (Suryanarayanan *et al.*, 2005; Zhang *et al.*, 2006; Li *et al.*, 2007) should be included in screening programmes. For example, the non-obligate, asymptomatic fungi occurring within lichen thalli appear to be an untapped source of secondary metabolites (Li *et al.*, 2007; Paranagama *et al.*, 2007). Similarly, the endophytic fungi associated with marine algae also appear to synthesize novel metabolites (Wang *et al.*, 2006a,b; Lösgen *et al.*, 2007; Zhang *et al.*, 2007).

5. Discovering novel chemicals in undiscovered endophytes

Not all endophytes are culturable (Higgins *et al.*, 2006) and these may produce useful metabolites. There are several methods to detect unculturable fungi and these include whole DNA analysis followed by DNA cloning (Guo *et al.*, 2001; Seena *et al.*, 2008), DGGE (Duong *et al.*, 2006; Tao *et al.*, 2008) or T-RLFP (Nikolcheva and Bärlocher, 2004, 2005). Therefore, apart from isolating culturable endophytes from different taxonomic groups of plants and plants growing in different habitats, shotgun metagenomics for endophyte community analysis and function-based screening of their metagenomic libraries could be used to harness the unculturable and truly cryptic endophytes from environmental samples for drug production. Such a metagenomic approach has been quite rewarding with soil samples (Kimura, 2006). Metabolomics of endophyte-infected and endophyte-free plant hosts could reveal junctions in secondary metabolite pathways which may be nudged into synthesizing novel chemical species or lead compounds – another possibility of exploiting these chemodiverse organisms (Jewett *et al.*, 2006). Furthermore, other novel techniques such as radiochemical labeling can be used for detecting products of genes with low expressions among endophytes (Miljkovic *et al.*, 2008). In addition, the biological potential of fungal secondary metabolites could also be fully realized by the application of combinatorial techniques (Pirrung *et al.*, 2006).

In fungi, genes coding for enzymes of secondary metabolic pathways usually occur as gene clusters being situated in the same locus and co-expressed (Keller and Hohn, 1997; Bok *et al.*, 2006). These gene clusters are known to evolve rapidly through multiple rearrangements, duplication and losses, and are capable of interspecific spread through horizontal gene transfer (Khaldi *et al.*, 2008). Such properties are conducive for synthesis of novel chemicals by organisms and provide reason to strengthen the need for targeting fungal endophytes for bioprospecting. It is important to screen fungal species for their secondary metabolite spectrum under different growth conditions; culture parameters such as composition of growth medium, aeration, pH and the presence of certain enzyme inhibitors change dramatically the secondary metabolite profile and even induce the synthesis of several new metabolites (Bode *et al.*, 2002). For example in *Aspergillus flavus*, the genes involved in secondary metabolism show higher levels of expression at 28 °C than at 37 °C (OBrian *et al.*, 2007).

As far as drug discovery is concerned, screening of libraries created by combinatorial synthesis once appeared to be more promising than natural products screening (Fehér and Schmidt, 2003; Ortholand and Ganesan, 2004). Though combinatorial synthesis can churn out molecules in enormous numbers, endophytic fungi can still be a good source of novel drugs and natural product-based scaffolds for combinatorial synthesis and libraries (Wang *et al.*, 2006a,b; Ge *et al.*, 2008). This is because the synthetic capability of endophytes, like in other organisms, has been fine tuned by natural selection over millions of years (Fehér and Schmidt, 2003; Taylor, 2008). Smith *et al.* (2008) combined sequence analysis with bioassay procedures to explore the endophyte diversity of the tropics. Their results suggest that tropical plants harbour a sizeable portion of undiscovered endophytes which may be vested with novel biochemical diversity. Hence the need for the inclusion of fungal endophytes in natural products discovery programmes. Testing endophytes isolated from different tissues of plant hosts (rather than those isolated from the same organ of different plant hosts) and from plants growing in unusual and less-studied habitats will be more productive. We suggest a global initiative involving fungal taxonomists, ecologists, and natural product chemists to evolve systematic and rapid screens for endophytic fungi by designing strategic bioassays that would indicate the production of novel bioactive compounds.

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