



## 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 (Stierle *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.



**Figure 1:** Photographs of pigmented endophytic fungi from different host plants cultured under standard laboratory conditions, From left to right: *Phyllosticta capitalensis*, *Fusarium* sp., *Fusarium oxysporum*, *Neosartorya hiratsukae* (red morph), *Neosartorya hiratsukae* (green morph), *Phyllosticta capitalensis*. All photographs were taken and credited to the authors.

### 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* (Stierle *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.

To 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 they produce 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 ways to study the biosynthesis of endophytic fungal pigments. The high throughput method is via genomic characterisation, but more commonly used are specific biochemical response tests for certain known pathways. For an investigation into melanin biosynthesis

(Suryanarayanan *et al.*, 2004), inhibitors were used to identify specific melanin synthesis pathways undertaken by a fungal strain. One group of inhibitors – tricyclazole, pyroquilon, and chlobenthiazole – 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 *P. 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 *P. 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 inefficiencies 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 (Martínková *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 syntheses for some isolates were strongly influenced by the nutrients which the fungi were exposed to; particularly starch (Radu and Ferders, 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 melanins, 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 persicinum* 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 pseudomonad. 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.

Name of pigment	Endophytic fungi	Host organism	Colour of pigment	Chemical class	Other functions	References
Rubropunctatin	<i>Monascus ruber</i>	<i>Zea mays</i> (Corn)	Orange		Antifungal, anti-inflammatory	Haws <i>et al.</i> (1959); Akihisa <i>et al.</i> (2005)
Monascorubrin	<i>Monascus spp.</i>	Red yeast rice	Orange		Antimicrobial, anti-inflammatory	Vendruscolo <i>et al.</i> (2015); Patakova (2013); Nukina and Marumo (1977)
Ankaflavin	<i>Monascus pilosus</i>	Red yeast rice	Yellow		Immunomodulative effects on mouse T-cells, anticholesterolemic effects	Patakova (2013); Lee <i>et al.</i> (2010)
Monascin	<i>Monascus purpureus</i>	<i>Zea mays</i> (Corn)	Yellow	Azaphilone	Antiinsectan, antiinflammation agent	Nishikawa (1932); Lee <i>et al.</i> (2006)
	<i>Monascus pilosus</i>	Red yeast rice	Yellow		Anti-tumor-initiating, anticholesterolemic effects	Akihisa <i>et al.</i> (2005)
Monascorubramine	<i>Monascus ruber</i>	Red yeast rice	Red		Antimicrobial	Hajjaj <i>et al.</i> (1997); Blanc <i>et al.</i> (1995); Martinková <i>et al.</i> (1995)
	<i>Monascus purpureus</i>	Wheat/pearl barley/rice	Purple		Antimicrobial	Martinková <i>et al.</i> (1995); Vendruscolo <i>et al.</i> (2015)
Rubropunctamine	<i>Monascus purpureus</i>	Wheat/pearl barley	Purple		Antimicrobial	Martinková <i>et al.</i> (1995)
Xanthomonasin A & B	<i>Monascus pilosus</i>	Red yeast rice	Yellow		Anti-inflammatory	Patakova (2013)
Monapilol A-D	<i>Monascus purpureus</i>	Red mold dioscorea	Orange		Anti-proliferative (against two cancer cell lines)	Feng <i>et al.</i> (2012); Hsu <i>et al.</i> (2011)
Monapurpyridine A	<i>Monascus purpureus</i>	Red yeast rice	Yellow		Anti-tumor	Hsu <i>et al.</i> (2012)
Lambertellin	<i>Lambertella sp.</i>	<i>Pinus koraiensis</i>	Orange-red		Anti-fungal	Armstrong and Turner (1965); Medentsev and Akimenko (1998); Seo <i>et al.</i> (2009)
Dimethoxy-1-methyl-2-(3-oxobutyl) anthrakunthone	<i>Fusarium sp.</i>	Mangrove	Yellow	Anthraquinone	Hepatotoxin	Huang <i>et al.</i> (2010)
Physcion	<i>Alternaria sp.</i>	<i>Aegiceras corniculatum</i>	Yellow		Antifungal	Huang <i>et al.</i> (2011)
Macrosporin	<i>Alternaria pori</i>	Various	Yellow		Antibacterial	Suemitsu <i>et al.</i> (1990)
Alterporriol K	<i>Alternaria sp.</i>	<i>Aegiceras corniculatum</i>	Red		Antitumor	Huang <i>et al.</i> (2011)

(fruit)						
Citromycetin	<i>Penicillium bilaii</i>	Marine algae	Yellow		Antifungal, neurodegenerative disease treatment	Capon <i>et al.</i> (2007); Castillo <i>et al.</i> (2004)
	<i>Penicillium striatisporum</i>	<i>Phyllostachys edulis</i>	Yellow			
2-O-methyleurotinone	<i>Eurotium rubrum</i>	<i>Hibiscus tiliaceus</i>	Brown		Antiangiogenic	Capon <i>et al.</i> (2007); Castillo <i>et al.</i> (2004); Shen <i>et al.</i> (2012)
Isodihydroauroglaucin	<i>Eurotium</i> sp.	<i>Porteresia coarctata</i>	Orange		Neurostimulant	Li <i>et al.</i> (2009)
Orevactaene	<i>Epicoccum nigrum</i>	Sugar cane	Orange		Anti-viral	Dnyaneshwar <i>et al.</i> (2002)
Monacolins	<i>Monascus pilosus</i>	Red yeast rice	Red	Polyketide	Inhibits cholesterol biosynthesis	Mapari <i>et al.</i> (2008); Shu <i>et al.</i> (1997)
Neosartorin	<i>Aspergillus fumigatiaffnis</i>	<i>Tribulus terrestris</i> (Zygophyllaceae)	Yellow		Antibacterial (broad spectrum Gram positive)	Patakova (2013)
	<i>Neosartorya fischeri</i>	<i>Macleaya cordata</i> (Roots)	Yellow		N.A.	Ola <i>et al.</i> (2014)
Purpurogenone	<i>Penicillium purpurogenum</i>	<i>Ginkgo biloba</i> L (twigs)	Red		Bio-leaching agent	Proksa <i>et al.</i> (1998); Shan <i>et al.</i> (2012)
Melanin	<i>Curvularia protuberata</i>	<i>Dicanthelium lanuginosum</i>	Brown to blackish		Associated with heat protection	King <i>et al.</i> (1970); Qiu <i>et al.</i> (2010); Medentsev and Akimenko (1998); Rezza <i>et al.</i> (2001)
	<i>Phyllosticta capitalensis</i>	Various	Black	Polyphenolic	Antioxidant	Rodriguez <i>et al.</i> (2008); Morsy <i>et al.</i> (2010)
Cycloleucomelone	<i>Aspergillus niger</i>	<i>Axinella damicornis</i>	Deep green		Apoptosis inducer	Suryanarayanan <i>et al.</i> (2004)
Sorbicillin	<i>Trichoderma</i> sp. PR-35	<i>Paeonia delavayi</i>	Yellow	Polyphenolic	Antimicrobial	Hiort <i>et al.</i> (2004)
Hypocrellin	<i>Penicillium chrysogenum</i>	<i>Fagonia cretica</i>	Purple	Quinonoid	Antifungal, algicidal, antibacterial, cytotoxic activity	Wu <i>et al.</i> (2011)
Xanthoviridicatin E & F	<i>Penicillium chrysogenum</i>	Unknown plant (leaves)	Yellow	Quinone	Anti-retroviral	Meng <i>et al.</i> (2011); José <i>et al.</i> (2009)
Monascumic acids	<i>Monascus pilosus</i>	Red yeast rice	Red	Amino acid derivative	Anti-tumor activity	Singh <i>et al.</i> (2003)
						Patakova (2013); Mostafa and Abbady (2014)

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 (Suryanarayanan *et al.*, 2004; Hiort *et al.*, 2004). Some of these compounds exhibiting anti-oxidant and anti-tumor properties were coincidentally pigments (Suryanarayanan *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- $\alpha$  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.

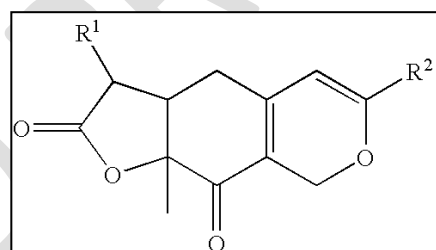
### Pigmentation and ability to survive in extreme conditions

According to Suryanarayanan *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 roumeguerii* 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)

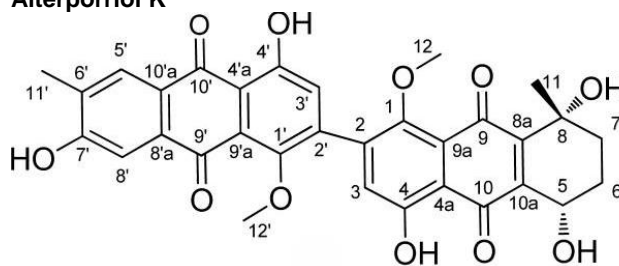
Endophytic fungi of the *Monascus* genus are noteworthy for their prevalent pigment production *in vitro* and *in planta*. There are six major pigments produced (Feng *et al.*, 2012), with colours ranging from yellow to orange and red. These pigments are all of the azaphilone class, sharing the same 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).

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 (*Alternaria* sp. ZJ9-6B), was found to be cytotoxic against some human cancer cell lines (Huang *et al.*, 2011).

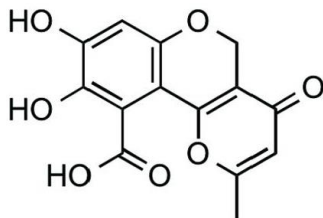
### Alterporriol K



**Figure 3:** Structure of alterporriol K (Huang *et al.*, 2011).

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 Abbadly (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 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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