Shades of fungi – A review of pigments from endophytic fungi

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ABSTRACT
Endophytic fungi are a unique group in the Fungi kingdom as they spend the majority of their life cycles within the living tissue of the host organism without causing apparent harm. The endophyte-host relationship is typically commensalism or mutualistic, with pathogenicity an issue only when either party is under stressed. The contribution of endophytic fungi to the host is mostly in the form of chemical protection – secondary metabolites with bioactivities against invading organisms which may harm the host and consequentially threaten the survival of the endophyte. Many of these chemical compounds have been found to be pigments. Due to easy visual identification, many pigments from fungal sources have been isolated and characterised. This review highlights the potential of endophytic fungi as a source of pigments; with additional focus on significant bioactivity, major chemical classes and biosynthesis. Existing and potential commercial applications of natural pigments by endophytes are also discussed.

Keywords: Endophytic fungi, pigments

INTRODUCTION
Endophytic fungi research has been gaining ground ever since the discovery of taxol isolated from an endophytic fungus in 1993 (Sterle et al., 1993). According to Petrini (1991), the term “endophyte” describes all organisms inhabiting plant organs that, at some time in their life, are able to colonize internal plant tissues without causing any apparent harm to their host. Varied ecological roles are played by endophytic fungi as they are often described as mutualists of forest ecosystems. Due to the symbiotic relationship between host plants and their associated endophytes, endophytic fungi often have unique biochemical pathways in order to produce biologically active secondary metabolites (Strobel and Daisy, 2003). Hence, endophytes are exciting potential sources of novel natural products such as antibiotics and anticancer compounds, many of which has been extracted and identified in the recent past (Strobel et al., 2004; Guo et al., 2008; Wong et al., 2015; Onn et al., 2016).

Pigments are chemical compounds that absorb light at different wavelengths of visible light and reflect those colours of non-adsorbed photons. Generally, all pigments are broadly placed into one of the following three groups according to their origin: natural, synthetic or inorganic (Delgado-Vargas et al., 2000). Natural pigments are produced by living organisms, including microorganisms which produce pigments as a survival mechanism (Rajagopal et al., 1997). Fig. 1 showcases some of the more stunning colours produced by pigmented endophytic fungi. Fungi have been historically associated with the large-scale production of stable pigments, and are viewed with positivity as the next generation of pigment producers as they can be cultivated at low cost, in large quantities, and under non-fastidious conditions. One such class of pigments are the anthraquinones, which have been later identified as prominent fungal secondary metabolites (Huang et al., 2011). Secondary metabolites are often correlated to defence mechanisms as they are biosynthetically derived from more basic primary metabolites for fungal defence in response to stresses (Rajagopal et al., 1997). Further work is still required to prove that secondary metabolite production is directly caused by fungal stress responses.

This review presents information about natural pigments produced by endophytic fungi; characterization of the types of natural pigments, as well as the biosynthesis of the pigments by endophytes. The significance of the pigments produced by these endophytes is based on their potential purposes rather than their chromophoric properties. Many studies have described extraction of these pigments to perform various assays as they are believed to be bioactive secondary metabolites (Strobel et al., 2004; Guo et al., 2008). Generally, fungal pigments are identified upon visual examination of fungal biomass or revealed as crude extracts of fungi are subjected to chromatography. This

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review will also consider existing and potential commercial applications of natural pigments by endophytes, as well as issues which may arise during fermentation and usage as consumer products.

**MECHANISM: BIOSYNTHESIS OF PIGMENTS**

There are many different pigments synthesised by endophytic fungi, many of which follow the same biosynthetic pathways as their plant hosts. For example, quinones and other polycyclic pigments are derivatives of chemical precursors generated by the well-studied Shikimic acid pathway (Ganem, 1978; Siegler, 1998). According to Tan and Zou (2001), endophytes which produce phytochemicals that were originally characteristic of the host may be related to genetic recombination of the endophytes with the host over many years of co-evolution. Such a hypothesis was formulated to explain how the anti-cancer metabolite paclitaxel (originally isolated from yew species Taxus spp.) was later isolated as a fermentation product of endophytic fungus Taxomyces andreanne (Sterle et al., 1993). In a similar way, the biosynthesis of pigments by endophytic fungi may have their coding sequences originate from their plant hosts during events of horizontal gene transfer in their co-evolutionary past.

By mycologists, the presence of pigments associated with endophytic fungi enables for preliminary identification of pure strains; to industry, the deep and vibrant colours can be potentially utilised as naturally derived food colorants and fabric dyes. But to the fungi, the pigments are not for show, those seemingly innocuous coloured compounds play a role in increasing survivability of the species and many pigments have been found to possess bioactivity against major human pathogens (Selvameenal et al., 2009). In a study by Redman et al. (2002) it was demonstrated that Dichanthelium lanuginosum plants were able to grow in geothermal soils only when they harbour a fungal endophyte which conferred thermal protection. They postulated that the thermal protection conferred by the endophyte could be due to fungal melanin which allowed for efficient dissipation of heat absorbed by host plant, as well as scavenging of oxygen radicals generated by plant tissue during heat stress.

There are many way to study the biosynthesis of endophytic fungal pigments. The high throughput method is via genomic characterisation, but more commonly used are specific biochemical response test for certain known pathways. For an investigation into melanin biosynthesis (Suryanarayanan et al., 2004), inhibitors were used to identify specific melanin synthesis pathway undertaken by a fungal strain. One group of inhibitors – tricyclazole, pyroquilon, and chlobenthiazone – inhibited the DHN pathway of melanin synthesis; another group of compounds – tropolone, kojic acid, and diethyldithiocarbamate – inhibited the DOPA pathway of melanin synthesis. The endophytic fungus Phyllosticta capitalensis was exposed to tricyclazole alone, the pigments produced were reddish brown instead of black, indicating that melanin biosynthesis was inhibited. When the Ph. capitalensis strain was exposed to kojic acid (a DOPA pathway inhibitor), melanin production was not affected. Exposure to tricyclazole in the first experiment successfully blocked melanin synthesis by inhibiting reductase enzyme used in the DHN pathway, thereby confirming that DHN pathway is used by that particular strain; whereas the DOPA pathway was not inhibited by kojic acid, therefore not an active pathway for melanin synthesis by that specific strain of Ph. capitalensis. Using this method of testing against distinct inhibitor classes, specific reactions of complex biosynthetic pathways can be verified or rejected.

Research into less common pigment synthesis pathways is on-going, and it is hoped that understanding of complex biochemical pathways can lead to the discovery of novel commercially valuable molecules.

**EXTRACTION OF PIGMENTS FROM ENDOPHYTES**

As this review addresses only pigments naturally produced by endophytic fungi, most methods of pigment extraction involve simple extraction procedures with organic solvents of different polarity. Sticher (2008) discusses conventional methods of natural product extraction such as the Soxhlet extraction, although disadvantages include long extraction times and yield inefficiency. Crude pigment extractions usually utilize solvent extraction before carrying out a purification step to separate crude compounds such as Thin Layer Chromatography (Sharma et al., 2011).

Recent advancements to improve extraction efficiencies involve hybrid techniques such as ultrasound assisted extraction technique which may
reduce extraction times and amounts of solvents consumed (Chemat et al., 2017).

INDUSTRIAL PRODUCTION OF ENDOPHYTIC FUNGI PIGMENTS

Endophytic fungi, especially the filamentous Ascomycota, have been long associated with microbial factories for production of food-grade pigments due to chemical and colour versatility of pigment profile (Vendruscolo et al., 2015). They are advantageous over other pigment sources due to ease of large-scale controlled cultivation as well as reliability. Pigment-producing endophytic fungi have a long history of industrially relevant strains for a great variety of biochemical compounds including for colorants (Dufossé et al., 2014).

In industrial scale pigment production, submerged liquid fermentation is often preferred for easier product recovery and purification. Temperature and pH play critical roles in a typical submerged culture medium by means of activating mechanisms related to genetic and metabolic control of the fungi (Dikshit and Tallapragada, 2013). Concentration and purity of pigments are regulated by the effects of these two factors coupled with the chemical regulation of substrates such as ATP, as well as physical parameters such as speed of agitation of medium.

An example of a suitable endophyte for large scale industrial pigment productions is the ascomycetous fungus of genus Monascus, widely used in Asia as food colorants for red rice and red soya bean (Martinkova et al., 1995; Patakova, 2013; Vendruscolo et al., 2015). By applying existing culture techniques, they grow relatively easily and produce high yields of pigments. Moreover, systematic evaluations carried out have shown that different cultivation media would even induce production of different pigments, hence making these fungal strains highly sought after for optimization of large-scale pigment production (Dufossé et al., 2014).

APPLICATIONS OF PIGMENTS FROM ENDOPHYTES

Based on current research endophytic pigments have many different uses and Table 1 summarises studies on pigments produced by endophytic fungi. All pigments produced are grouped based on their chemical class, such as azaphilones, anthraquinones, polyketides, etc.

Several endophytic pigments have antibacterial and antifungal properties (Table 1) which makes these pigments important as pharmaceutical products. Some of these pigments are industrially important as food colorants and there is evidence that the pigments present in endophytic fungi provide plants the ability to survive extreme environmental conditions such as high salinity and temperature as discussed above. The various uses are discussed in more detail in the following.

Based on past studies (Strobel and Daisy, 2003; Qiu et al., 2010; Dufossé et al., 2014), this review proposes that some fungi actively synthesize secondary metabolites even under non-stressful conditions by overexpression of certain genes throughout its growth cycle. This may be the reason why endophytic fungi are often found to be naturally pigmented even when axenically grown on general purpose medium such as potato dextrose media. Other studies have found that pigment synthesises for some isolates were strongly influenced by the nutrients which the fungi were exposed to; particularly starch (Radu and Fders, 2011).

Use of pigments as colorants

Pigments that have been derived from natural sources such as algae or plants are currently available on the market and certain groups of consumers are inclined towards these products due to their natural origins when compared with completely synthetic colorants (Chattopadhyay et al., 2008). Fungi are capable of producing a wide range of pigments that include melamins, carotenoids, quinines and flavins. Pigments such as Arpink red from Penicillium oxalicum and riboflavin from Ashbya gossypii are already available in the market (Dufossé et al., 2014). Similar to ArpinkRed from Penicillium oxalicum, many other species of Penicillium such as Penicillium herquei, Penicillium persiscium and Penicillium fagi have all been explored as potential sources of natural pigments (Qiu et al., 2010). As can be seen in Table 1, many different types of pigments isolated from a wide variety of endophytic fungi possess the ability of being converted into commercially useable natural colorants. However, any toxic effects of these pigments must be assessed before using them as food products. Among the advantages of using fungal pigments as colorants are the following. Fungi are a readily available source of raw material. Also, the pigments produced possess versatility in their colour and chemical profiles. It is also possible to carry out large scale controlled cultivation using fungal species (Dufossé et al., 2014).

Use of pigments as antibiotic and antifungal compounds

The endophytic fungus Cryptosporiopsis quercina produces cryptocandin and pseudomycins which are a group of antifungal compounds produced by a plant associated pseudomonomad. Both these compounds are obtained from endophytic fungi (Strobel and Daisy, 2003). However, according to Visalakchi and Muthumary (2009), the SVJM139 pigment isolated from the endophytic fungus M. Castaneae possesses antibiotic abilities. This is one of the rare occasions where the pigment produced by the fungus has the capability of acting as an antibiotic and endophytic fungi have since gained attention as antibiotic producers due to their ability to produce coloured pigments with high chemical stability due to their resonance-stabilized chemical structures (Reid, 1965). The SVJM139 pigment exerted varied levels of inhibition against the four pathogenic bacteria Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi, and Vibrio cholerae. It was found to be more effective in controlling these bacteria compared to the antibiotic...
Table 1: Characterization of pigments (all known structures) from endophytic fungi and other relevant information.

<table>
<thead>
<tr>
<th>Name of pigment</th>
<th>Endophytic fungi</th>
<th>Host organism</th>
<th>Colour of pigment</th>
<th>Chemical class</th>
<th>Other functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubropunctatin</td>
<td>Monascus ruber</td>
<td>Zea mays (Corn)</td>
<td>Orange</td>
<td>Antifungal, anti-inflammatory</td>
<td>Haws et al. (1959); Akihisa et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>Ankaflavin</td>
<td>Monascus pilosus</td>
<td>Red yeast rice</td>
<td>Yellow</td>
<td>Immunomodulative effects on mouse T-cells, anticholesterolemic effects</td>
<td>Patakova (2013); Lee et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Monascin</td>
<td>Monascus purpureus</td>
<td>Zea mays (Corn)</td>
<td>Yellow</td>
<td>Antinsectan, anti-inflammation agent</td>
<td>Nishikawa (1932); Lee et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>Monascorubramine</td>
<td>Monascus ruber</td>
<td>Red yeast rice</td>
<td>Red</td>
<td>Antimicrobial</td>
<td>Hajij et al. (1997); Blanc et al. (1995); Martínková et al. (1995)</td>
<td></td>
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<tr>
<td>Rubropunctamine A &amp; B</td>
<td>Monascus purpureus</td>
<td>Wheat/pearl barley/rice</td>
<td>Purple</td>
<td>Antimicrobial</td>
<td>Martínková et al. (1995); Vendruscolo et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>Monapilol A-D</td>
<td>Monascus purpureus</td>
<td>Red yeast rice</td>
<td>Yellow</td>
<td>Anti-inflammatory (against two cancer cell lines)</td>
<td>Patakova (2013); Feng et al. (2012); Hsu et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>Monapurpyridine A</td>
<td>Monascus purpureus</td>
<td>Red mold dioscorea</td>
<td>Orange</td>
<td>Anti-tumor</td>
<td>Hsu et al. (2012)</td>
<td></td>
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<tr>
<td>Lambertellin</td>
<td>Lambertella sp.</td>
<td>Pinus koraiensis</td>
<td>Orange-red</td>
<td>Anti-fungal</td>
<td>Armstrong and Turner (1965); Medentsev and Akimenko (1998); Seo et al. (2009)</td>
<td></td>
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<tr>
<td>Physcion</td>
<td>Alternaria sp.</td>
<td>Aegiceras corniculatum</td>
<td>Yellow</td>
<td>Anthraquinone</td>
<td>Huang et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>Macrosporin</td>
<td>Alternaria pori</td>
<td>Various</td>
<td>Yellow</td>
<td>Anthraquinone</td>
<td>Huang et al. (2011)</td>
<td></td>
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<tr>
<td>Alterporriol K</td>
<td>Alternaria sp.</td>
<td>Aegiceras corniculatum</td>
<td>Red</td>
<td>Antitumor</td>
<td>Suemitsu et al. (1990); Huang et al. (2011)</td>
<td></td>
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<tr>
<td>(fruit)</td>
<td>Penicillium bilaii</td>
<td>Marine algae</td>
<td>Yellow</td>
<td>Antifungal, neurodegenerative disease treatment</td>
<td>Capon et al. (2007); Castillo et al. (2004)</td>
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<tr>
<td>Citromycetin</td>
<td>Penicillium</td>
<td>Phyllostachys edulis</td>
<td>Yellow</td>
<td>Anti-angiogenic</td>
<td>Capon et al. (2007); Castillo et al. (2004)</td>
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<tr>
<td></td>
<td>striatiscoporum</td>
<td></td>
<td></td>
<td>Neurostimulant</td>
<td>Shen et al. (2012)</td>
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</tr>
<tr>
<td>2-O-methylurotine</td>
<td>Eurotium rubrum</td>
<td>Hibiscus tiliaceus</td>
<td>Brown</td>
<td>Anti-angiogenic</td>
<td>Li et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>Isodihydroauroglaucin</td>
<td>Eurotium sp.</td>
<td>Porteresia coarctata</td>
<td>Orange</td>
<td>Neurostimulant</td>
<td>Dnyaneshwar et al. (2002)</td>
<td></td>
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<tr>
<td>Orevaactaene</td>
<td>Epicoccum nigrum</td>
<td>Sugar cane</td>
<td>Orange</td>
<td>Neurostimulant</td>
<td>Capon et al. (2007); Castilo et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>Monacolins</td>
<td>Monascus pilosus</td>
<td>Red yeast rice</td>
<td>Red</td>
<td>Anti-viral</td>
<td>Shu et al. (1997)</td>
<td></td>
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<tr>
<td></td>
<td>Aspergillus</td>
<td>Tribulus terrestris</td>
<td>Yellow</td>
<td>Inhibits cholesterol biosynthesis</td>
<td>Patakova (2013)</td>
<td></td>
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<tr>
<td></td>
<td>fumigatiafns</td>
<td>(Zyophyllaceae)</td>
<td></td>
<td>Antibacterial (broad spectrum Gram positive)</td>
<td>Ola et al. (2014)</td>
<td></td>
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<tr>
<td>Neosartorin</td>
<td>Neosartorya fischeri</td>
<td>Macleaya cordata</td>
<td>Yellow</td>
<td>N.A.</td>
<td>Proksa et al. (1998); Shan et al. (2012)</td>
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<td></td>
<td></td>
<td>(Roots)</td>
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<td>King et al. (1970); Qiu et al. (2010)</td>
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<td></td>
<td>Medentsev and Akimenko (1998); Rezza et al. (2001)</td>
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<td></td>
<td>purpurogenum</td>
<td></td>
<td></td>
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<tr>
<td>Melanin</td>
<td>Curvularia protuberata</td>
<td>Dicantheium</td>
<td>Brown to blackish</td>
<td>Associated with heat protection</td>
<td>Rodríguez et al. (2008); Morsy et al. (2010)</td>
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<tr>
<td></td>
<td></td>
<td>lanuginosum</td>
<td></td>
<td>Antioxidant</td>
<td>Suryanarayanan et al. (2004)</td>
<td></td>
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<tr>
<td></td>
<td>Phyllosticta</td>
<td>Various</td>
<td>Black</td>
<td>Apoptosis inducer</td>
<td>Hiort et al. (2004)</td>
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<td></td>
<td>capitalensis</td>
<td></td>
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<tr>
<td>2-O-methylurotine</td>
<td>Aspergillus niger</td>
<td>Axinella damicornis</td>
<td>Deep green</td>
<td>Polyphenolic</td>
<td>Wu et al. (2011)</td>
<td></td>
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<tr>
<td>Sorbicillin</td>
<td>Trichoderma sp. PR-35</td>
<td>Paenia delavayi</td>
<td>Yellow</td>
<td>Antimicrobial</td>
<td></td>
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<tr>
<td>Hypocrellin</td>
<td>Penicillium</td>
<td>Fagonia cretica</td>
<td>Purple</td>
<td>Antifungal, algicidal, antibacterial, cytotoxic activity</td>
<td>Meng et al. (2011); José et al. (2009)</td>
<td></td>
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<tr>
<td>Xanthoviridicatn E &amp; F</td>
<td>Penicillium chrysogenum</td>
<td>Unknown plant (leaves)</td>
<td>Yellow</td>
<td>Anti-retroviral</td>
<td>Singh et al. (2003)</td>
<td></td>
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streptomycin. According to the authors, further studies should be carried out on the effects of said pigment against a wider range of pathogens and on its mode of action, in order to fully realise its potential as a commercial antibiotic compound (Visalakchi and Muthumary, 2009).

**Pigments as anti-oxidants and anti-inflammatory**

Several studies have identified bioactive secondary metabolites from endophytic fungi with the potential to inhibit tumour progression (Huang et al., 2011; Feng et al., 2012; Patakova, 2013) and/or carcinogenesis (Suryanarayan et al., 2004; Hiort et al., 2004). Some of these compounds exhibiting anti-oxidant and anti-tumor properties were coincidentally pigments (Suryanarayan et al., 2004; Feng et al., 2012; Patakova, 2013). Certain Monascus pigments have exhibited potent inhibitory effects against inflammations, significant disruption of tumor necrosis factor-α and interleukin 6 at both protein and mRNA levels, as well as bind and interfere with expression of inflammatory genes (Akihisa et al., 2005, Hsu et al., 2012).

An example of a noteworthy study reported that blue azaphilone pigments extracted from the endophyte Monascus purpureus NTU 568 exhibited cytotoxic effects to breast cancer cell lines as well as reducing inflammatory responses on lipopolysaccharide-stimulated RAW 264.7 cells (Hsu et al., 2012). Hsu et al. (2012) also described the structure of the pigment relating to its apoptosis-related mechanisms.

**Pigmentation and ability to survive in extreme conditions**

According to Suryanarayan et al. (2004), the ability of the endophyte Phyllosticta capitalensis to have a wide geographical and host range is due to the presence of the pigment melanin in its hyphae. It is believed that the presence of melanin increases its fitness. However, this theory requires further studies through melanin synthesis gene disruption or with mutants that lack the ability to synthesise melanin. The results of these studies will confirm the hypothesis that melanin pigment is in fact the compound providing the endophyte with the above mentioned abilities. Also in a separate study conducted by Sun et al. (2011), it was observed that several common pigmented endophytic species Alternaria spp., Cladosporium spp., and Stemphylium spp. were largely present in plants obtained from several sampling locations. Therefore, it is believed that these pigment-producing fungi give the plants the ability to survive in the halophytic environment. It is also mentioned that similar pigmented fungi were found in several other halophytes. Examples include Camarosporium spp., Colletotrichum gloeosporioides from stems and leaves of Suaeda fruticosa, the salt-marsh fungus Camarosporium roumegueri that produces a dark green pigment from Chenopodiaceae plants (Sun et al., 2011). We believe that pigments produced by endophytic fungi play a significant role in the stress tolerance response of halophytes.

**BRIEF STRUCTURE ANALYSIS ON SELECTED EXAMPLES (BASED ON FUNCTION)**

Endophytic fungi of the Monascus genus are noteworthy for their prevalent pigment production in vitro and in planta. There are six major pigments produced (Feng et al., 2012), with colours ranging from yellow to orange and red. These pigments are all of the azaphilone class, sharing the same parent structure as shown in Fig. 2. The central three-membered ring structure plays an active role in modulating nuclear hormone receptors, whereas the length of the side chains influences the overall efficacy in terms of hydrophobicity and steric hindrance. This class of pigments has been extensively studied ever since the initial discovery by Salomon and Karrer (1932) and is commonly used (intentionally or otherwise) as food colorants in several East Asian countries.

![Figure 2: General structure of an azaphilone class pigment (Chen et al., 2015).](image)

The second most studied class of pigments are the anthraquinones, typified by their aromaticity and multiple fused-ring systems. These compounds are widely occurring in nature as the conjugated electronic structures act as good electron acceptors (Reid, 1965). The basic anthraquinone structure acts as a very suitable chemical precursor in several semi-synthetic dye production (Sutter and Aeschlimann, 2005); many natural derivatives of anthraquinones possessing photochemical properties have also been documented (Del Giacco et al., 2003). Alterporriol K (Fig. 3), an anthraquinone isolated from marine endophytic fungus Alteraria sp. JZ9-6B, was found to be cytotoxic against some human cancer cell lines (Huang et al., 2011).

![Figure 3: Structure of alterporriol K (Huang et al., 2011).](image)
The third most significant class of endophytic fungi pigments are the polyketides, some of which are more notable as mycotoxins as opposed to being edible food colouring agents. Polyketide compounds are structurally diverse and typically have high bioactivity; some may be large closed-chain molecules (e.g. macrolides) although more common are smaller structures composed of fused-rings. Citromycetin (Fig 4) is an example of a fused-ring polyketide pigment. It has been isolated from endophytic fungi of marine algae as well as soil fungi (Capon et al., 2007), indicative of an early development of the synthesis pathway in fungal evolution. This compound and its analogues have been found to have a wide range of bioactivity including anti-fungal, anti-bacterial, and mild cytotoxicity (Tian et al., 2015).

Citromycetin

Figure 4: Structure of citromycetin (Mori et al., 2015).

ISSUES CONCERNING USAGE OF PIGMENTS FROM ENDOPHytic FUNGI

Even though fungal pigments provide many important uses, several issues arise with their use as commercial products. It is known that the success of a product manufactured by fermentation depends largely on its appeal to consumers, investment required and regulatory approval (Dufossé, 2006). The initial issue with the development of these pigments industrially was the large capital investment that was required (Dufossé et al., 2014).

Another problem related to the use of fungal products as food colorants is the public perception of fungal products. However, currently this perception is changing and consumers are more inclined towards buying food that contains natural ingredients (Qiu et al., 2010). The toxicity of some fungal pigments is also an issue that is related to the commercial use of these products.

As an example, pigments produced by Monascus spp., although used as natural food colorants in Asia for centuries, are not authorized for use in the European Union and the United States due to risk of mycotoxin co-production (Mapari et al., 2008). The compound citrinin is a common side-product of fungal pigment production, it is a mycotoxin which will result in serious food poisoning and is given significant attention by regulating bodies of more developed countries.

However, through manipulation of culture conditions, development of strains that are incapable of producing the toxins and also by screening for species that are not harmful, issues related to toxicity can be solved (Dufossé et al., 2014). According to Mostafa and Abbady (2014), in order to prevent mycotoxin by-products, pigment production must occur under suitable nutritional and environmental conditions. For example, Monascus pilosus IFO4520 used in the study of Miyake et al. (2005), does not produce citrinin as a by-product when grown within a specific range of physico-chemical conditions.

Alternatively, on-going bioprospecting efforts (Mapari et al., 2008) offer another solution to this issue. Screening for novel Monascus-like pigments producers which do not generate mycotoxins will potentially result in the discovery of acceptable sources of Monascus-like pigments as food colorants in the USA and EU.

CONCLUSION

There is still much to explore in the area of endophytic fungi research. Endophytes are a unique class of microbes which have undergone a long period of mutualistic coevolution with their host plants, receiving nutrients and shelter while giving back chemical protection to the host. This review attempts to bring awareness to the diversity of endophytic fungi beyond taxonomy and biology, but to an area which might seem so obvious that some scientists take for granted. Pigments derived from endophytic fungi have been found to be unique compared to their non-endophytic counterparts, leading to a greater diversity in colour profile as well as chemical structures. And with higher probability of novel chemical structures, come even more exciting paths to explore in terms of bioactive compounds and bioprospecting niches.

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