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Research review paper

A critical review on exploiting the pharmaceutical potential of plant endophytic fungi

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ABSTRACT

The escalating demand for secondary metabolites in international markets poses a severe threat to many plant species. An unscrupulous collection is also the immediate challenge to the survival of many unthreatened as well as vulnerable plants. Fungal endophytes have emerged in recent years as a promising substitute for sources of plant secondary metabolites. Many appealing secondary metabolites with potent antibacterial, antifungal, insecticidal, antioxidant, cytotoxic and anticancer properties have been discovered from endophytic fungi. Concerning their distinctive genetic and metabolic diversity and promising activities, they hold a plausible application in medicine and industry. However, there is little success in utilizing the pharmaceutical potential of fungal endophytes. Cutting-edge research is desirable to establish and bolster *in vitro* biosynthetic proficiency of fungal endophytes. Modern biotechnological techniques [such as multilocus sequence typing (MLST), metabolomics, metagenomics and next-generation sequencing (NGS) technologies] and bioinformatics approaches can fill a gap in fungal endophyte research. The present review focuses on how advanced chemical, biotechnological and computational molecular biology methods can be used for robust exploitation of bioactive compounds from these microorganisms.

1. Introduction

Microorganisms can affect almost every process in the biosphere and alter the environment around them. The swiftly varying microbial community has imposed some challenges to humans such as drug-resistant pathogenic microbes and the emergence of new diseases (Chang et al., 2015; Bengtsson-Palme, 2018). Also, due to the side-effects caused by synthetic drugs during the treatment of a disease has led to an increasing demand for natural drugs world-wide. These circumstances prompted scientists to investigate different natural sources to counter these contemporary challenges. The rate of discovery of novel bioactive metabolites particularly antibiotics and anticancer agents is descending (Lam, 2007; Rodriguez et al., 2009; Hesterkamp, 2017). Furthermore, several medicinal plants are unique, have slow growth rate, are found in high altitudes or in unusual habitats and are facing a persistent threat by humankind (Kala et al., 2006; Chen et al., 2016). The population of the world is progressing at an alarming scale, and the exploitation of medicinal plants for medicine can lead to the loss of biodiversity. Therefore, it is important to inspect different sources and habitats in order to discover novel bioactive metabolites of natural origin.

Fungi have been proved to be a valuable source of important natural products such as penicillin, a breakthrough discovery by Alexander Fleming (Fleming, 1929). This motivated other researchers to isolate novel antimicrobial molecules from fungi. Several recent discoveries have been in extracting bioactive compounds from a distinctive group of microbes known as endophytes, which primarily comprise of fungi (Petrini and Fisher, 1986), bacteria (Rogers et al., 2012), actinobacteria (Zhao et al., 2011) and algae (Bast et al., 2014). Endophytic microorganisms inhabit inside different tissues of the host plant, without inducing any apparent expression of any disease (Bacon and White, 2000; Currie et al., 2014). Endophytic fungi have been reported in fossilized tissues of plants, which reveals that their relationship with plants is primitive (Redecker et al., 2000; Tan and Zhou, 2001; Krings et al., 2007). The association between fungal endophytes and their host plant is mutualistic since they assist each other in a number of ways. Plants provide the fungi with habitation and nourishment, whereas endophytic fungi in return assist their host to withstand biotic (pathogen damage) and abiotic stresses (such as high salinity, temperature and drought) by the production of many bioactive metabolites (Rodriguez et al., 2009; Larriba et al., 2015). There are nearly 300,000

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plant species and each plant hosts one or more endophyte (Strobel and Daisy, 2003; Zimmerman and Vitousek, 2012). Although scientists claim the discovery of one-hundred thousand fungal species to date, there could however even be over one million species. Bioactive molecules, especially from the plant-associated microorganisms such as endophytes are considerably unexplored (Staniek et al., 2008).

Consequently, there is an excellent possibility to explore novel biomolecules from such microorganisms among countless plants present in various niches moreover ecosystems. Over the last several years, the endophytic fungi are a rich source of natural bioactive metabolites with potential benefits in the pharmaceutical industry (Higginbotham et al., 2013; Mousa and Raizada, 2013). Many of these secondary metabolites are intriguingly the same as produced by their respective hosts and could serve as a substitute of significant plant-based compounds (Mohana Kumara et al., 2012). Despite numerous research reports on bioactive compounds from fungal endophytes, endophyte research is still in its infancy as there is little success in commercial production of such bioactive compounds. Therefore, in order to recognize the true potential of endophytic fungi, a clear understanding of ecological, physiological, biochemical, biotechnological, chemical and bioinformatical aspects is needed. The present review primarily summarizes the past and current research (1993–2019) that is being done on endophytes comprising ecology, interaction, establishment in the host plant as well as their isolation. A recent approach to characterize and study the endophytic fungi using 'omics' methods and high-throughput sequencing is also discussed. This review also sheds light on different classes of secondary metabolites produced by endophytic fungi along with their impending applications. The present review will help researchers to obtain a clear understanding of the plant-endophyte relationship and to make better use of this symbiosis and for obtaining excellent resources which may be utilized by pharmaceutical industries.

2. Ecology of fungal endophytes

Fungal endophytes have been reported in every plant species that have been studied so far. Detailed investigations of the internal microbiota of plants frequently uncover novel taxa and reveal new distributions of known fungal species. They are typically located in the intercellular (apoplast) and intracellular (symplast) spaces of different plant species (Belanger, 1996). In plants, their occurrence is comprehensive with some fungi being organ-specific (leaf, petiole, stem or root). This could be due to the adaptation to microecological and physiological conditions present in an organ (Carroll et al., 1977). A large number of fungal endophyte species are generally found during the process of isolation; however, only a few host-specific strains are dominant. The host-specificity is sometimes affected by the site or habitat of the host plant (Petrini et al., 1992). Plants growing at similar sites may also have distinct fungal assemblages (Petrini and Fisher, 1988). Recently, novel high-throughput sequencing (HTS) or next-generation sequencing (NGS) methods (discussed later) are used by many workers for fungal ecology studies (Buée et al., 2009; Jumpponen and Jones, 2009; Öpik et al., 2009). A standard description of NGS datasets of fungal communities has been proposed by Nilsson et al. (2011). These methods facilitate the identification and relative quantification of plant-associated fungal endophytes in various tissues and different geographic locations (Peršoh, 2015).

The endophytic fungi belong to ascomycetes, basidiomycetes, zygomycetes or oomycetes, however; ascomycetes (chiefly Diaporthales and Helotiales in angiosperms and gymnosperms, respectively) are predominant compared to the other groups (Sinclair and Cerkauskas, 1996; Sieber, 2007). Endophytic fungi can be categorized on the basis of the mode of reproduction (sexual or asexual) (Brem and Leuchtmann, 2001), mode of transmission (vertically- and horizontally-transmitted endophytes; Saikkonen et al., 2002), source of nutrition (necrotrophs, or biotrophs; Delaye et al., 2013); expression of infection (symptomatic and asymptomatic; Pinto et al., 2000); host plant part that is affected by the fungal endophytes (e.g. root and foliar endophytes; Behie et al., 2015) or on an ecological basis (Rodriguez et al., 2009). Based on ecology, diversity and functional roles the endophytic fungi are classified into four classes and are divided into two groups; clavicipitaceous (Class 1) and non-clavicipitaceous endophytes (Class 2, 3 and 4) (Rodriguez et al., 2009). The clavicipitaceous endophytic fungi are monophyletic and have a narrow host range. They are phylogenetically related and infect cool and warm-season grasses, whereas, the non-clavicipitaceous endophytic fungi have a comprehensive range of host plants and are polyphyletic (Bischoff and White, 2005; Rodriguez et al., 2009). Every class of fungal endophyte follows a special mode of transmission. Class 1 and 2 can be transmitted by horizontal or vertical transmission, whereas Classes 3 and 4 only follow vertical transmission. In vertical transfer, the transmission occurs from parent to offspring by penetrating their hyphae into the embryo of the seeds and therefore is true-to-type, whereas horizontal transfer occurs amongst individual plants by developing asexual or sexual spores (Suryanarayanan, 2013). These spores enter into the host plant through the stomatal apertures (Roos and Hattingh, 1983), hydathodes (Horino, 1984), cuts and wounds (Lamb et al., 1996), lenticels (Fox et al., 1971) or by insects and animal vectors (Juniper, 1991). Germinating spores enter a host plant by penetrating the epidermis or cuticle with enzymatic digestion (Petrini et al., 1992).

3. Plant endophyte interactions

Endophytic fungi have a close interaction with their host, thus facing a lesser amount of competition for space and nutrients and are shielded from unfavorable environmental conditions such as ultraviolet light, extreme temperatures and water deficits (Card et al., 2016). In return, the endophytes protect their host from pathogenic microorganisms and also promotes their growth, which is detailed later. The fungal endophytes also provide tolerance to plants against toxic/heavy metals by their degradation, sequestration or chelation of these metals (Weyens et al., 2009). In some plants such as orchids and ryegrass, the germination and further development of seeds are dependent on fungal endophytes (Stone et al., 2000). In the inadequacy of these fungal endophytes, plant growth ceases beyond a particular stage. It has been proposed that the relationship among fungal endophytes and their host is balanced pathogen-host antagonism rather than synergism (Schulz et al., 1999). According to the balanced antagonism hypothesis, the fungal endophyte manages to grow within the plant by avoiding the activation of the host's defense mechanism without causing any disease, and this association remains asymptomatic (Schulz et al., 1999). This association between the endophytic fungi and its host is complex and mutualistic; however, this interaction may turn pathogenic under a state of imbalance, or saprophytic during senescence (Arnold, 2007; Slippers and Wingfield, 2007; Palencia et al., 2010). The expression of certain intrinsic factors such as stress- and mitogen-activated protein kinase gene (sakA) can lead to pathogenicity (Arnold, 2008; Eaton et al., 2011). It has also been recommended that the plant endophyte relationship is much more complicated than just being an equilibrium as suggested by the balanced antagonism hypothesis (Kusari and Spiteller, 2012). The intimate relationship between fungal endophytes and their host plant results in the production of diverse secondary metabolites (Strobel, 2003a, 2003b). These metabolites produced by plants also include many antifungal compounds, yet these endophytic fungi live in harmony within the plant tissues. It has been observed that the endophytic fungi resist toxicity conferred by the host plant by specific amino acid residue alterations in the secondary metabolite-binding site (Kusari et al., 2011). Alternatively, it is possible that the fungal endophytes biodegrade these toxic compounds with the help of specific enzymes (Kusari et al., 2011). The plant endophyte interactions are complex involving multi-species communications and vary from host to host and endophyte to endophyte. Multidisciplinary approaches are thus needed to understand such complex interactions. Modern

genomic studies involving 'omics' approaches can be helpful in understanding the basic physiological and biochemical aspect of the plant endophyte interaction and for the effective use of the symbiotic association between the two partners (Kaul et al., 2016). For instance, whole genome analysis of fungal endophytes has provided an insight to diligently assess the hereditary traits that directly or indirectly influence the diverse bioactivities and reveal the essential features in colonizing the host plants. Genes for nitrogen fixation, virulence proteins/effectors, phytohormone (Indole-3-acetic acid, gibberellic acid, etc.) production, mineral acquisition (iron, potassium, phosphorous etc.), biotic and abiotic stress tolerance, adhesion and other colonization related genes have been reported from fungal endophytes (Rafiqi et al., 2013; Firrincieli et al., 2015). The comparative multigenome analysis is rather helpful in understanding the genetic and metabolic diversity of fungal endophytes involved in diverse interactions with their host plants (Ye et al., 2017).

Recently, microarray-based techniques are used to analyze gene profiling and investigation of plant-endophyte interactions (Felitti et al., 2006). The potential of *Epichloe* endophyte cDNA microarrays (Nchip™ and Endochip™ microarrays) for genome-wide high-throughput transcriptome analysis has been described by Felitti et al. (2006). These microarrays allow studies related to genome-specific gene expression, profiling of novel endophyte genes, and examination of the plant-endophyte interaction. The factors for the establishment and maintenance of the mutualistic association between fungal endophytes and its host plant can be better understood by using such novel approaches.

4. Criteria for plant selection and effective isolation of fungal endophytes

The initial step in dealing with fungal endophytes is the assortment of a suitable and promising plant for study, followed by isolation of the fungal endophyte. Broadly, the phanerogams (angiosperms and gymnosperms) are studied for their endophytic diversity and novel secondary metabolites. Endophytes related to cryptogams (viz. algae, lichens, bryophytes and pteridophytes) should also be considered because they too hold great potential (Suryanarayanan et al., 2005; Wang et al., 2006; Li et al., 2007a; Paranagama et al., 2007). While isolating the endophytic fungi, a large number of species can be isolated from the hosts, which can show the presence of some dominant and rare species. The rare genera could be more crucial than the dominant fungal endophytes. Isolation of endophytic fungi is important for their characterization, for studying population dynamics and diversity, to isolate novel bioactive secondary metabolites and also for improved plant growth and biocontrol agents (Hallmann et al., 2006; Amin, 2016; Nassimi and Taheri, 2017). Several considerations should be taken into account during isolation studies such as sampling from various plant segments of diverse age, sample size, site of collection, the season of collection, growth media and surface sterilization of plant tissues.

4.1. Site of collection

Innovative and ingenious strategies can narrow down the search for fungal endophytes exhibiting bioactivity (Mittermeier et al., 1999). Plants with unusual biology (i.e. at very high altitudes, deserts, swamps and marshes, saline habitats, rainforests), having medicinal and ethnobotanical history, growing in hotspots and that are under endangered categories could be auspicious for bioprospecting. The habitats which are not yet explored might also contain novel fungal endophyte isolates of pharmaceutical interest. A good physical state of a sampling site with no sign of the plant disease is recommended for selecting the plants (Suryanarayanan et al., 2005). The identified plant should be deposited in the local herbarium as a voucher specimen. The field data that includes location [must be marked using a Global Positioning System (GPS)], soil pH, temperature, humidity, light intensity, soil type, salinity, soil microbes and other relevant information can help in relating the data obtained and their influence on the fungal endophytic community (Suryanarayanan et al., 2005).

4.2. Sample size

One of the most significant challenges in endophytic fungal diversity studies is their measurement. A promising isolation strategy can help in obtaining maximum endophytic fungal diversity from a given plant. The number of plant samples and size of explant can influence the diversity of the endophytic fungal community. According to Petrini et al. (1992), approximately 40 plants of a given species with 30 to 40 sampling units per tissue is adequate for the recovery of 80% fungal endophytes present in a given host. An increase in the recovery of endophytic fungal isolates was observed when the leaf explant size was reduced, and the number of samples was increased (Carroll, 1995; Gamboa et al., 2002). This indicates that the size of plant parts should be optimized to achieve factual diversity of endophytic fungal isolates.

4.3. Age dependent isolation

It is important to discern whether young, mature or old segments (viz. bark, twigs and leaves, petioles) have more fungal endophytes, as the younger plant parts are biochemically different from mature and old plant parts. For example, the frequency of endophyte establishment improved with the leaf age in leaves of *Calotropis procera* (Nascimento et al., 2015) and *Coscinium fenestratum* (Goveas et al., 2011). The younger leaves may contain lower concentrations of antifungal and anti-herbivore substances compared to mature leaves, which could be the reason for the low rate of colonization (Coley and Barone, 1996; Jeffrey et al., 2012). An increase in the number of fungal endophytes in mature leaves might be due to the extended period of tissue exposure to the surroundings (Arnold and Herre, 2003).

4.4. Seasonal influence on fungal endophytes

The variation in a seasonal pattern of colonization might be notably associated with the seasonal behavior of fungal endophytes. For example, a greater endophytic fungal diversity was observed in the twigs and bark of *Terminalia arjuna* (Tejesvi et al., 2005) and leaves of *Centella asiatica* (Gupta and Chaturvedi, 2017) during rainy seasons. It has been suggested that high moisture and temperature during the rainy seasons favour growth and dispersal of spores of fungal endophytes (Mishra et al., 2012). However, some studies reported that the endophytic fungal diversity was considerably higher in the winter seasons in comparison to the rainy and summer seasons (Naik et al., 2008; Fang et al., 2013). The variation in the diversity of fungal endophytes in different seasons might be due to the fact that the levels of secondary metabolites vary throughout the year (Fang et al., 2013).

4.5. Localization of fungal endophytes in plant tissues

Localization of fungal endophytes in plant tissue can help in detecting inter or intracellular colonization, which can help to understand endophyte-host interaction (Garcia et al., 2012). Microscopic (electron or light microscopy) visualization can be considered as direct confirmation to assign the exact location and colonization pattern of fungal endophytes in the internal tissues of plants, especially in leaves (Clark et al., 1983; Petrini and Fischer, 1990; Kowalski and Kehr, 1992; James, 2000; Kuldau and Yates, 2000; Christensen et al., 2002; Hahn et al., 2003; Baum et al., 2003; Higgins et al., 2007; Oses et al., 2008; Bernardi-Wenzel et al., 2010; Garcia et al., 2012; Raja et al., 2016). The distribution of fungal endophytes can also be determined by a rapid, inexpensive and reproducible tissue immunoblot technique (well associated with histological staining, followed by microscopic analysis) (Gwinn et al., 1991; Hiatt et al., 1999).

4.6. Growth media and isolation of fungal endophytes

Different modifications of standard growth media [potato dextrose agar (PDA), malt extract agar (MEA), corn meal agar (CMA), Czapek's agar (CZA), synthetic nutrient-poor agar (SNA), oatmeal agar (OA), V-8 agar (V8A), sabouraud agar (SDA), modified melin-norkrans (MMN) medium, and water agar (WA)] with varying factors such as pH, temperature, light, aeration and length of incubation and addition of plant extract should be tested for the optimal recovery of fungal endophytes (Matsushima, 1971; Arnold and Herre, 2003; Crous et al., 2009a; VanderMolen et al., 2013). It is generally recommended to use half-strength media for isolating fungal endophytes as there are chances that emerging endophytes might experience osmotic shock (Murphy et al., 2015).

Effective isolation is the most critical step in studying endophytic fungi. The proper surface-sterilization of the plant tissues is, therefore, necessary to eliminate epiphytic fungi and other microorganisms (Gagné et al., 1987; Misaghi and Donndelinger, 1990; Bills, 1996; Mahaffee and Kloepper, 1997; Greenfield et al., 2015; Yang et al., 2018). Concentration and duration of exposure of sterilizing agents should be standardized as the sterilizing agent may penetrate the plant tissue and kill endophytes, affecting the results. The efficiency of the surface-sterilization must be ensured (Gagné et al., 1987; McInroy and Kloepper, 1994; Hallmann et al., 2006).

The media can be supplemented with antibiotics (streptomycin, penicillin, novobiocin, oxytetracycline and/or chloramphenicol) to eliminate bacterial growth. Instead of cutting, plant parts can also be ground in a sterile mortar and pestle or with mechanical devices such as a Klecco tissue pulveriser (Mahaffee and Kloepper, 1997) or by using a Polytron homogeniser (Zinniel et al., 2002) depending on the sample size and toughness of the plant material (Sieber, 2002; Hallmann et al., 2006). Fungal endophytes should be recovered as early as possible and transferred to a new solid medium as they emerge from the edges of an explant. For further purification, the isolates should be subcultured using a single spore (Choi et al., 1999; Crous, 2002). The purified isolates should be immediately stored using 15% glycerol at −70°C. The strains must be submitted to fungal repositories.

4.7. Identification of fungal endophytes

Endophytic fungal isolates can be identified both morphologically (using a standard manual) or by molecular techniques (DNA barcoding). In the morphological methods, the number of phenotypic characters of endophytic fungal isolates is noted viz. colony shape, colour of colony, elevation of colony and characteristics of spores (Arnold, 2002; Promputtha et al., 2005; Barnett and Hunter, 2006; Kirk et al., 2008; Seifert and Rossman, 2010). Several online databases [Fungal Databases, Systematic Mycology and Microbiology Laboratory (ARS, USDA), MycoBank, Index Fungorum, Fungal Planet, Bibliography of Systematic Mycology, Tree of Life Web Project] are helpful in identification of fungal species. Many isolates are not able to sporulate during cultivation and are thus categorized as ‘sterile fungi’ or ‘sterile mycelia’ (Suryanarayanan et al., 1998; Kumaresan and Suryanarayanan, 2001). Endophytic fungi including ‘sterile fungi’ are obscure to identify at genus or species level (Gamboa and Bayman, 2001). Molecular techniques are much more reliable compared to morphological identification, as phenotypic characters are unstable and may alter according to the environmental conditions (Schulz and Boyle, 2005). The pure endophytic fungal isolates are subjected to DNA isolation using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987; Arnold et al., 2007) or by using a DNA isolation kit (Tejesvi et al., 2011). Alternatively, the endophytic fungi can be isolated directly from the plant tissue using laser microdissection and pressure catapulting (LMPC) technique (Kerk et al., 2003; Duong et al., 2006). This technique is useful in isolating uncultivable fungal endophytes (Guo et al., 2001; Kerk et al., 2003; Nikolcheva and Bärlocher, 2004; Seena et al., 2008). LMPC is a promising technique where the cells of interest are viewed under a microscope and are cut out using a laser beam, and the DNA is extracted from the collected sample to identify the fungal endophyte (Kerk et al., 2003). Internal transcribed spacer (ITS) is the most widely used DNA barcode for molecular identification of fungal species (Schulz and Boyle, 2005; U'ren et al., 2009; Sun et al., 2011). In DNA barcoding techniques the endophytic fungal isolates are subjected to genomic DNA isolation and PCR, targeting the adjacent sequences of the 18S ribosomal RNA gene, ITS 1, 5.8S ribosomal RNA gene, ITS 4, 28S ribosomal RNA gene, using the ITS 1/4 primers (White et al., 1990). Amplified PCR products are analyzed by electrophoresis in agarose gel, stained with ethidium bromide or SYBR Green, and visualized underneath UV (ultraviolet) light using a Gel Documentation (gel image) system (Arnold et al., 2007). Large-scale investigations of microfungi frequently depend on Basic Local Alignment Search Tool (BLAST) matches with the National Center for Biotechnology Information (NCBI) GenBank database for identification. To validate the accuracy of identification based on BLAST matches, comparison of taxonomic matches at the genus and family levels made for the similar isolates based on BLAST searches. Identification and characterization of various endophytic fungal species can also be achieved meaningfully employing curated fungal barcoding databases (Yahr et al., 2016) such as Barcode of Life Database, BOLD (http://www.boldsystems.org/index.php/IDS_OpenIdEngine); Fungal Barcoding (http://www.fungalbarcoding.org); User-friendly Nordic ITS Ectomycorrhiza Database, UNITE (https://unite.ut.ee/); RefSeq Target Loci, RTL (http://www.ncbi.nlm.nih.gov/refseq/targetedloci/); The International Society for Human and Animal Mycology, ISHAM (http://its.mycologylab.org); Naïve Bayesian Classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp); Fungal MLST database Q-Bank (http://www.q-bank.eu/Fungi/); International Subcommission on Hypocrea and Trichoderma (ISHT) TrichoKey and TrichoBLAST (Trichoderma) (http://www.isth.info/tools/blast/). An identity level of more than 98% ITS rDNA sequence similarity can be used for species identitification and between 95% and 98% as a genus identity (Arnold et al., 2007; Higgins et al., 2007; Kõljalg et al., 2013; Garnica et al., 2016). However, to have a higher confidence of the endophytic fungal identity at species level additional molecular markers such as cytochrome c oxidase subunit I (CO1), β-tubulin (TUB) and translation elongation factor (TEF) can also be used along with morphology/physiology of the isolate (Seifert et al., 2007; Roe et al., 2010; Porras-Alfaro and Bayman, 2011). If an endophytic fungus is not displaying any close match in GenBank, rigorous literature survey pertaining to its morphology/physiology might promise to be instrumental in proposing a new taxon. Consensus tree is generally produced with sequences identified by BLASTN search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) reflecting the highest identity and maximum query coverage. Finally, the amplified ITS sequence of fungal isolates is submitted to the NCBI GenBank database using the web-based data submission tool BankIt (www.ncbi.nlm.nih.gov/

WebSub/?tool=genbank) or the stand-alone program Sequin (https://www.ncbi.nlm.nih.gov/Sequin/).

4.8. Cultivation-independent approach in identification of endophytic fungi

The traditional methods of identification are subject to many technical limitations and the results obtained are not sufficient to provide a complete overview of the fungal endophytes in their host (Hawksworth, 1985). There are many endophytic fungal isolates which are difficult to culture on artificial media or are slow growers or non-sporulating sterile mycelia and less competitive to fast-growing strains. A cultivation-independent approach can overcome such technical limitations and can help to obtain these uncultivable endophytic fungi (Guo et al., 2001; Oono et al., 2015; Liu et al., 2017; Dissanayake et al., 2018). In *Livistona chinensis* it has been reported that some endophytic fungal species of basidiomycetes and Herpotrichiellaceae could only be identified with culture-independent molecular techniques (Guo et al., 2000b; Guo et al., 2001). Direct evaluation of environmental samples containing mycobiomes offers unique details of community diversity,

composition, taxonomy and interactions (Purahong et al., 2016). Metagenomics or environmental genomics is a molecular technique which involves analysis of genetic material recovered directly from environmental samples (e.g. plant tissue) and allows unculturable species to be detected. The putative functional characteristics of the fungal endophytes can be unravelled based on metagenome analysis (Sessitsch et al., 2012).

In fungal metagenomics approach, DNA is extracted from the surface-sterilized plant tissue [environmental PCR (polymerase chain reaction)] and investigated for its gene content (Jones, 2010). However, it is a tedious procedure to separate fungal DNA from host plant DNA, since plant DNA is more abundant than fungal DNA, making it difficult to isolate and sequence the fungal metagenome (Porras-Alfaro and Bayman, 2011; Cuadros-Orellana et al., 2013). The first step in fungal metagenome analysis is a sampling of plant material and surface sterilization of plant samples (Gao et al., 2005; Yang et al., 2018). The samples can be stored at −80 °C before DNA extraction (Arnold et al., 2007). To confirm the removal of surface DNA contamination, PCR can be performed with sterilized distilled water in which the sterilized tissue is rinsed last (Gao et al., 2005). Additionally, the water sample can be tested under a light microscope to confirm the presence of any contamination. The samples, after the homogenization step, can be divided into replicates to observe the consistency in fungal community composition of the independently analysed replicates (Kauserud et al., 2012). Subsequently, the total genomic DNA is extracted. PCR amplification of ITS rDNA (White et al., 1990) is performed (Schoch et al., 2012) and PCR products are ligated to a cloning vector (e.g. pGEM-T vector, pMD 18-T vector) using a cloning kit (Gao et al., 2005; Arnold et al., 2007). The recombinants are then transformed into *Escherichia coli* (TG1, DH5α) competent cells with ampicillin and blue/white screening (Gao et al., 2005; Tejesvi et al., 2011). The positive transformants of rDNA libraries are then screened by PCR amplification of inserts. PCR amplification products comprising the correct size of the insert are then digested using restriction enzyme, and digested DNA fragments can be analysed using DNA fingerprinting techniques such as RFLP (Restriction Fragment Length Polymorphism). Alternatively, separation of PCR products (bands) can be done using DGGE (denaturing gradient gel electrophoresis; Tao et al., 2008; Sun and Guo, 2012) or T-RFLP (Terminal restriction fragment length polymorphism; Nikolcheva and Bärlocher, 2005). The plasmid DNA from recombination clones with different fingerprinting patterns and DGGE bands representing different taxa are then extracted and purified for sequencing (Yuan et al., 2004). The DNA sequences from an environmental sample can be rapidly studied using HTS [e.g. Illumina (Solexa) sequencing, 454 pyrosequencing sequencing, ion torrent: proton/ion personal genome machine (PGM) sequencing and/or sequencing by oligonucleotide ligation and detection (SOLiD)] (Jumpponen et al., 2010; Toju et al., 2013; Akinsanya et al., 2015). The NGS data from representative clones with different fingerprinting patterns and DGGE bands are analysed and identified into various taxonomic levels based on phylogenetic analysis and sequence similarity comparison with highly customizable bioinformatic tools (Thompson et al., 1997; Swofford, 2000; Tamura et al., 2007; Kauserud et al., 2012; Tamura et al., 2013; Bálint et al., 2016; Kumar et al., 2016; Kumar et al., 2018). Software such as MG-RAST (Keegan et al., 2016), QIIME (Caporaso et al., 2010) and CloVR (Angiuoli et al., 2011) can ease extensive metagenomics analysis resulting in a faster investigation of large datasets. The statistical analyses can be performed using the R statistical package or PAST software (Hammer et al., 2001).

4.9. Identification of fungal endophytes using multi-locus sequence analysis/typing (MLSA/MLST)

The taxonomic identification of endophytic fungi at the species level is achieved by multilocus sequence analysis/typing (MLSA/MLST) (Santos et al., 2010; Udayanga et al., 2012; Gomes et al., 2013; Soares et al., 2018). MLSA is a phylogenetic analysis of multiple internal fragments of housekeeping genes that are universal to the studied taxon (Polonio et al., 2016). The primers for rDNA ITS (ITS1 and ITS4; White et al., 1990), partial sequences of translation elongation factor 1-α (EF 1-α; Carbone and Kohn, 1999), partial sequences of β-tubulin (TUB; O'Donnell and Cigelnik, 1997; Glass and Donaldson, 1995), actin (ACT; Carbone and Kohn, 1999), partial sequences of glyceraldehyde-3-phosphate dehydrogenase (GPDH; Myllys et al., 2002), histone H3 (HIS; Crous et al., 2004; Glass and Donaldson, 1995), mitochondrial small subunit (SSU) (mtSSU; Li et al., 1994), partial large subunit nuclear ribosomal DNA (nrDNA) (LSU; Crous et al., 2009b; Vilgalys and Hester, 1990) and calmodulin (CAL; Carbone and Kohn, 1999) and several other house-keeping gene segments can be used for amplification and sequencing. The combined analysis of amplified genomic regions of such loci are more informative and can provide robust species identification, from which phylogenetic relationships can be inferred (Polonio et al., 2016; Soares et al., 2018). The DNA sequences are compared with pertaining data from publically available databases such as GenBank and UNITE (Polonio et al., 2016; Yahr et al., 2016). Sequence alignment and consensus generation is achieved through tools like MAFT (Katoh and Toh, 2008) and CLUSTAL (Thompson et al., 1994). Phylogenetic analysis is accomplished with software packages like MEGA (Tamura et al., 2007; Tamura et al., 2013; Kumar et al., 2016; Kumar et al., 2018), RAxML (Randomized Axelerated Maximum Likelihood; Stamatakis, 2014) and MrBayes (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012).

5. Characterization of metabolites from fungal isolates

Purification and characterization of bioactive substances from endophytic fungi are done once the appropriate growth conditions are achieved. The purified endophytic fungus is cultured in liquid media or solid media before extraction. Various liquid or solid media can be chosen for culturing endophytic fungi. For liquid media, Czapek Dox Broth (CDB), Malt Extract Broth (MEB), Potato Dextrose Broth (PDB), Yeast powder Soluble starch (YpSs) broth, and Peptone Yeast Glucose (PYG) broth can be used for cultivating endophytic fungi (VanderMolen et al., 2013). For solid media cultivation, different media can be prepared using rice, maize, oatmeal, wheat germ, potato, carrot and/or wood shavings (VanderMolen et al., 2013; Bogner et al., 2017; Zhu et al., 2017; Zhou et al., 2018). The flasks/Petri dishes from liquid/solid fermentation can be incubated at room temperature and/or 25 °C in the light/dark under a static condition or constant agitation for 14-28 days (Sharma et al., 2016).

Extraction of the secondary metabolite from an endophytic fungus can be undertaken using solvent partitioning. The effectiveness of extraction is a critical factor for the assessment of functional activities of an endophytic fungal extract. For extraction of secondary metabolites from fungal mycelia (from solid media), the dry-pressed mycelia are placed in the solvent of choice (such as ethyl acetate, methanol, n-butanol) over-night at room temperature with constant agitation (VanderMolen et al., 2013; Gao et al., 2017). Alternatively, the solvent can be added directly to the Petri dishes (which should be glass- or plastic-resistant to the solvent of choice) after the fungus has grown and can then be incubated with gentle shaking for 20–30 min. The solvent is then recovered by filtration. The efficiency of extraction is much higher if the fungal mycelia are homogenized using glass/steel bead cell disruptor or mortar and pestle with liquid nitrogen directly in the solvent. Such a protocol also helps to analyze intracellular secondary metabolites which could be sequestered inbetween the mycelium layers. For extraction of extracellular secondary metabolites from liquid media, the culture broths should be separated into mycelia and filtrates using filter paper or by vacuum filtration. Equal volumes of filtrate and solvent of choice are added to extract the secondary metabolites from the filtrate (Verma et al., 2014). The mycelia can be dried and ground and can then be subjected to extraction (Zhang et al., 2012). The pooled filtrates are

stirred for 10–15 min and left to stand for 5–10 min until transparent immiscible layers are formed. Besides, the extracts of the mycelia and filtrates can be treated separately to determine the presence of bioactive compounds in the interior or exterior of the mycelia of the isolated fungal endophyte (Zhang et al., 2012). The extraction process (for both fungal mycelium and broth filtrate) is usually repeated with a second and third cycle of extraction. The organic solvent layer containing the target compounds is separated from the aqueous phase in a separatory funnel. Finally, the solvent can be evaporated to dryness under reduced pressure at 40 °C in a rotary vacuum evaporator to yield the crude metabolite (Bhardwaj et al., 2015). The crude extract can be dissolved in a solvent [such as methanol (MeOH) or dimethyl sulphoxide (DMSO)] (preferably at a concentration of 1 mg/ml) and stored at 4 °C (Sharma et al., 2016).

Immunoassays are also used for screening of fungal endophyte crude extracts by using monoclonal antibodies specific for a metabolite (Strobel et al., 1996b). Bioassay- or (bio)activity-guided fractionation interconnects multidisciplinary instrumental and analytical techniques including fractionation, separation and purification with each step being assisted by an appropriate biological detection assay (Weller, 2012).

Initial purification can be attained by successively partitioning the crude extract between solvent(s) (such as n-hexane and methanol; ethyl acetate and methanol) (Sharma et al., 2016). A preliminary bioassay (antimicrobial, anticancer, antiviral, antidiabetic, antioxidant etc.) of the organic layers (n-hexane and methanol; ethyl acetate and methanol) can be performed to assess the bioactivity of the fractions. Furthermore, compound isolation can be carried out using the fraction owning bioactivity. The solvent fraction(s) can be subjected to column chromatography using silica gel or Sephadex LH20 as stationary phase and eluted with different solvents such as MeOH; petroleum ether; acetone; petroleum ether-acetone gradient; petroleum ether and ethyl acetate gradient; n-hexane-ethyl acetate gradient; chloroform-methanol gradient; methanol-acetone gradient to yield various fractions (Lu et al., 2000; Verma et al., 2014; Bogner et al., 2017). The fractions obtained can again be evaluated for bioactivity. Preparative TLC can also be used for the isolation and purification of the target compound(s) (Verma et al., 2014). The fractions showing bioactivity are further analysed using TLC where the crude extract is spotted (10-30 μL) on a TLC plate and chromatography is performed by employing a solvent system such as dichloromethane: MeOH (90:10 v/v); EtOAc:MeOH:water (30:5:4). Fractions having similar colours and locations are scrapped from the TLC plates and pooled (Verma et al., 2014). Separation of compounds can also be done using size exclusion chromatography (over Sephadex LH 20) or vacuum liquid chromatography (VLC) on silica gel using a gradient solvent system (such as MeOH-H_2O, petroleum ether-ethyl acetate) (Wang et al., 2017b; Zhu et al., 2017). Detection of specific compound(s) on the TLC plates is achieved by observing bands under a UV lamp in UV inspection cabinet or by visualizing compounds by spraying with a specific spray reagent(s) (such as dragendorff reagent, ninhydrin, folin-ciocalteu reagent, aluminium chloride, anisaldehyde or ceric sulfate) (Pyka, 2014). Developed chromatograms are air-dried for complete removal of solvent and silica residue is extracted and centrifuged.

Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) or preparative reversed-phase ultra-performance liquid chromatography (RP-UPLC) can also be conducted on extracts using a mobile phase of choice (such as MeOH with 0.1% trifluoroacetate in water; MeOH-H_2O) for qualitative as well as quantitative analysis i.e. for purification and purity determination of compound(s) (Gao et al., 2017). The mobile system program and the UV wavelength for the target compounds are set according to the retention time of eluate. Target peaks are collected manually or with the help of a fraction collector during the program run.

The structure of the isolated compounds is determined following both spectrometric and spectroscopic analyses. UV-visible spectroscopy provides useful information about the electronic features of a molecule, such as a characteristic chromophore (Qin et al., 2009). Infrared (IR) spectroscopy is relevant in terms of ascertaining the functional groups of a molecule. The absolute configuration of the compound can be obtained using optical rotation and circular dichroism (CD) data which indicates optical purity (Qin et al., 2009; Yang and Li, 2013; Ju et al., 2016; Gao et al., 2017). Furthermore, quantitative analysis of pure compounds can be achieved using hyphenated techniques such as liquid chromatography-mass spectrometry [electrospray ionisation mass spectrometry (ESI-MS); high-resolution electrospray ionisation mass spectrometry (HR-ESI-MS)] (Qin et al., 2009; Ju et al., 2016). These techniques provide mass data including molecular weights. Finally, structure elucidation of an unknown organic compound can be achieved via combination of independent methods such as one- (1D) and two-dimensional (2D) proton (^1H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography (Qin et al., 2009; Gao et al., 2017; Wang et al., 2017b; Zhu et al., 2017). Infrared (IR) spectroscopy can reveal the functional groups present in the compound (Qin et al., 2009). X-ray crystallography is absolute in its outcome in that it provides the exact structure and stereochemistry of a molecule (Qin et al., 2009; Ju et al., 2016). NMR confers information on the carbon and hydrogen atoms that make up a molecule and allows extensive interaction profiling in 3D space (Gao et al., 2017). In the case of known compounds, the spectroscopic data is verified against an authentic standard or by comparing with the literature data (Dias et al., 2016).

6. Genome mining approach in screening endophytic fungi

Efficient and HTS techniques nowadays are being used extensively in endophyte studies due to their sensitivity, specificity and precision. The genetic, developmental and environmental factors affect the production of secondary metabolites from fungal endophytes. Also, the identification of potential fungal isolates producing target metabolites is a cumbersome process and needs to screen hundreds of isolates (Xiong et al., 2013). The endophytic fungi can be screened for target secondary metabolites using a genome mining approach employing PCR (Kusari and Spiteller, 2011; Vasundhara et al., 2016). The *in silico* detection of conserved cryptic genes cluster of multidomain and multimodular enzymes such as polyketide synthases (PKSs), nonribosomal peptide synthetases (NRPSs), prenyltransferases and terpene cyclases involved in the biosynthesis of various secondary metabolites might facilitate in subsequent isolation of bioactive metabolites from a respective fungal endophyte (Scherlach and Hertweck, 2006). The presence of key genes encoding target secondary metabolites can be used as molecular markers to screen endophytic fungi. However, it is crucial to select the suitable genes as markers for screening fungal endophytes for production of bioactive secondary metabolites. For example, gene encoding 10-deacetylbaccatin III-10-O-acetyltransferase (DBAT) and baccatin III 13-O-(3-amino-3-phenylpropanoyl) transferase (BAPT) are used as molecular markers to screen taxol (paclitaxel) producing endophytic fungi (Walker and Croteau, 2000). The primers for target secondary metabolites are designed and synthesized according to published reports and fungal isolates are screened using polymerase chain reaction (PCR) for the key genes. Subsequently, the amplified fragments are evaluated by agarose gel electrophoresis and endophytic fungi showing amplicons for the target genes are selected for sequencing. The sequence identification of positive samples is then endeavored by searching for databases using the NCBI BLAST sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/).

7. Endophytic fungi as source of novel biomolecules

Fungal endophytes are proficient of fabricating various bioactive compounds which have the potential to be used as curative agents against a surfeit of problems (Kusari and Spiteller, 2010; Kusari and Spiteller, 2011). An endophytic fungus can be isolated from diverse plant species belonging to different families and plant groups under

Table 1
Illustrative list of endophytic fungi producing plant secondary metabolites (1993 to 2019).

Endophytic fungi	Secondary metabolite	Chemical nature	Activity	Host	Host family	Reference
Taxomyces andreanae	Taxol (**1**)	Diterpene	Anticancerous	*Taxus brevifolia*	Taxaceae	Stierle et al. (1993)
Phoma sp.	Altersolanol A (**10**), 2- hydroxy- 6 methyl benzoic acid (**11**)	Anthraquinone	Antibacterial (*Bacillus subtilis*)	*Taxus wallachiana*	Taxaceae	Yang et al. (1994)
Fusarium subglutinans	Subglutinol A (**12**) and B (**13**)	Diterpene pyrones	Immunosuppressive	*Tripterygium wilfordii*	Celastraceae	Lee et al. (1995)
Pestalotiopsis microspora	Torreyanic acid (**14**)	Quinone dimmer	Cytotoxic (Human cancer cell lines)	*Torreya taxifolia*	Taxaceae	Lee et al. (1996)
Microsphaeropsis sp.	S 39163/F-I (**15**)	Cyclic ester	Antiviral (Herpes viruses)	*Buxus sempervirens*	Buxaceae	Tscherter et al. (1988)
Pseudomassaria sp.	Demethylasterriquinone B-1 (L-783,281) (**16**)	Asterriquinone	Antidiabetic	Undetermined plant	-	Zhang et al. (1999a)
Alternaria sp.	Vinblastine (**3**)	Alkaloid	Anticancerous	*Catharanthus roseus*	Apocynaceae	Guo et al. (1998)
Rhizopus oryzae	α-Irone (**17**), β-Irone (**18**)	Irones	Odorants, flavor enhancer	*Iris germanica*	Iridaceae	Zhang et al. (1999b)
Cryptosporiopsis cf. *quercina*	Cryptocandin (**19**)	Lipopeptide	Antibiotic, antimycotic	*Tripterigeum wilfordii*	Celastraceae	Strobel et al. (1999)
Fusarium sp.	CR377 (**20**)	Pentaketide	Antifungal (*Candida albicans*)	*Selaginella pallescens*	Selaginellacea	Brady and Clardy (2000)
Cytonaema sp.	Cytonic acid A (**21**) and B (**22**)	Tridepside	Antiviral [Human Cytomegalovirus (hCMV)], protease inhibitor	*Quercus* sp.	Fagaceae	Guo et al. (2000a)
Cryptosporiopsis cf. *quercina*	Cryptocin (**23**)	Tetramic acid	Antifungal (*Pyricularia oryzae*)	*Triptergyium wilfordii*	Celastraceae	Li et al. (2000)
Colletotrichum sp.	6-isoprenylindole-3-carboxylic acid (**24**)	Indole derivative	Antifungal (*Phytopthora capsici, Rhizoctonia cerealis,* and *Gaeumannomyces graminis*)	*Artemisia annua*	Asteraceae	Lu et al. (2000)
Hormonema sp.	Enfumafungin (**25**)	Triterpenoid glycoside	Antifungal (*C. albicans*, *C. tropicalis*, *Aspergillus fumigatus* and *Saccharomyces cerevisiae*)	*Juniperus communis*	Cupressaceae	Pelaez et al. (2000)
Rhinocladiella sp.	Cytochalasin 1-3 (**26-28**), E (**29**),	Cytochalasins	Cytotoxic (A2780S- ovarian tumor cell line, SW-620-colon tumor cell line and HCT-116 colon tumor cell line)	*Terygium wilfordii*	Celastraceae	Wagenaar et al. (2000)
Fusarium oxysporum	Vincristine (**4**)	Alkaloid	Anticancerous	*Catharanthus roseus*	Apocynaceae	Zhang et al. (2000)
Colletotrichum gloeosporioides	Colletotric acid (**30**)	Tridepside	Antimicrobial (*B. subtilis*, *Staphylococcus aureus*, *Sarcina lutea* and *Helminthsporium sativum*)	*Artemisia mongolica*	Asteraceae	Zou et al. (2000)
Pestalotiopsis jesteri	Jesterone (**31**) and Hydroxy-jesterone (**32**)	Cyclohexenone epoxides	Antifungal (*Pythium ultimum*, *Aphanomyces* sp., *Phytophthora citrophthora* and *P. cinnamomi*)	*Fragraea bodenii*	Gentianaceae	Li and Strobel (2001)
Pestalotiopsis microspora	Ambuic acid (**33**)	Cyclohexenone	Antifungal (*Fusarium* sp., *Diplodia natalensis, Cephalosporium gramineum* and *P. ultimum*)	*Torreya taxifolia*	Taxaceae	Li et al. (2001)
Rhizoctonia sp.	Rhizoctonic acid (**34**)	Monomethylsulochrin	Anti-helicobacter pylori (Monomethylsulochrin)	*Cynodon dactylon*	Poaceae	Ma et al. (2004)
Aspergillus parasiticus	Sequoiatones C–F (**35-38**)	Similar to Azaphilones	Antiviral	*Sequoia sempervirens*	Cupressaceae	Stierle et al. (2001)
Guignardia sp.	Guignardic acid (**39**)	Dioxolanone	Antibacterial	*Spondias mombin*	Anacardiaceae	Rodriguez-Heerklotz et al. (2001)
Pestalotiopsis microspora	Pestacin (**40**) and Isopestacin (**41**)	Isobenzofuran	Antioxidant, antifungal	*Terminalia morobensis*	Combretaceae	Strobel et al. (2002); Harper et al. (2003)
Wardomyces anomalus	2,3,6,8-tetrahydroxy-1-methylxanthone (42) 5-(hydroxymethyl)-2-furanocarboxylic acid (**43**)	Xanthone derivative	Antioxidant, tyrosine kinase inhibitor	*Enteromorpha* sp.	Ulvaceae	Abdel-lateff et al. (2003)
Entrophospora infrequens	Camptothecin (**2**)	Alkaloid	Antileukemic	*Nothapodytes foetida*	Icacinaceae	Puri et al. (2005)
Microsphaeropsis olivacea	Graphislactone A (**44**) and Botrallin (**45**)	Benzopyranones	Cytotoxic and Acetylcholinesterase inhibitor	*Pilgerodendron uviferum*	Cupressaceae	Hormazabala et al. (2005)
Strain JJF006 (Unidentified)	Rutin (**46**)	Flavonoid	Antibacterial and antioxidant	*Pteris multifida*	Pteridaceae	Fan et al. (2007)
Acremonium sp.	Huperzine A (**47**)	Alkaloid	Memory booster, anti-Alzheimer	*Huperzia serrata*	Huperziaceae	Li et al. (2007b)
Fusarium sp.	Fusapyridon A (**48**)	Pyridone alkaloid	Antibacterial (*S. aureus* and *Pseudomonas aeruginosa*)	*Maackia chinensis*	Fabaceae	Tsuchinari et al. (2007)
Fusarium oxysporum	Podophyllotoxin (**6**)	Lignan	Antineoplastic, antiviral	*Juniperus recurva*	Cupressaceae	Kour et al. (2008)
Chaetomium globosum	Hypericin (**49**)	Naphthodianthrone	Antimicrobial	*Hypericum perforatum*	Hypericaceae	Kusari et al. (2008)
Xylaria sp.	7-amino-4-methylcoumarin (**50**)	Benzopyrone	Antibacterial (*S. aureus*, *Escherichia coli*, *Salmonella typhimurium*, *S. enteritidis*, *Shigella* sp.)	*Ginkgo biloba* L.	Ginkgoacaea	Liu et al. (2008)
Xylaria sp.	Xylarenones A, B (**51-52**) and Xylarenic acid (**53**)	Sesquiterpenes	Antimicrobial (*B. subtilis*, *C. albicans*, *E. coli*, *S. aureus*, *S. cerevisiae*)	*Torreya jackii*	Cephalotaxaceae	Hu et al. (2008)
Phomopsis sp.	Phomopsin A (**54**)	Hexapeptide	Antifungal (*C. albicans* and *Fusarium oxysporum*)	*Excoecaria agallocha*	Euphorbiaceae	Huang et al. (2008)
Colletotrichum dematium	Colutellin A (**55**)	lipopeptide	Immunosuppressive	*Pteromischum* sp.	Araceae	Ren et al. (2008)
Neurospora crassa	Camptothecin (**2**)	Alkaloid	Antileukemic	*Nothapodytes foetida*	Icacinaceae	Rehman et al. (2008)

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Table 1 (*continued*)

Endophytic fungi	Secondary metabolite	Chemical nature	Activity	Host	Host family	Reference
Aspergillus clavatonanicus	Clavatol (**56**)	Phenone	Antifungal (*Botrytis cinerea, Didymella bryoniae, F. oxysporum, Rhizoctonia solani*, and *P. ultimum*)	*Torreya mairei*	Taxaceae	Zhang et al. (2008)
	Patulin (**57**)	Polyketide lactone				
Corynespora cassiicola	Corynesidones A and B (**58-59**)	Depsidone	Antioxidant	*Lindenbergia philippensis*	Scrophulariaceae	Chomcheon et al. (2009)
Fungal culture, PM0651480	Ergoflavin (**60**)	Pigment	Anticancerous	*Mimusops elengi*	Sapotaceae	Deshmukh et al. (2009)
Alternaria sp.	Berberine (**61**)	Alkaloid	Antibiotic	*Phellodendron amurense*	Rutaceae	Duan (2009)
Blastomyces sp.	Huperzine A (**47**)	Alkaloid	Memory booster, anti-alzheimer's	*Phlegmariurus cryptomerianus*	Lycopodiaceae	Ju et al. (2009)
Chaetomium globosum	Chaetomugilin D (**62**)	Azaphilone	Activity against brine shrimp (*Artemia salina*), antifungal	*Ginkgo biloba*	Ginkgoacaea	Qin et al. (2009)
Nodulisporium sp.	Camptothecin (**2**)	Alkaloid	Antineoplastic	*Nothapodytes foetida*	Icacinaceae	Rehman et al. (2009)
Chloridium sp.	Javanicin (**63**)	Napthaquinone	Antibacterial (*Pseudomonas* sp.)	*Azadirachta indica*	Meliaceae	Kharwar et al. (2009)
Fusarium solani	Camptothecin (**2**)	Alkaloid	Antineoplastic	*Camptotheca acuminata*	Nyssaceae	Kusari et al. (2009a)
Thielavia subthermophila	Hypericin (**49**) and emodin (**64**)	Anthraquinone	Photodynamic cytotoxicity (Human acute monocytic leukemia cell line)	*Hypericum perforatum*	Hypericaceae	Kusari et al. (2009b)
Chaetomium globosum	Chaetomugilin D (**62**)	Azaphilone	Antifungal (*Mucor miehei*)	*Ginkgo biloba*	Ginkgoacaea	Qin et al. (2009)
Botryosphaeria parva	Camptothecin (**2**)	Alkaloid	Antineoplastic	*Nothapodytes nimmoniana*	Icacinaceae	Gurudatt et al. (2010)
Fusarium solani	Camptothecin (**2**)	Alkaloid	Antineoplastic	*Apodytes dimidiata*	Icacinaceae	Shweta et al. (2010)
Cochliobolus nisikadoi	Borneol (**65**)	Terpene	Anti-inflammatory, antioxidant	*Cinnamomum camphora*	Lauraceae	Chen et al. (2011)
Fusarium solani, Fusarium proliferatum	Cajaninstilbene acid (**66**)	Stilbene	Antioxidant	*Cajanus cajan*	Fabaceae	Gao et al. (2011)
Trichoderma atroviride	Tanshinone I (**67**), Tanshinone IIA (**68**)	Diterpenoids	Anti-inflammatory	*Salvia miltiorrhiza*	Lamiaceae	Ming et al. (2011)
Aspergillus niger, Alternaria alternata	Lapachol (**69**)	Phenolic metabolite	Antioxidant	*Tabebuia argentea*	Bignoniaceae	Sadananda et al. (2011)
Aspergillus flavus	(8E,12Z)-10,11-dihydroxyoctadeca-8,12-dienoic acid (**70**)	Oxylipin	Acetylcholinesterase inhibitory activity	*Corallina officinalis*	Corallinaceae	Qiao et al. (2011)
	3β,4α-dihydroxy-26-methoxyergosta-7,24(28)-dien-6-one (**71**)	Steroid				
Fusarium oxysporum	Ginkgolide B (**72**)	Diterpene lactones	Anti-allergic and anti-inflamatory	*Ginkgo biloba*	Ginkgoacaea	Cui et al. (2012)
Fusarium proliferatum	Rohitukine (**7**)	Alkaloid	Anticancerous Immuno-modulatory	*Dysoxylum binectariferum*	Meliaceae	Mohana Kumara et al. (2012)
Eupenicillium parvum	Azadirachtin (**8**)	Alkaloid	Insecticidal	*Azadirachta indica*	Meliaceae	Kusari et al. (2012)
Aspergillus niger	Monacolin K or Lovastatin (**73**)	Statin drug	Hypocholesterolemic	*Taxus baccata*	Taxaceae	Raghunath et al. (2012)
Aspergillus niger, Fusarium oxysporum	Coumarin (**74**)	Benzo-pyrone	Antioxidant	*Crotalaria pallida*	Fabaceae	Umashankar et al. (2012)
Aspergillus glaucus	2,14-dihydroxy-7-drimen-12,11-olide (**75**)	Sesquiterpene	Antioxidant	*Ipomoea batatas*	Convolvulacea	Asker et al. (2013)
Aspergillus fumigatus	Phloroglucinol (**76**)	Phenol derivative	Antioxidant	*Garcinia* sp.	Clusiaceae	Karmakar et al. (2013)
Aspergillus sydowii	Sydoxanthone A and B (**77**,**78**), and 13-*O*-acetylsydowinin B (**79**)	Xanthone derivatives	Immunosuppressive	*Scapania ciliata*	Scapaniaceae	Song et al. (2013)
Colletotrichum gloeosporioides	Piperine (**9**)	Alkaloid	Antimicrobial, antidepressant, anti-inflammatory and anticancerous	*Piper nigrurm*	Piperaceae	Chithra et al. (2014)
Xylaria sp.	Seco-Cytochalasin E (**80**), Cytochalasin Z18 (**81**), Cytochalasin Z27 (**82**), Cytochalasin Z28 (**83**)	Cytochalasins	Brine shrimp toxicity, phytotoxicity and antifungal	*Toona sinensis*	Meliaceae	Zhang et al. (2014)
Lachnum sp.	Salidroside (**84**) and *p*-tyrosol (**85**)	Phenolics	Antioxidant	*Rhodiola crenulata*	Crassulaceae	Cui et al. (2015)
Xylaria sp.	Cytochalasin D (**86**)	Alkaloid	Antitumor, antibiotic	*Bostrychia tenella*	Rhodomelaceae	de Felício et al. (2015)
Eutypella scoparia	Scoparasin C (**87**)	Cytochalasin	Cytotoxic	*Hevea brasiliensis*	Euphorbiaceae	Kongprapan et al. (2015)
Fusarium redolens	Peimisine (**88**) and Imperialine 3-β-D-glucoside (**89**)	Alkaloid	Get rid of sputum, cough and antitumor	*Fritillaria unibracteata* var. wabuensis	Liliaceae	Pan et al. (2015)
Penicillium chrysogenum	Penochalasin I (**90**), Penochalasin J (**91**),	Chaetoglobosins	Cytotoxic	*Myoporum bontioides*	Scrophulariaceae	Huang et al. (2016)
Penicillium sp.	Peniphenone (**92**) and Methyl peniphenone (**93**)	Polyketide benzannulated spiroketal	Immunosuppressive	*Sonneratia apetala*	Lythraceae	Liu et al. (2016)
Talaromyces sp.	Z -roquefortine C (**94**), Viridicatol (**95**), Penitrem A (**96**), Penijanthine A (**97**)	Alkaloids	Antibacterial (*S. aureus*)	*Sargassum* sp.	Phaeophyceae	Yang et al. (2016)

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Table 1 (*continued*)

Endophytic fungi	Secondary metabolite	Chemical nature	Activity	Host	Host family	Reference
Aspergillus tamarii	Malformin E (**98**)	Cyclic pentapeptide	Cytotoxic	*Ficus carica*	Moraceae	Ma et al. (2016)
Fusarium chlamydosporiums	Fusarithioamide A (**99**)	Benzamide	Antimicrobial and cytotoxic	*Anvillea garcinii*	Asteraceae	Ibrahim et al. (2016)
Setosphaeria rostrata	Exserohilone (**100**)	Thiodiketopiperazine derivatives	Alpha–glucosidase inhibitory activity	*Costus speciosus*	Costaceae	Centko et al. (2017)
	Rostratazine B (**101**)		Porcine pancreatic alpha amylase inhibitory activity			
Gaeumannomyces sp.	Stemphol C and D (**102-103**)	Glycosylated dialkylresorcinol derivatives	Nitric oxide reduction activity in lipopolysaccharide-stimulated microglia BV-2 cells	*Phragmites communis*	Poaceae	Lee et al. (2017)
	1-O-methyl-6-O-(α-D-ribofuranosyl)-emodin (**104**)	Anthraquinone derivatives				
Gibberella moniliformis	2-hydroxy-1, 4-naphthoquinone (Lawsone) (**105**)	Napthoquinone	Dye, antibacterial, antioxidant	*Lawsonia inermis*	Lythraceae	Sarang et al. (2017)
Pestalotiopsis sp.	Pestalone (**106**)	Chlorinated benzophenone	Antibacterial (*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Candida glabrata*)	*Melia azedarach*	Meliaceae	Song et al. (2017)
Phomopsis sp.	Cytosporone U (**107**)	Octaketide phenolic lipids	Antiviral (Tobacco mosaic virus)	*Brucea javanica*	Simaroubaceae	Tan et al. (2017)
Chaetomium globosum	Podophyllotoxin (**6**)	Lignan	Antineoplastic, antiviral	*Sinopodophyllum hexandrum*	Berberidaceae	Wang et al. (2017a)
Mycosphaerella nawae	(-) Mycousnine (**108**)	Dibenzofurane	Immunosuppressive	*Smilax china*	Smilacaceae	Wang et al. (2017b)
Penicillium decumbens	Peniproline A (**109**)	Phenylamino pyrrolidone	Cytotoxic	*Cephalotaxus mannii*	Cephalotaxaceae	Wang et al. (2017c)
Penicillium chrysogenum	Penochalasin K (**110**)	Chaetoglobosin	Cytotoxic	*Myoporum bontioides*	Scrophulariaceae	Zhu et al. (2017)
Colletotrichum gloeosporioides	Asiaticoside (**111**)	Triterpene	Memory enhancement	*Centella asiatica*	Apiaceae	Gupta et al. (2018)
Fusarium sp.	Fusaripeptide A (**112**)	Cyclodepsipeptide	Antifungal and anti-malarial	*Mentha longifolia*	Lamiaceae	Ibrahim et al. (2018)
Neosartorya fischeri	Sartorypyrone E (**113**)	Meroditerpene	Neuroprotective	*Glehnia littoralis*	Apiaceae	Bang et al. (2019)

various ecological and geographical conditions (Petrini, 1996). From the year 1999 to 2010 there has been an upsurge in US patents registered on fungal endophytes, which are capable of producing important secondary metabolites with various pharmacological applications. A comprehensive study showed that about 51% of bioactive substances sequestered from fungal endophytes were formerly undefined (Strobel and Daisy, 2003). There are millions of endophytic fungi present ubiquitously in plants (Shekhawat et al., 2010). Secondary metabolites from fungal endophytes could be acquired at a superior level as they can be mass multiplicated in bioreactors, which can be economically viable. The great interest for some of the plant metabolites [such as taxol (**1**), camptothecin (**2**) etc.] and its scarcity in natural plant sources has led to varied approaches of bioprospecting of fungal endophytes as alternate sources of biologically active molecules. The first anticipation for this was confirmed by Stierle et al. (1993), who isolated an endophytic fungus *Taxomyces andreanae* from the bark of Pacific yew, *Taxus brevifolia* that produces taxol (a diterpene alkaloid), the multibillion-dollar potent anticancerous drug used successfully in the treatment of a wide variety of cancers. The anticancer drug taxol (generic name paclitaxel) was thought to be uniquely produced by *Taxus* genus (yew trees) of Taxaceae family belonging to order Coniferales of Gymnosperms and no other plant species has yet been reported to produce taxol. However, since Stierle et al. (1993) reported the unprecedented isolation of taxol by an endophytic fungus, correspondingly many other workers reported and confirm this phenomenon of biosynthesis of taxol and other secondary metabolites [including vinblastine (**3**), vincristine (**4**), diosgenin (**5**), podophyllotoxin (**6**), camptothecin (**2**), rohitukine (**7**), azadirachtin (**8**), piperine (**9**), etc.] by fungal endophytes (Tables 1 and 2, Fig. 1A-E).

The production of taxol is not only restricted to a specific fungus from *Taxus* species, but several genera of fungi from various other plant species produce taxol. Out of the 73 genera of endophytic fungi studied in this review (reported to produce taxol from the year 1993 to 2019), 44 genera were isolated from Taxaceae alone and one each from Acanthaceae, Araucariaceae, Betulaceae, Magnoliaceae, Moringaceae, Pinaceae, Podocarpaceae, Rhizophoraceae, Rubiaceae, Sapindaceae, Plantaginaceae and two each from Cupressaceae, Ginkgoaceae, Malvaceae, Rutaceae, three from Combretaceae and seven from Celastraceae (Table 2). Most of these plant families belong to Gymnosperms (69.86%) and few to Angiosperms (30.13%). The maximum isolates from these plants from various families were isolated from leaves (23) and bark (16) (Table 2).

To date, it has not been established why some endophytic fungi produce host secondary metabolites such as taxol, despite many reports on secondary metabolite production by endophytic fungi. It is significant to illuminate the metabolome in fungal endophytes correlating to their host plants. A case study of taxol is discussed here to understand how the biogenetic gene clusters are regulated and their expression is affected *in vivo* and *in vitro*.

In *Taxus* species taxol is formed from geranylgeranyl diphosphate (GGPP), the universal precursor of diterpenoids (which is synthesized from three isopentenyl diphosphate (IPP) molecules and a molecule of dimethyl pyrophosphate (DMAPP) by the enzyme geranylgeranyl diphosphate synthase (Eisenreich et al., 1996) (Fig. 2). Dimethyl pyrophosphate is an isomer of IPP and is an intermediate product of both 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP, plastid derived) pathway and mevalonic acid (MVA, cytosol derived) pathway. In *Taxus* species the plastidic pathway is involved in forming GGPP (Eisenreich et al., 1996), however, studies have also shown the involvement of cytosolic pathway (Lansing et al., 1991; Cusidó et al., 2007; Expósito et al., 2009). The biosynthesis of taxol involves the initial cyclization of GGPP to taxa-4(5),11(12)-diene (a diterpene olefin), by enzyme TS (taxa-4(5),11(12)-diene synthase or taxadiene synthase) followed by its extensive oxidative modification, acylations and oxetane ring formation. The phenylalanine aminomutase (PAM) is a rate-limiting enzyme which converts α-phenylalanine to β-

phenylalanine and thus executes the first step in the side chain assembly, resulting eventually in the synthesis of taxol (Fig. 2). Endophytic fungi do not possess plastids and synthesize IPP and DMAPP using the MVA pathway which functions in cytosol (Kuzuyama and Seto, 2012). Endophytic fungi possess the major pathway genes for MVA pathway, however, they seem to lack some crucial taxol biosynthetic gene as well as other downstream genes. Most of the studies reporting the genes for taxol production from fungal endophytes detect a few key genes [TS (Staniek et al., 2009; Kumaran et al., 2012; Xiong et al., 2013; Kasaei et al., 2017; de Andrade et al., 2018); DBAT: 10-deacetylbaccatin III-10-O-acetyl transferase (Zhang et al., 2009a; Zhang et al., 2009b; Xiong et al., 2013; Roopa et al., 2015; de Andrade et al., 2018); BAPT: C-13 phenylpropanoid side chain-CoA acyltransferase (Roopa et al., 2015; Xiong et al., 2013); T5αH: taxa-4(5),11(12)-diene-5α-hydroxylase (Yang et al., 2014); T10βH: taxane-10β-hydroxylase (Yang et al., 2014); PAM (Yang et al., 2014)] involved in taxol biosynthesis (Table 2, Fig. 2). Some studies showed low homology (< 50% sequence similarity) and others showed high homology (> 95% sequence similarity) of taxol biosynthesis genes in fungal endophytes with the plant counterpart (Zhang et al., 2009b; Staniek et al., 2009; Flores-Bustamante et al., 2010; Xiong et al., 2013). Wildung and Croteau (2016) reported that amino acid sequence from TS has a putative plastidial transit peptide (approximately 137 amino acids), this suggests that endophytic fungi might have a distinctive enzyme catalyzing the reaction in the direction of taxadiene. The taxol biosynthetic genes in *Penicillium aurantiogriseum* (an endophytic fungus producing taxol) were quite different from those found in *T. baccata* and showed only 19% to 65% identical amino acid sequences (Yang et al., 2014).

It has been anticipated that the genetic recombination of endophytic fungus and its host plant might have occurred via horizontal gene transfer (HGT) during their collective evolutionary history and later that the endophytic fungus might have transferred the gene(s) to new plant hosts (Pirozynski and Malloch, 1975). Richards et al. (2009) investigated the incidence and evolutionary history of HGT between plants and fungi and identified five fungi-to-plant HGTs and four plant-to-fungi HGTs. Also, the phylogenetic analyses showed that the subtilisin gene was assimilated by *Colletotrichum* spp. through HGT from plants to a *Colletotrichum* ancestor (Armijos Jaramillo et al., 2013). The physical interaction and genetic recombination between the host and endophyte are thought to be responsible for HGT, which possibly occurred during the long period of coevolution and coexistence (Strobel and Daisy, 2003; Gogarten, 2003; Richardson and Palmer, 2007). However, it is ambiguous that the endophytic fungus can synthesize all the enzymes required for the synthesis of secondary metabolites such as taxol. The transfer of the whole pathway for taxol biosynthesis consisting of more than 20 steps (located in different cellular compartments viz. plastid, endoplasmic reticulum and cytosol) via HGT to multifarious endophytic fungi seems challenging (Croteau et al., 2006). Moreover, the presence of taxol-producing endophytic fungi in plants other than *Taxus* species (Table 2) and different homology with taxol biosynthetic genes from those found in *Taxus* suggests that it might have its own taxane biosynthesis genes and indicates that HGT is an implausible justification. These genes in endophytic fungi seem to have evolved independently. This can be correlated with the gibberellin biosynthesis pathway which is dissimilar in plants and fungi (Tudzynski and Hölter, 1998; Bömke and Tudzynski, 2009).

Some studies have confirmed the taxol production in endophytic fungi despite the absence of key genes for taxol production. Heinig et al. (2013) were not able to identify any fungal genomic sequence related to known taxane-specific sequences from *Taxus* species and thus, concluded that taxane biosynthesis in endophytes might have evolved independently. Due to the low yield and attenuation of taxol/paclitaxel in endophytic fungi, there is a possibility that they might not synthesize taxol independently. Nevertheless, they might accrue taxol (which is highly lipophilic) in their cell wall from their plant counterpart *Taxus* or store them in vesicles through passive transport (Heinig et al., 2013). However, this theory does not hold true for the taxol synthesizing endophytic fungi found on non-taxol host plants (Stierle et al., 1993; Strobel et al., 1996b). The cell fractionation based on density gradient centrifugation followed by organelle analysis through assay development may be used to determine taxol quantification and localization in endophytic fungi.

The fabrication of useful bioactive metabolites by fungal endophytes holds immense potential, which can be exploited by pharmaceutical industries. However, low yield and attenuation of secondary metabolites in axenic conditions is a major problem. The absence of host stimulus in the culture media and/or silencing of genes in axenic cultures could be responsible for the attenuation of metabolites (Hertweck, 2009; Sachin et al., 2013). Brakhage et al. (2008) inferred that secondary metabolite biosynthesis gene clusters remain silent until triggers for their induction are identified. The biosynthesis gene cluster for a specific metabolite can be discovered using modern gene targeting techniques followed by a comparative metabolic profile analysis (Chiang et al., 2008). Various strategies to stimulate silent gene clusters of fungal secondary metabolites have been proposed (Chiang et al., 2009; Hertweck, 2009; Brakhage and Schroeckh, 2011). Many workers have tried to optimize the culture conditions to obtain secondary metabolites from fungal endophytes at a commercial scale. Ji et al. (2006) extrapolated that taxol yield from endophytic fungi up to the level of 1000 μg/L is commercially feasible. Qiao et al. (2017) reported an elevation in taxol yield from 334.92 to 1337.56 μg/L by using copper sulphate ($CuSO_4$), salicylic acid ($C_7H_6O_3$) and sodium acetate (CH_3COONa). A significant increase in taxol production from 20 to 225.2 g/L after strain improvement [by protoplast mutation through UV radiation and diethyl sulfate (DES)] and media optimization was reported by Xu et al. (2006). In a recent study, it was observed that light could be a crucial factor affecting taxol production as these fungal endophytes reside in the dark inner bark microenvironment (Soliman and Raizada, 2018). The fungal endophyte *Paraconiothyrium* species when incubated in light for a week prior to inoculation in liquid yeast-peptone-dextrose (YPD) media, showed loss of taxol in the culture media (Soliman and Raizada, 2018). Moreover, the expression of genes involved in fungal taxol biosynthesis [HMGCoA reductase (HMGR) and 3-dehydroquinate synthase (DHQ)] was found to be considerably reduced (Soliman et al., 2011; Soliman and Raizada, 2018). Such instances highlight that fungal endophytes require a similar atmosphere as their original habitat [light, darkness, pH, temperature, water availability and solutes such as NaCl (sodium chloride), KCl (potassium chloride), glucose, glycerol and sorbitol] to enhance the production of host/novel secondary metabolites.

Modern gene editing techniques, such as zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) systems offer the opportunity to modify the genome of fungal endophytes (Urnov et al., 2010; Chen and Gao, 2013; Doudna and Charpentier, 2014; Pohl et al., 2016; El-Sayed et al., 2017). Gene expression modulation using CRISPR/Cas9 can be employed in the improvement of selected endophytic fungal strains and subsequently, explore the riddles of the plant-endophyte and endophyte-endophyte interactions. Through cost-effective CRISPR/Cas9 genome editing technology, it is plausible to enhance the production of selected bioactive compounds by specific fungal endophyte (El-Sayed et al., 2017; Yan et al., 2018). Additionally, the genes encoding for target secondary metabolites can also be identified using CRISPR/Cas9 (Nielsen et al., 2017). Recently, a new gene in *Talaromyces atroroseus* responsible for the production of polyketide-nonribosomal peptide hybrid products was identified through the CRISPR/Cas9 based genome editing approach (Nielsen et al., 2017). CRISPR/Cas9 genome editing technology offers novel scopes to optimize and modulate target genes from endophytic fungi efficiently.

8. Pharmacological potential of fungal endophytes

Endophytic fungi are capable of producing a broad range of novel bioactive compounds. The pharmacological properties of these fungal endophytes are elaborated in the following sections.

Table 2
A list of taxol-producing endophytic fungi (cited from 1993-2019).

Endophytic fungi	Host plant	Family	Plant part	Reported genes[a]	Quantity	Identification method[b]	Reference
Taxomyces andreanae	*Taxus brevifolia*	Taxaceae	Inner bark	–	24–50 ng/L	TLC, HPLC, MS	Stierle et al. (1993)
Pestalotiopsis microspora	*Taxus wallichiana*	Taxaceae	Inner bark	–	60–70 μg/L	MS and NMR	Strobel et al. (1996a)
Pestalotiopsis microspora	*Taxodium distichum*	Cupressaceae	Phloem	–	14–1487 ng/L	TLC, HPLC, MS	Li et al. (1996)
Pestalotiopsis guepinii	*Wollemia nobilis*	Araucariaceae	Stem	–	485 ng/L	UV, TLC, EIA	Strobel et al. (1997)
Periconia sp.	*Torreya grandifolia*	Taxaceae	Inner bark	–	30–821 ng/L	MS, NMR	Li et al. (1998)
Kitasatospora sp.	*Taxus baccata*	Taxaceae	Wood	–	1.3 μg/L	HPLC, LC-MS, EIA	Caruso et al. (2000a)
Alternaria sp.	*Taxus baccata*	Taxaceae	Leaf	–	20–50 ng/L	EIA	Caruso et al. (2000b)
Aspergillus sp.	*Taxus baccata*	Taxaceae	Wood	–	10–20 ng/L	EIA	Caruso et al. (2000b)
Beauveria sp.	*Taxus baccata*	Taxaceae	Wood	–	10–20 ng/L	EIA	Caruso et al. (2000b)
Epicoccum sp.	*Taxus baccata*	Taxaceae	Leaf	–	20–50 ng/L	EIA	Caruso et al. (2000b)
Fusarium sp.	*Taxus baccata*	Taxaceae	Wood	–	20–50 ng/L	EIA	Caruso et al. (2000b)
Gelasinospora sp.	*Taxus baccata*	Taxaceae	Wood	–	20–50 ng/L	EIA	Caruso et al. (2000b)
Geotrichum sp.	*Taxus baccata*	Taxaceae	Leaf	–	20–50 ng/L	EIA	Caruso et al. (2000b)
Phoma sp.	*Taxus baccata*	Taxaceae	Leaf	–	20–50 ng/L	EIA	Caruso et al. (2000b)
Phomopsis sp.	*Taxus baccata*	Taxaceae	Wood	–	10–20 ng/L	EIA	Caruso et al. (2000b)
Mycelia sterilia	*Taxus baccata*	Taxaceae	Wood	–	50–100 ng/L	EIA	Caruso et al. (2000b)
Tubercularia sp.	*Taxus mairei*	Taxaceae	Inner bark	–	–	UV, TLC, HPLC, MS	Wang et al. (2000)
Pestalotiopsis microspora	*Taxus wallichiana*	Taxaceae	Stem	–	0.03–0.20 ng/mL	TLC, LC-MS	Shrestha et al. (2001)
Sporormia minima	*Taxus wallichiana*	Taxaceae	Stem	–	0.02 ng/mL	TLC, HPLC, ESI-MS	Shrestha et al. (2001)
Trichothecium sp.	*Taxus wallichiana*	Taxaceae	Stem	–	0.17 ng/mL	TLC, HPLC, ESI-MS	Shrestha et al. (2001)
Nodulisporium sylviforme	*Taxus cuspidata*	Taxaceae	Phloem	–	51.06–125.7 μg/L	HPLC	Zhou and Ping (2001)
Ozonium sp.	*Taxus chinensis* var. *mairei*	Taxaceae	Inner bark	–	4 – 7 μg/L	HPLC, LCMS, CIEIA	Guo et al. (2006)
Alternaria alternata	*Taxus chinensis* var. *mairei*	Taxaceae	Inner bark	–	84.5 μg/L	HPLC, MS	Tian et al. (2006)
Fusarium maire	*Taxus mairei*	Taxaceae	Bark	–	20 μg/L	HPLC, MS, CIEIA	Xu et al. (2006)
Fusarium solani	*Taxus celebica*	Taxaceae	Stem cutting	–	1.6 μg/L	TLC, HPLC, LC-ESI-MS	Chakravarthi et al. (2008)
Pestalotiopsis pauciseta	*Cardiospermum helicacabum*	Sapindaceae	Leaf	–	113.3 μg/L	HPLC	Gangadevi et al. (2008)
Bartalinia robillardoides	*Aegle marmelos*	Rutaceae	Leaf	–	187.6 μg/L	TLC, UV, HPLC	Gangadevi and Muthumary (2008a)
Colletotrichum gloeosporioides	*Justicia gendarussa*	Acanthaceae	Leaf	–	163.4 μg/L	HPLC	Gangadevi and Muthumary (2008b)
Pestalotiopsis terminaliae	*Terminalia arjuna*	Combretaceae	Leaf	–	211.1 μg/L	UV, TLC, HPLC	Gangadevi and Muthumary (2009a)
Chaetomella raphigera	*Terminalia arjuna*	Combretaceae	Leaf	–	79.6 μg/L	UV, IR, FAB-MS, and NMR	Gangadevi and Muthumary (2009b)
Phyllosticta citricarpa	*Citrus medica*	Rutaceae	Leaf	–	265 μg/L	HPLC, NMR	Kumaran et al. (2008a)
Phyllosticta melochiae	*Melochia corchorifolia*	Malvaceae	Leaf	–	274 μg/L	IR, HPLC, LC-MS, NMR	Kumaran et al. (2008b)
Phyllosticta spinarum	Cupressus sp.	Cupressaceae	Needle	–	235 μg/L	UV, IR,TLC, HPLC	Kumaran et al. (2008c)
Aspergillus fumigatus	*Podocarpus* sp.	Podocarpaceae	–	–	0.56 mg/L	TLC, HPLC	Sun et al. (2008)
Botryodiplodia theobromae	*Taxus baccata*	Taxaceae	–	–	280.50 μg/L	IR, TLC, HPLC,MS	Venkatachalam et al. (2008)
Fusarium solani	*Taxus chinensis*	Taxaceae	Bark	–	163.35 μg/L	HPLC	Deng et al. (2009)
Phyllosticta dioscoreae	*Hibiscus rosa-sinensis*	Malvaceae	Leaf	–	298 μg/L	HPLC	Kumaran et al. (2009)
Phomopsis sp.	*Larix leptolepis*	Pinaceae	Leaf	–	334 μg/L	UV, IR, HPLC, LC-MS, NMR	Kumaran and Hur (2009)
Phomopsis sp.	*Taxus cuspidata*	Taxaceae	Leaf	–	418 μg/L	UV, IR, HPLC, LC-MS, NMR	Kumaran and Hur (2009)
Phomopsis sp.	*Ginkgo biloba*	Ginkgoaceae	Leaf	–	372 μg/L	UV, IR, HPLC, LC-MS, NMR	Kumaran and Hur (2009)
Metarhizium anisopliae	*Taxus chinensis*	Taxaceae	Bark	–	846.1 ng/L	HPLC, LC-MS, ESI-MS	Liu et al. (2009)
Mucor rouxianus	*Taxus chinensis*	Taxaceae	Bark	DBAT and Baccatin III	30 μg/L	LC-MS, ELISA	Miao et al. (2009)
Aspergillus candidus	*Taxus* x media	Taxaceae	Inner bark	DBAT	112 μg/g dry weight	UV, LC-MS, NMR	Zhang et al. (2009a)
Cladosporium cladosporioides	*Taxus media*	Taxaceae	Inner bark	DBAT	800 μg/L	HPLC, MS, NMR	Zhang et al. (2009b)
Aspergillus niger var. taxi	*Taxus cuspidata*	Taxaceae	Inner bark	–	273.46 μg/L	LC-MS	Zhao et al. (2009)
Pestalotiopsis versicolor	*Taxus cuspidata*	Taxaceae	Bark/leaf	TS	478 μg/L	HPLC, LC-MS, NMR	Kumaran et al. (2010)

(*continued on next page*)

Table 2 (*continued*)

Endophytic fungi	Host plant	Family	Plant part	Reported genes[a]	Quantity	Identification method[b]	Reference
Lasiodiplodia theobromae	*Morinda citrifolia*	Rubiaceae	Leaf	–	245 μg/L	UV, IR, HPLC, NMR, FAB-MS	Pandi et al. (2011)
Paraconiothyrium sp.	*Taxus media*	Taxaceae	Bark	–	80 μg/L	LC-MS	Soliman et al. (2011)
Didymostilbe sp.	*Taxus chinensis* var. *mairei*	Taxaceae	Bark	–	8-15 μg/L	LC-MS, EIA	Wang and Tang (2011)
Stemphylium sedicola	*Taxus baccata*	Taxaceae	Inner bark	–	6.9 μg/L	HPLC	Mirjalili et al. (2012)
Fusarium oxysporum	*Rhizophora annamalayana*	Rhizophoraceae	Leaf	–	172.3 μg/L	IR, TLC, HPLC	Elavarasi et al. (2012)
Phoma betae	*Ginkgo biloba*	Ginkgoaceae	Leaf	TS	795 μg/L	UV, IR, NMR, TLC, HPLC	Kumaran et al. (2012)
Chaetomium sp.	*Michelia champaca*	Magnoliaceae	Leaf	–	77.23	HPLC, UV	Rebecca et al. (2012)
Fusarium redolens	*Taxus baccata* L. subsp. *wallichiana* (Zucc.) Pilger	Taxaceae	Bark	BAPT	66 μg/L	LC-MS	Garyali et al. (2013)
Guignardia mangiferae	*Taxus* x media	Taxaceae	Bark/leaf	TS, DBAT, BAPT	720 ng/L	HPLC-MS	Xiong et al. (2013)
Fusarium proliferatum	*Taxus* x media	Taxaceae	Bark /leaf	TS, DBAT, BAPT	240 ng/L	HPLC-MS	Xiong et al. (2013)
Colletotrichum gloeosporioides	*Taxus* x media	Taxaceae	Bark/leaf	TS, DBAT, BAPT	120 ng/L	HPLC-MS	Xiong et al. (2013)
Penicillium aurantiogriseum	*Corylus avellana*	Betulaceae	Nut	TS, DBAT, BAPT, T5αH, T10βH, PAM	70 μg/L	LC-MS, NMR	Yang et al. (2014)
Cladosporium oxysporum	*Moringa oleifera*	Moringaceae	Leaf	DBAT	550 μg/L	UV, HPLC, LC-MS, IR, NMR	Gokul Raj et al. (2015)
Botryosphaeria rhodina,	*Salacia oblonga*	Celastraceae	Bark	DBAT, BAPT	–	Genomic mining	Roopa et al. (2015)
Trichoderma longibrachiatum	*Salacia oblonga*	Celastraceae	Bark	DBAT	–	Genomic mining	Roopa et al. (2015)
Lasiodiplodia theobromae	*Salacia oblonga*	Celastraceae	Bark	DBAT	–	Genomic mining	Roopa et al. (2015)
Aspergillus niger	*Salacia oblonga*	Celastraceae	Bark	DBAT	–	Genomic mining	Roopa et al. (2015)
Coriolopsis caperata	*Salacia oblonga*	Celastraceae	Bark	DBAT	–	Genomic mining	Roopa et al. (2015)
Phomopsis sp.	*Salacia oblonga*	Celastraceae	Bark	DBAT	–	Genomic mining	Roopa et al. (2015)
Fusarium solani	*Salacia oblonga*	Celastraceae	Bark	DBAT	–	Genomic mining	Roopa et al. (2015)
Paraconiothyrium variabile	*Taxus baccata*	Taxaceae	Twig	–	1.75 μg/L	HPLC, LC-MS/MS	Somjaipeng et al. (2015)
Epicoccum nigrum	*Taxus baccata*	Taxaceae	Twig	–	1.32 μg/L	HPLC, LC-MS/MS	Somjaipeng et al. (2015)
Cladosporium sp.	*Taxus baccata*	Taxaceae	–	Exon No. 1 of TS	129 mg/kg dry wt.	HPLC	Kasaei et al. (2017)
Phoma medicaginis	*Taxus wallichiana* var. *mairei*	Taxaceae	Bark	–	PDB culture: 1.215 mg/L, spent culture medium: 0.936 mg/L and dry mycelium: 20 mg/kg	LC-MS	Zaiyou et al. (2017)
Aspergillus aculeatinus	*Taxus chinensis* var. *mairei*	Taxaceae	Bark	–	334.92 - 1337.56 μg/L	HPLC, ESI-MS	Qiao et al. (2017)
Colletotrichum gloeosporioides	*Plantago major*	Plantaginaceae	Leaf	TS and DBAT	5.24 μg/mL - 4.4 μg/mL	UV, LC-MS	de Andrade et al. (2018)
Alternaria brassicicola	*Terminalia arjuna*	Combretaceae	Leaf	–	140.8 μg/L	UV, HPLC, FTIR and LC–ESI–MS	Gill and Vasundhara (2019)

[a] DBAT: 10-deacetylbaccatin III-10-O-acetyl transferase; TS: Taxadiene synthase; BAPT: Baccatin III 13-O-(3-amino-3-phenylpropanoyl) transferase; PAM: Phenylalanine aminomutase; T5αH: Taxa-4(5),11(12)-diene-5α-hydroxylase; T10βH: Taxane-10β-hydroxylase

[b] UV: Ultraviolet–visible spectroscopy; IR: Infrared spectroscopy; MS: Mass spectrometry; TLC: Thin-layer chromatography; MPLC: Medium-pressure liquid chromatography; HPLC: High-performance liquid chromatography; LC-MS: Liquid chromatography–mass spectrometry; ESI-MS: Electrospray ionisation mass spectrometry; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; EIMS: Electron impact mass spectrometry; FAB-MS: Fast atom bombardment mass spectrometry; NMR: Nuclear magnetic resonance spectroscopy; EIA: Competitive inhibition enzyme immunoassay

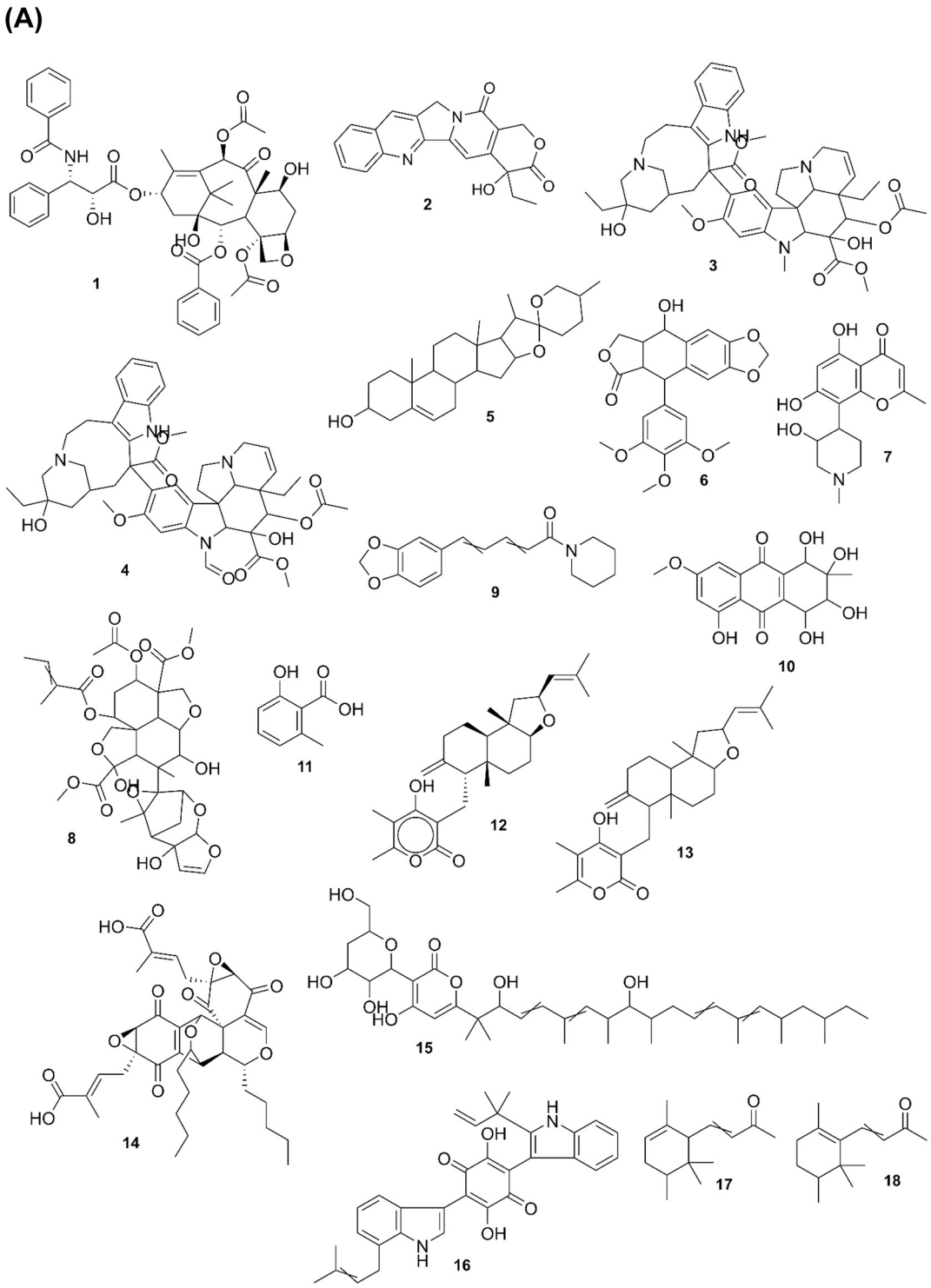


Fig. 1. A: Bioactive metabolites (1-18) isolated from fungal endophytes.

8.1. Cytotoxic compounds from fungal endophytes

Cancer is one of the major cause of death globally, with an increasing number of cases being reported annually (Global Burden of Disease Cancer Collaboration, 2015). The discovery of first anticancer drug [taxol (**1**)] from endophytic fungi (*Taxomyces andreanae*) become a hot topic. Since then many bioactive metabolites [such as camptothecin (**2**), vinblastine (**3**), vincristine (**4**), podophyllotoxin (**6**), rohitukine (**7**), piperine (**9**), torreyanic acid (**14**), cytochalasin 1-3 (**26-28**), cytochalasin E (**29**), graphislactone A (**44**), botrallin (**45**), hypericin (**49**), ergoflavin (**60**), emodin (**64**), scoparasin C (**87**), penochalasin I (**90**), penochalasin J (**91**), malformin E (**98**), fusarithioamide A (**99**), peniproline A (**109**), penochalasin K (**110**)] possessing cytotoxic activity have been isolated from fungal endophytes. The cytotoxic compounds isolated from endophytic fungi belong to various chemical classes (for example: aldehydes, alkaloids, chromones, benzo[*j*]fluoranthenes, cyclohexanones, depsidones, depsipeptides, ergochromes, esters, lignans, polyketides, quinones, spirobisnaphthalenes, diterpenes, sesquiterpenes and xanthones) (Kharwar et al., 2011; Chen et al., 2016; Uzma et al., 2018). More than 100 anticancer compounds have been described from

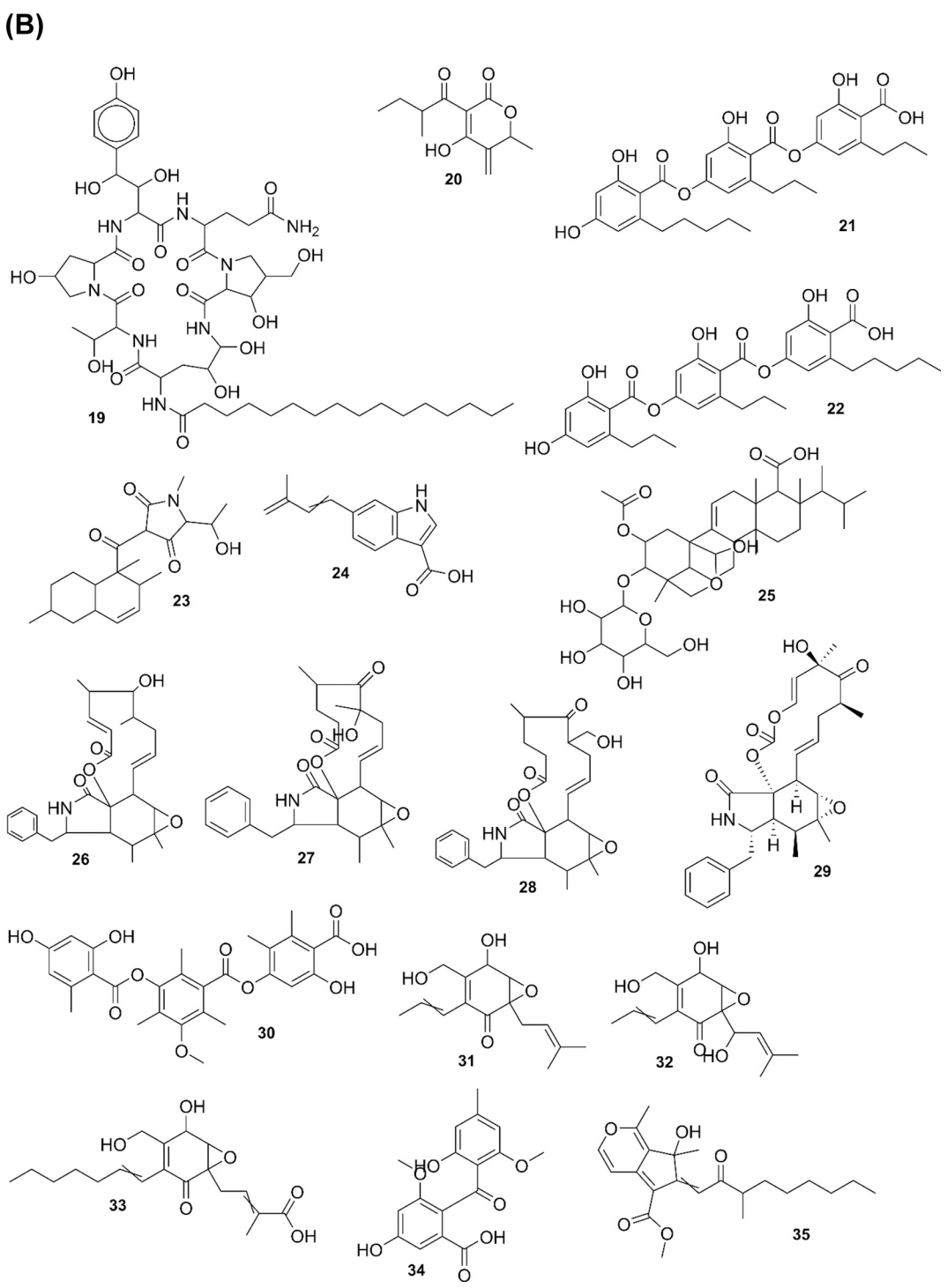


Fig. 1. B: Bioactive metabolites (19-35) isolated from fungal endophytes.

endophytic fungi between the period of 1990–2010 (Kharwar et al., 2011). These compounds have been tested for *in vitro* cytotoxicity screening against various cell lines (for example Human acute monocytic leukemia cell line, A2780S- ovarian tumor cell line, SW-620-colon tumor cell line and HCT-116 colon tumor cell line, MCF-7 breast cancer cell lines, A549- a lung cancer cell line, Vero cell line, human U87MG and U138MG glioblastoma cell lines) using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Mosmann, 1983) and are reported to show potent cytotoxic activity (Kharwar et al., 2011; Uzma et al., 2018; Zaferanloo et al., 2018). The IC_{50} values of many anticancerous compounds reported from endophytic fungi are analogous to those of the standard reference drugs (taxol, vincristine, vinblastine, and podophyllotoxin), signifying potential of fungal endophytes in anticancer drug discovery (Kharwar et al., 2011). Few studies have tried to gain insight mechanisms of such compounds from fungal endophytes against a crystal structures of varied protein targets [such as cyclin-dependent kinases: CDK1, CDK2, CDK4 (protein bank database PDB IDs- 4YC3, 1FVT, 2W96), c-Src tyrosine kinase (PDB 3G5D), estrogen receptor DNA binding protein (PDB 1GWQ), human oxidosqualene cyclase (PDB 1W6K), ERα nuclear receptor (PDB 3ERT), p38a MAP kinase (PDB 3HEG)] of diverse cell lines using *in-silico* molecular docking (Rollando and Hariono, 2017; Moussa et al., 2019). Improved culture and

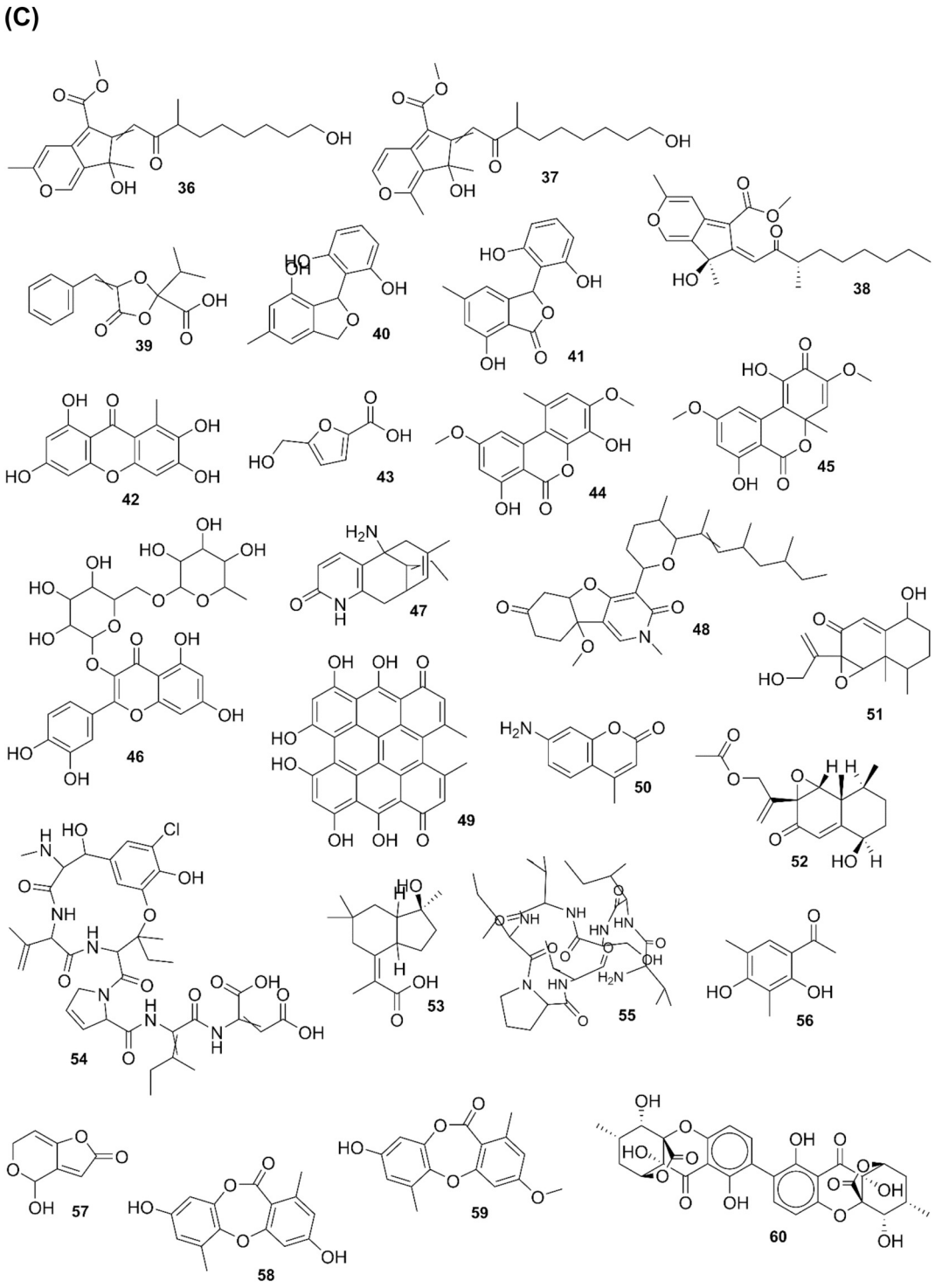


Fig. 1. C: Bioactive metabolites (36-60) isolated from fungal endophytes.

fermentation conditions along with *in vitro*, *in vivo* and *in silico* evaluation, will boost the chances of success in anticancer drug discovery efforts from fungal endophytes.

8.2. Antioxidant compounds from fungal endophytes

Antioxidants are also known as oxidation inhibitors (Pokorny et al., 2001) and are extremely active in combating against reactive oxygen species (ROS). The discovery of novel and safe antioxidants from natural resources to combat or prevent diseases is a continuing process. The secondary metabolites [such as pestacin (**40**), isopestacin (**41**), 2,3,6,8-tetrahydroxy-1-methylxanthone (**42**) 5-(hydroxymethyl)-2-furanocarboxylic acid (**43**), rutin (**46**), corynesidones A and B (**58-59**), borneol (**65**), lapachol (**69**), coumarin (**74**), 2,14-dihydroxy-7-drimen-12,11-olide (**75**), phloroglucinol (**76**), salidroside (**84**) and *p*-tyrosol (**85**)] from fungal endophytes can be a potential source of both known and novel natural antioxidants (Harper et al., 2003; Huang et al., 2007). These natural antioxidants may possess anti-inflammatory, antitumor, antimutagenic or anticarcinogenic activities. Antioxidants are considered as the most promising chemopreventive agents against various human cancers (Kawanishi et al., 2005). The antioxidant compounds from endophytic fungi are of great significance which can decrease the risk of diseases caused by oxidative damage.

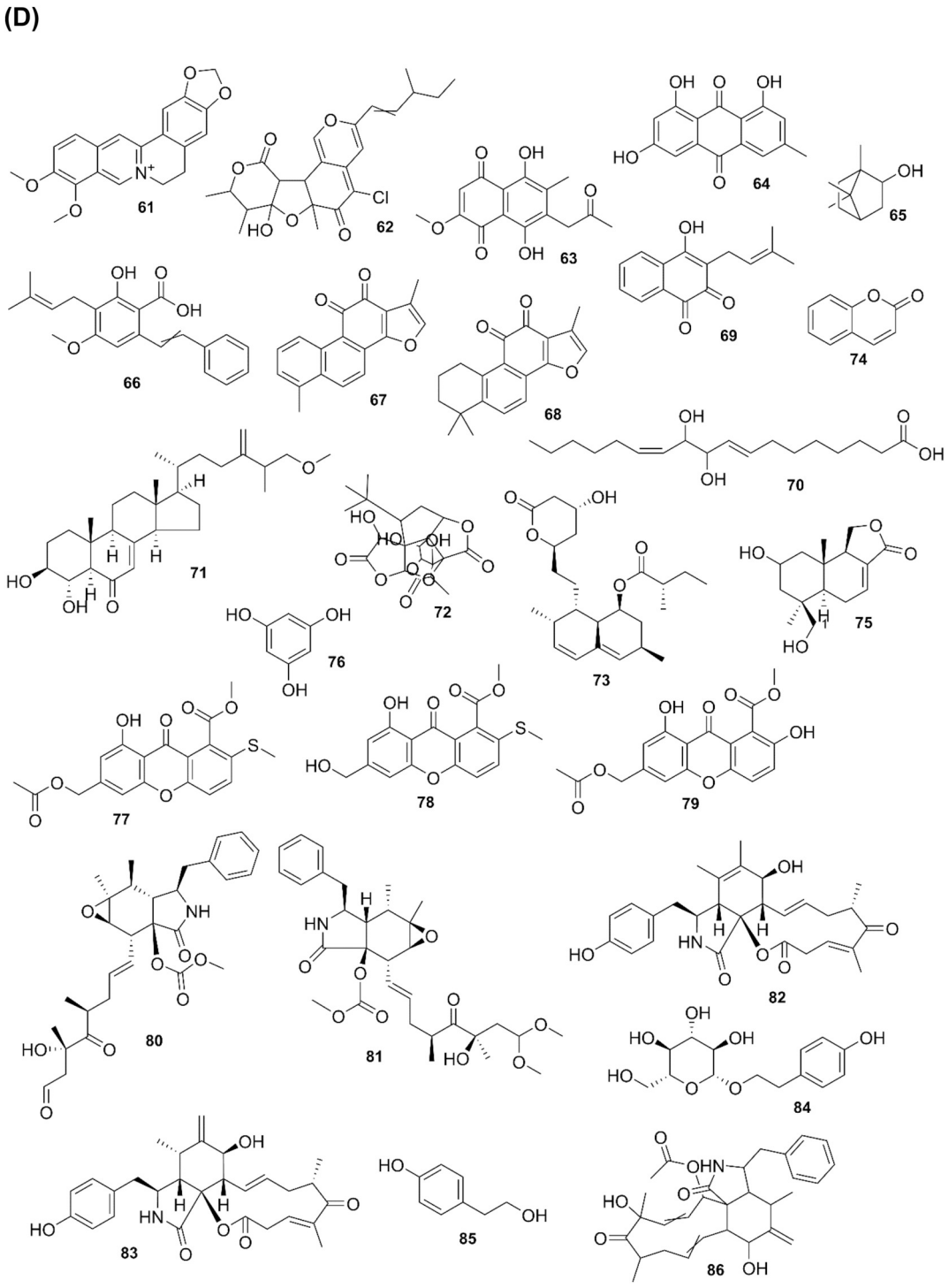


Fig. 1. D: Bioactive metabolites (61-86) isolated from fungal endophytes.

8.3. Antimicrobial compounds from fungal endophytes

The endophytic fungi safeguard their host plant from a variety of pathogens (bacteria, fungi, virus and protozoa) by producing bioactive metabolites (Zhang et al., 2010; Alvin et al., 2014; Zhang et al., 2015a; Pamphile et al., 2017). Compounds such as piperine (**9**), altersolanol A (**10**), 2- hydroxy- 6 methyl benzoic acid (**11**), enfumafungin (**25**), colletotric acid (**30**), jesterone (**31**), hydroxy-jesterone (**32**), guignardic acid (**39**), pestacin (**40**), isopestacin (**41**), rutin (**46**), fusapyridon A (**48**), hypericin (**49**), 7-amino-4-methylcoumarin (**50**), xylarenone A (**51**), xylarenone B (**52**), xylarenic acid (**53**), phomopsin A (**54**), clavatol (**56**), chaetomugilin D (**62**), javanicin (**63**), Z-roquefortine C (**94**), viridicatol (**95**), penitrem A (**96**), penijanthine A (**97**), fusarithioamide A (**99**) pestalone (**106**), fusaripeptide A (**112**) having antimicrobial activity have been isolated from fungal endophytes (Table 1). These antimicrobial compounds from endophytes have anti-bacterial, anti-fungal, anti-viral and anti-protozoan activities (Table 1). Dreyfuss and Chapela (1994) reported that xylotropic fungal endophytes (living in association with woody plant organs) are better producers of antimicrobial compounds. Novel antibiotic sources are always sought for or required due to the increasing drug resistance capacity in bacteria after prolonged usage of a known drug. Many endophytic fungi yield

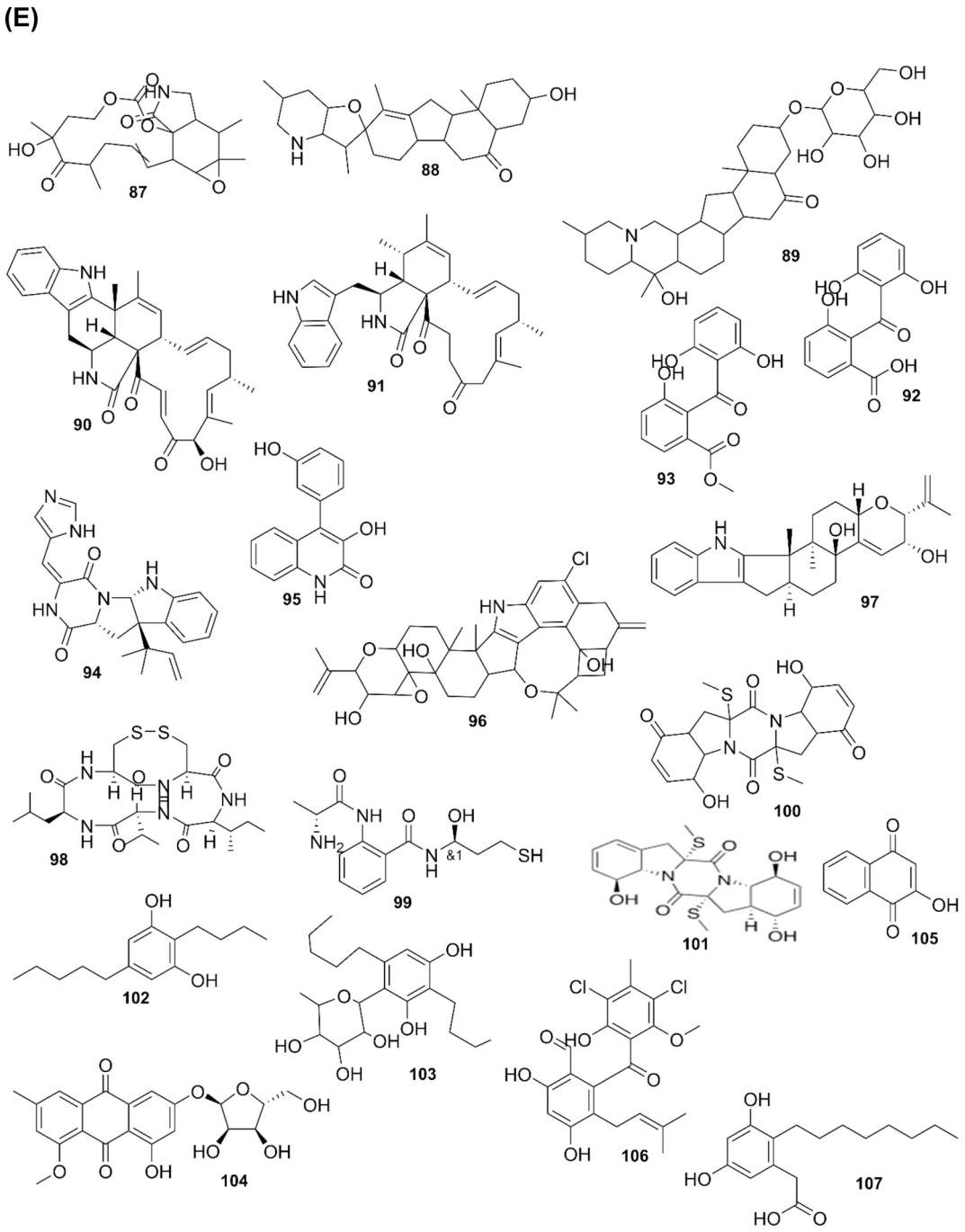


Fig. 1. E: Bioactive metabolites (87-107) isolated from fungal endophytes.

antibiotic compounds in an axenic culture having activity against human and plant pathogenic bacteria. Natural products obtained from endophytic fungi have broad-spectrum activity and lower toxicity as compared to synthetic products.

8.4. Antiviral compounds from fungal endophytes

There is a global need for new antiviral compounds to solve drug resistance problems. The inhibition of viruses by bioactive metabolites from fungal endophytes is intriguing. The potential for discovery of antiviral compounds from fungal endophytes is in its infancy. The lack of appropriate antiviral screening schemes in most compound discovery programs is a major constraint in discovery of antiviral compounds from endophytic fungi. Nevertheless, few promising antiviral compounds [for example podophyllotoxin (**6**), S 39163/F-I (**15**), CR377 (**20**), cytonic acid A (**21**) and B (**22**), sequoiatones C–F (**35-38**), cytosporone U (**107**)] have been reported from fungal endophytes. The antiviral compounds from fungal endophytes are reported to show activity against viruses such as Human Cytomegalovirus (hCMV) (Guo et al., 2000a); Influenza A (H1N1) virus (Zhang et al., 2011); Human Immunodeficiency (HIV) virus (Zhang et al., 2015b) and Dengue virus (Raekiansyah et al., 2017).

8.5. Immunosuppressive compounds from fungal endophytes

Immunosuppressive drugs are used to prevent allograft rejection in organ transplantation and might be used to treat autoimmune diseases such as insulin-dependent diabetes and rheumatoid arthritis (Dangroo

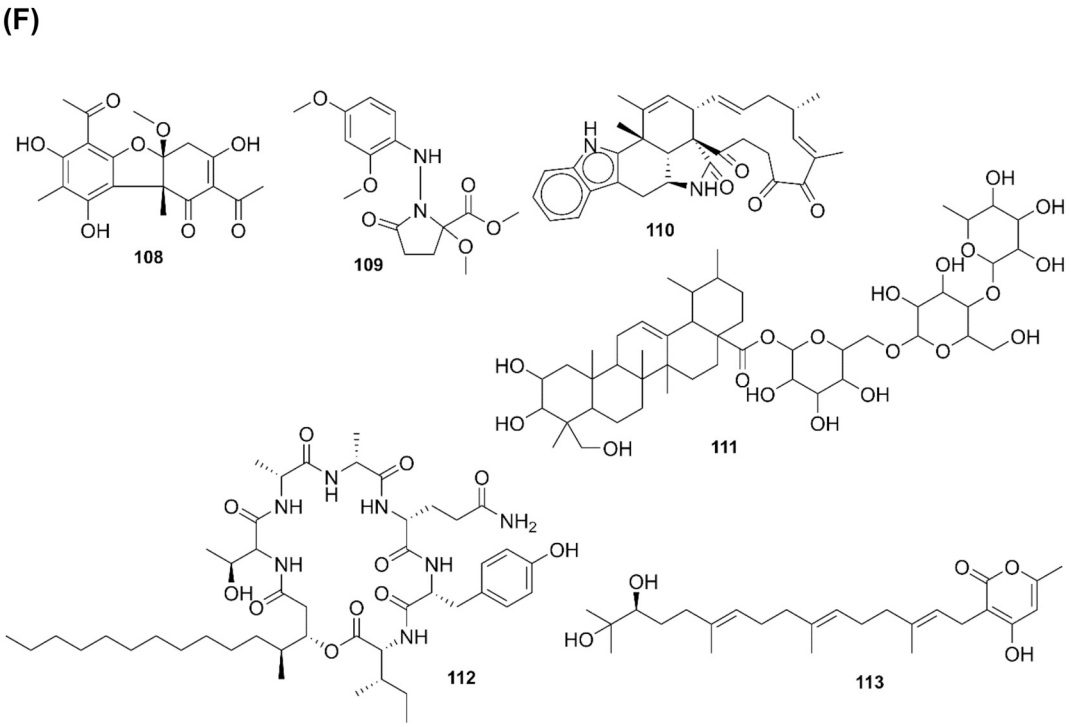


Fig. 1. F: Bioactive metabolites (108-113) isolated from fungal endophytes.

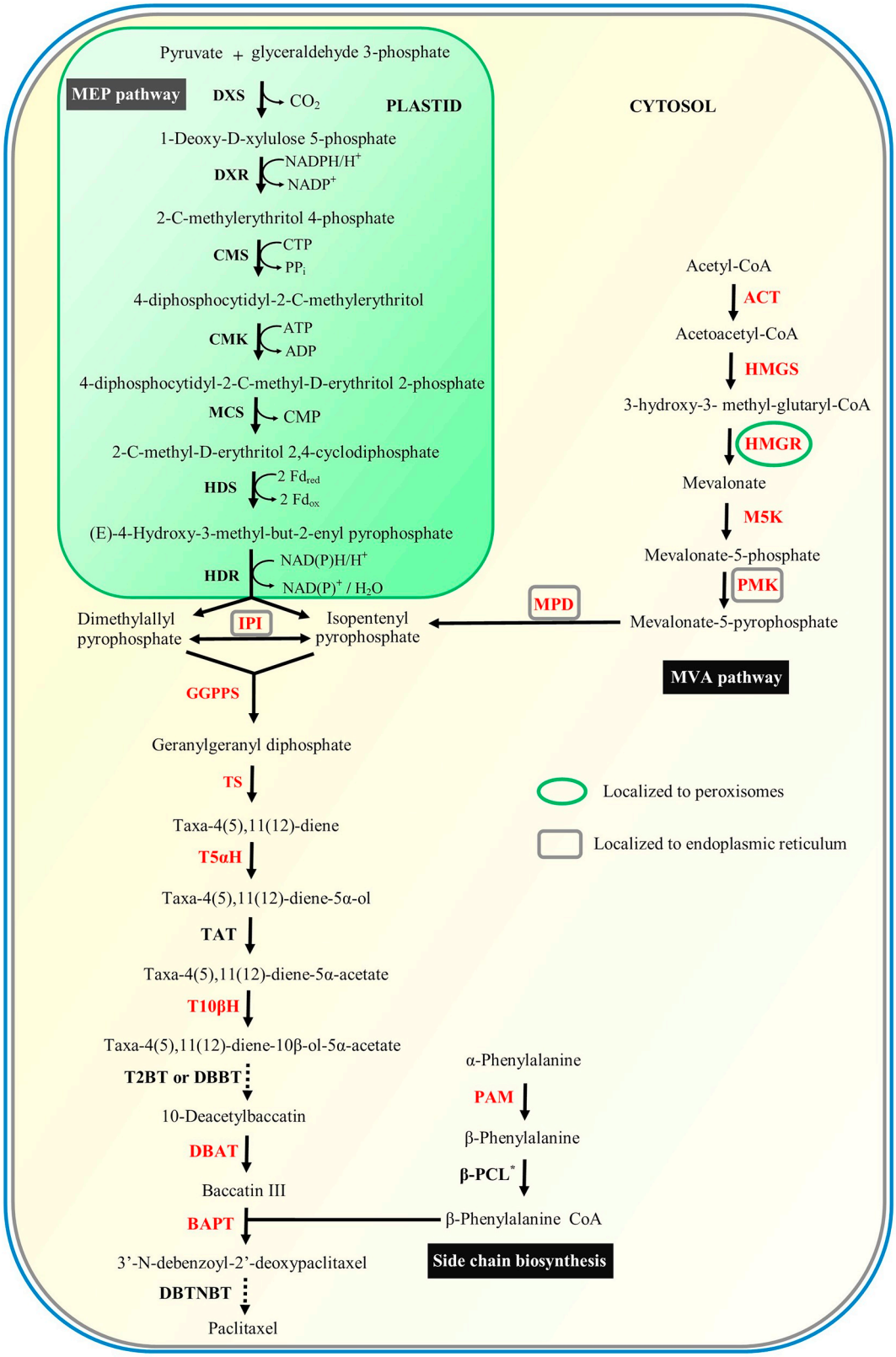
et al., 2016). Most of the chemical immunosuppressive drugs have vague and heterogeneous modes of action, and the consequential adverse effects are challenging in the long-term treatment of the disease (Niethammer et al., 1999). Since the discovery of cyclosporin A from *Trichoderma polysporum* (isolated from soil) it has been the principal immunosuppressive agent (Dreyfuss et al., 1976). However, it possesses serious side effects including nephrotoxicity and neurotoxicity, and the risks of infection, hyperlipidemia and hypertension (Tedesco and Haragsim, 2012). Therefore there is an urgent need to find effective and benign immunosuppressive drugs. Researchers have recently discovered novel immunosuppressant drugs from fungal endophytes, such as subglutinol A (**12**) and B (**13**, benzophenone derivatives), colutellin A (**55**, lipopeptide), sydoxanthone A (**77**) and B (**78**, xanthone derivatives), 13-O-acetylsydowinin B (**79**, xanthone derivative), peniphenone (**92**, polyketide benzannulated spiroketal), methyl peniphenone (**93**, polyketide benzannulated spiroketal) and (-) mycousnine (**108**, dibenzofurane) (Table 1). In a study, it was found that subglutinol A effectively blocks T-cell (T lymphocytes) proliferation and survival *in vitro* and *in vivo* (Lin et al., 2014). Ren et al. (2008) showed that colutellin A inhibited CD4 (cluster of differentiation 4)—T cell activation of IL-2 (Interleukin 2) production with an IC_{50} (half maximal inhibitory concentration) of 167.3 ± 0.38 nM and showed no toxicity on human peripheral blood mononuclear cells. Natural bioactive compounds from endophytic fungi such as subglutinol A and colutellin A may have a great potential for safe immunosuppressive drugs for autoimmune diseases. However, the molecular target(s) and clear mode of action of these drugs are yet to be identified. These important compounds from endophytic fungi may fulfill the crucial demand for new immunosuppressive drugs which might be useful for the treatment of autoimmune diseases and for post-transplantation care.

8.6. Antidiabetic compounds from fungal endophytes

Due to the side effects (viz. diarrhoea, abdominal discomfort) of glycosidase inhibitors (acarbose, voglibose and miglitol) there is a need for new compounds having less or no side effects (Bischoff et al., 1985; Lebovitz, 1997). The biosynthetic products of the endophytic fungi exhibit strong antidiabetic activity and are economically viable. Singh et al. (2015) isolated an endophytic fungus *Cladosporium* sp. from *Tinospora cordifolia*, having strong inhibitory activity against alpha-glucosidase. Govindappa et al. (2015) isolated lectin from *Alternaria* species from the plant *Viscum album* and found that the endophytic fungal lectin possesses potent antidiabetic activity in diabetic rats. A non-peptidal fungal metabolite demethylasterriquinone B-1 (L-783,281) (**16**) from an endophytic fungus *Pseudomassaria* sp. showed insulin-like activity (Zhang et al., 1999a). The oral administration of L-783,281 to two mouse models [db/db (hypoinsulinemic) and ob/ob (hyperinsulinemic) mice, two models of NIDDM (Non-insulin-dependent diabetes mellitus)] of diabetes resulted in significant lowering in blood glucose levels. Uzor et al. (2017) purified (S)-(+)-2-cis-4-trans-abscisic acid, 7'-hydroxy-abscisic acid and 4-des-hydroxyl altersolanol A from an endophytic fungus *Nigrospora oryzae* isolated from leaves of *Combretum dolichopetalum* which exhibited strong antidiabetic activity by significantly reducing the fasting blood sugar of the diabetic mice. A bioactive compound, 8-hydroxy-6,7-dimethoxy-3-methylisocoumarine produced by an endophytic fungus, *Xylariaceae* sp. isolated from the stem of *Quercus gilva* Blume showed high α-glucosidase inhibitory activity (Indrianingsih and Tachibana, 2017). These studies indicate that the endophytic fungi can be a potential source of antidiabetic compounds.

9. Biotransformation by fungal endophytes

The endophytic fungi not only produce novel bioactive compounds but they are also capable of transforming natural products by changing their structures and bioactivities. Biotransformation or bioconversion can be defined as the use of biological systems to produce chemical changes to compounds that are not amongst their natural substrates (Borges et al., 2009). The biotransformation process provides a number of advantages over chemical synthesis. A molecule can be modified by transforming functional groups, with or without degradation of the carbon skeleton. Such amendments result in the development of novel and useful products which is difficult to formulate by chemical methods (Borges et al., 2009). Biotransformation experiments can also be carried



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out for those metabolites which show weak activity on target enzymes to generate potentially bioactive analogues. Microbial transformation is a very efficient and economical way to access active metabolites and novel drugs having biological activity useful to the pharmaceutical industry. Endophytic fungi are able to produce many enzymes (such as oxidases and reductases and hydrolases) that can act as biocatalysts in the chemical transformation of bioactive compounds.

The biotransformation reactions such as hydrolysis, reduction, oxidation and isomerization reactions are involved in drug metabolism in microorganisms (Sih and Chen, 1984). Their ability to modify chemical

Fig. 2. Prevaling consent for taxol biosynthetic pathway showing the 2-C-methyl-D-erythritol-4-phosphate (MEP, present in higher eukaryotes and some bacteria) and mevalonate (MVA, present in all eukaryotes including fungi) pathway leading to the production of taxol by *Taxus* species. (The taxol biosynthetic pathway is proposed to have about 20 different enzymatic steps in *Taxus* plants) Abbreviations: MEP, 2-C-methyl-D-erythritol-4-phosphate; DXS, 1-Deoxy-D-xylulose 5-phosphate synthase; DXR, 1-Deoxy-D-xylulose 5-phosphate reductoisomerase; CMS, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; CMK, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; MCS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate; HDR, (E)-4-Hydroxy-3-methyl-but-2-enyl reductase; MVA, mevalonic acid; IPI, isopentenyl pyrophosphate isomerase; ACT, Acetoacetyl-CoA thiolase; HCS, HMG-CoA synthase; HCR, HMG-CoA reductase; M5K: Mevalonate-5-kinase; PMK, Phosphomevalonate kinase; MPD, Mevalonate-5-pyrophosphate decarboxylase; GGPPS, geranylgeranyldiphosphate synthase; TS, taxa-4(5),11(12)-diene synthase (catalyzes the committed step of taxol pathway); T5αH, taxa-4(5),11(12)-diene-5α-hydroxylase; TAT, taxa-4(5),11(12)-diene-5α-ol-O-acetyltranseferase; T10βH, taxane-10β-hydroxylase; T2BT or DBBT, debenzoyltaxane-20-a-O-benzoyltransferase; DBAT, 10-deacetylbaccatin III-O-acetyltransferase; BAPT, baccatin III 13-O-(3-amino-3-phenylpropanoyl) transferase; DBTNBT, 30-N-debenzoyl-20-deoxytaxol-Nbenzoyltransferase; PAM, phenylalanine aminomutase; βPCL*, β-phenylalanine coenzyme A ligase. * indicates unknown genes and their corresponding enzymes. Dotted arrows indicate more than one biosynthetic step. The red colour represents the genes reported in endophytic fungi (The taxol biosynthesis genes present in endophytic fungi have low homology compared to taxol biosynthesis found in *Taxus* species).

structures with a high degree of stereospecificity and to produce known or novel enzymes facilitate the production of compounds of interest (Azerad, 1999). The endophytic fungus *Diaporthe* sp. (=*Phomopsis* sp.) from *Viguiera arenaria* transformed (-)-grandisin (a tetrahydrofuran lignin) to 3,4-dimethyl-2-(4'-hydroxy-3',5'-dimethoxyphenyl)-5-methoxy-tetrahydrofuran. This compound showed trypanocidal activity similar to its natural corresponding precursor against *Trypanosoma cruzi,* the causative agent of Chagas disease (Verza et al., 2009). Carrão et al. (2011) documented that endophytic fungus *Penicillium crustosum* isolated from *Viguiera robusta* can metabolize albendazole to albendazole sulfoxide. Bier et al. (2017) performed biotransformation of limonene (terpene) by submerged fermentation method using an endophytic fungus *Diaporthe* sp. (=*Phomopsis* sp.) isolated from *Pinus taeda.* Their study produced a wide variety of limonene derivatives (α-terpineol, carvone and limoneno-1,2-diol) at a high concentration. Endophytic fungus *Alternaria eureka* 1E1BL1 isolated from *Astragalus angustifolius* resulted in the conversion of cyclocanthogenol (a sapogenin) in eight metabolites by hydroxylation, oxidation, epoxidation, O-methylation, ring-expansion and methyl migration reactions on the triterpenoid skeleton (Ekiza et al., 2018). The endophytic fungi have potent biotransformation activity and can be efficiently used for biotransformation of natural products and other toxic substances to change their structures and bioactivities.

10. Future prospects and challenges

In recent years, there has been a great interest in bioprospecting fungal endophytes due to their biochemical diversity, which can be utilized for novel natural products or biotransformed products having pharmaceutical applications. However, there is very little information regarding the ecology, evolution, interaction and cross-talks of endophytic fungi with their host plants and other microbial communities. It is important to know how the environmental conditions and host plants transform fungal endophytes to pathogens and/or saprotrophs. A clear understanding of isolation and selection of various endophytes, their mode of entry into the host plants, mechanisms of growth promotion and inhibition, and resistance to disease can immensely contribute to plant sciences and its allied fields. Emerging omics technologies (genomics, proteomics, metabolomics, secretomics, transcriptomics) and associated know-how may help in establishing biochemical and physiological relationships of the host plant and their respective endophytic fungi (Greenbaum et al., 2001). This might facilitate realization of mutualistic association of plant and its endophyte and add value to sustainable production of bioactive compounds (Kusari and Spiteller, 2011). Many endophytic fungi are constantly being discovered which are capable of producing the same bioactive metabolites as that of their host plants or novel metabolites, however, there is a lack of commercial implementation (Kusari and Spiteller, 2011). The biosynthetic pathways of the desired novel bioactive compounds from fungal endophytes also need to be further clarified and resolved for their further pharmaceutical utility. The major bottleneck in extracting the biosynthetic potential of endophytic fungi are low yield and attenuation of the secondary metabolites on repeated subculturing under *in vitro* conditions. No study has yet claimed the cost-effective yield of bioactive metabolites from fungal endophytes. Hence, there is a need to establish the methods that are feasible for commercial production of secondary metabolites in a cost-effective way. Modern techniques such as genome mining, metagenomics and *in silico* profiling of signature genes can help in optimizing the production of bioactive compounds from fungal resources. Molecular and epigenetics-based (development of specific traits by interaction of genes and its environmental factors) protocols along with imitation of the natural habitat as present in host plant may activate silent gene clusters and promote upregulation of novel metabolites (Brakhage and Schroeckh, 2011; Vasundhara et al., 2016). The emergence of several CRISPR/Cas9 based genome editing methods promises to aid discovery of novel natural bioactive compounds and their biosynthetic pathways. Gene targeting techniques aided by comparative metabolic scrutiny can be employed for identification of imperative gene cluster(s). However, this technique is not applicable when the gene cluster of interest is silent and is limited to secondary metabolites that are produced under standard conditions (Brakhage and Schroeckh, 2011). The sequencing of target genes present in an endophytic fungus can also be performed under various growth conditions to determine their expression and function. In natural conditions, interaction of endophytic fungi with surrounding microbes and communication with associated plant host drives the production of bioactive secondary metabolites (Hertweck, 2009). Therefore, simulation of natural conditions such as cocultivating two or more endophytic fungal strains together, and/or addition of plant extract in the culture media might induce the generation of novel metabolites (Huang et al., 2018). Also, there is a need to recognise the mode of action of novel bioactive metabolites from fungal endophytes, their preclinical and clinical development, which can only be achieved by developing precise methodology dealing with ecology, biology, biochemistry, biotechnology and bioinformatics.

11. Concluding remarks

Endophytic fungi are a treasure trove of novel secondary metabolites with immense potential applications in pharmaceutical industries. A clear understanding of how these fungal endophytes associate and communicate with the host plant and other microbial endophytes can be helpful in harnessing their true potential. Intensive studies on impact of host plants and environmental factors in stimulating fungal endophytes to produce bioactive secondary metabolites might be of commercial application. Modern chemical techniques and advanced biotechnological methods aided by sophisticated high-throughput genomic/proteomic approaches promise to aid robust exploitation of secondary metabolite production by the vital fungal endophytic communities.

Declaration of Competing Interest

It is declared that the authors have no conflict of interest in the publication of this article. Neither the manuscript nor its main contents have been published or submitted elsewhere.

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