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A review on pigment producing soil fungi and its applications

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Abstract

Much interest has been brought to microbial pigments owing to their broad-spectrum of industrial applications coupled with their safe and eco-friendly characteristics. Soil fungi have been explored as a feasible resource of natural colouring agents and have been used as a safer alternative to synthetic hues. In addition to the colouring ability, many of the fungal pigments are known to possess antimicrobial, antioxidant and cytotoxic properties, which extend their use in a multidimensional angle. The present review discusses the different pigments derived from soil fungi, the influence of culture conditions on pigment production, various methods of extraction techniques, diverse pigment characterization techniques and their applications. In addition to the advantages of fungal pigments, a lot of challenges pertaining to the use of fungal pigments are also delineated.

Key words – antimicrobial – aromatic polyketides – natural colouring agents – pigment characterization

Introduction

The story of artificial colouring agents began with the synthesis of mauveine by Sir William Henry Perkin in 1856 (Walford 1980). Since then, the synthetic colour industry witnessed a rapid revolution and captured the market due to its ease of production, low cost, lack of unwanted flavours imparted to food, superior hi-quality colouring properties, and requirement for a small quantity to impart colour (Downham & Collins 2000). Nevertheless, synthetic colouring agents often bring about hazardous side effects to human health and the environment. Bowel cancer is developed as a potential side effect of benzidine dyes (Choudhary 1996). In addition to this, carbon black is considered a potential carcinogen (Gardiner et al. 1993). The long term stability of hazardous synthetic dye effluents released as a result of the industrial process brought forth the persistence of toxic contaminants in nature (Babitha 2009). The undesirable side effects of artificial colouring agents and increasing demand for safe colorants stimulated the search for natural sources of pigments.

Plants and microorganisms are considered feasible sources of natural pigments (Sen et al. 2019). Major pigments synthesized by plants include anthocyanins, beta carotenes, betalains, and chlorophyll (Fernández-López et al. 2020, Schoefs 2005). Plant-based pigments are broadly categorized into four classes depending on their chemical structure: alkaloids, carotenoids, polyphenolic compounds and tetra pyrrols (Schoefs 2005). Nowadays, several commercial industries use these plant-derived pigments; for example, chlorophyll pigments extracted from the leaves are used for chewing gums, beverages and cheese spreads (Rodriguez-Amaya 2019).

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Betalains are employed to impart colour to flavoured milk products, frozen food items, etc. (Janiszewska 2014). However, the instability of plant pigments limits the usage of plant-based pigments significantly (Narsing Rao et al. 2017). The stability of plant pigments is considerably controlled by light, pH, temperature, harvesting period and processing method. For example, chlorophyll pigments exhibit low resistance and low stability in response to higher temperature, pH change and light activity (Qaisar et al. 2019). Moreover, the seasonal supply of plants resulted in a limited supply of plant material throughout the year for the extraction of pigments (Kalra et al. 2020). To address the shortcomings of plant-based pigments, microorganisms such as bacteria and fungi are explored for the production of natural pigments (Kalra et al. 2020). Large scale production of high quality pigments within a short period of time and ease of processing made microbes a superior choice for producing natural colouring agents (Lagashetti et al. 2019). Diverse biological activities make fungi potent pigment producers (Akilandeswari & Pradeep 2016). Most pigment-producing fungi are included under the genera *Aspergillus*, *Monascus, Paecilomyces* and *Penicillium* (Gunasekaran & Poorniammal 2008, Mendez et al. 2011). Some of the commercially exploited pigments derived from fungi included red coloured anthraquinones isolated from *Penicillium oxalicum* (Caro et al. 2017), blood-red coloured naphthoquinone from *Cordyceps unilateralis* (Nematollahi et al. 2012), monascorubramin derived from *Monascus* spp. (Mostafa & Abbady 2014) and yellow-tinted riboflavin obtained from *Ashbya gossip* (Tuli et al. 2015). The majority of the pigments synthesized by fungi are quinones, flavonoids, melanins and azaphilones, that form part of the aromatic-polyketide chemical group (Dufosse 2006, Pastre et al. 2007, Venil et al. 2020), and has been described widely for their medicinal and dyeing properties (Kongruang 2011, Teixeira et al. 2012). *Monascus*, a typical ascomycete that produces red pigments, has been widely utilized in the food industry (Fabre et al. 1993). Several species of *Aspergillus* produce azaphilones, aspergillin, asperenone and melanin (Pattenden 1969, Pattenden 1970, Ray & Eakin 1975). Various species of *Monascus* are significant in pigment production and serve as a source of natural polyketide red pigment (Kim et al. 1999, Feng et al. 2012). The major pigment-producing *Monascus* species are *M. froridanus*, *M. purpureus*, *M. pilosus* and *M. ruber* (Mukherjee et al. 2017). *Fusarium oxysporum* synthesises pink-tinted anthraquinone (Gessler et al. 2013). *Fusarium verticillioides* generates yellow-tinted naphthoquinone (Boonyapranai et al. 2008).

Pigment producing fungi have been isolated from various habitats, including soil (Vancov & Keen 2009, Kulkarni & Gupta 2013), water (Gomes et al. 2008, Hussain et al. 2010), endophytic fungi inhabiting in the tissues of healthy plants (Guimaraes et al. 2010, Debbab et al. 2011), marine organisms (Wiese et al. 2011, Liu et al. 2012) and even from arctic glacial ice (Sonjak et al. 2006, 2007). As many of the synthetic hues are found to be hazardous, and the occurrence of a limited number of permitted dyes of interest has been observed in fungi. In this review, we summarise the research on pigment production by soil fungi, as a potential source of natural colouring agents possessing a vast array of applications in diverse industries.

Soil Fungi as Pigment Producers

Soil inhabiting fungi generally consist of four phyla viz. Deuteromycota, Zygomycota, Basidiomycota and Ascomycota (Guiraud et al.1995, Buée et al. 2009, Richardson 2009). Soil fungi play crucial roles in the recycling and redistribution of soil nutrients (Frąc et al. 2018). Fungal hyphae form a filamentous network with rock, soil particles and roots, which secrete enzymes that can digest soil organic matter composed of lignin and cellulose (Gupta et al. 2017). Soil dwelling fungi are highly active in their metabolic processes, and hence they may provide bioactive compounds like organic acids, pigments, extracellular enzymes and antimicrobial compounds (Akilandeswari & Pradeep 2016). Even though fungi are ubiquitous, they can survive in a vast range of temperatures and pHs. Fungi prefer to live in a slightly acidic environment (Frąc et al. 2018). Initially, soil-borne fungi were investigated randomly by researchers for bioactive compounds. Jancic et al. (2016) hypothesised that extreme habitats act as hotspots of microorganisms producing novel bioactive compounds. It is suggested that the successful establishment of fungi in the extreme environment occurs because of the production of unique

secondary metabolites, which have potential uses in commercial industries (Frisvad 2005). For instance, *Aspergillus glaucus* isolated from mangrove sediments in Fujian Province produced yellow coloured (+)-Variecolorquinones A and red coloured Aspergiolide B (Du et al. 2008). Studies have shown that desert habitats also harbour many fungal species, including novel strains (Santiago et al. 2018). Wallimidione is a specialised metabolite extracted from *Wallemia sebi*, inhabiting the Atacama Desert (Jancic et al. 2016). Also, fungi have been found from arctic and antarctic permafrost soils (Bridge & Spooner 2012). *Aspergillus ochraceopetaliformis* excavated from Antarctic soil reported with five new sesquiterpenoids named ochraceopones A–E. Ochraceopones A exhibited antiviral activity against H1N1 as well as the H3N2 virus (Wang et al. 2016). The above-cited literature suggests that unusual habitats are a good choice for investigating soil fungi that can produce novel bioactive compounds.

The research on fungal pigments made through various biosynthetic processes of soil fungi received enormous usage in textile dyeing, food tinting, medical and cosmetics fields (Akilandeswari & Pradeep 2016). Microorganisms that are able to synthesize natural pigments with applications in the dye industry have been identified (Gunasekaran & Poorniammal 2008). In addition to the pigments, different pharmacologically active molecules were also isolated from soil fungi (Takahashi et al. 2008). The different types of pigments extracted from soil fungi along with its applications are reported in Table 1.

Table 1 Examples of different pigments isolated from soil fungi and their biological activity

Fungi	Soil habitat	Pigment	Colour	Activity and Uses	Reference
Aspergillus bridgeri	*Eucalyptus* tree plantation in Lucknow, India	Melanin	Black	Antioxidant activity	Kumar et al. (2011)
Aspergillus niger	Women's Christian College premises, Chennai India	Aspergillin	Black Brown	Antimicrobial activity, Textile dyeing	Ray & Eakin (1975), Atalla et al. (2011), Anchanadevi (2014)
Aspergillus sclerotiorum	Amazonia Brazil	Neoaspergillic acid	Yellow	Antibacterial activity	Teixeira et al. (2012)
Aspergillus versicolor	Mud of the South China Sea, China	Asperversin	Yellow	Antifungal activity	Miao et al. (2012) Li et al. (2018)
Fusarium oxysporum	Not specified	Anthraquinone	Pink / violet	Antibacterial Activity, Textile dyeing	Gessler et al. (2013)
Fusarium verticillioides	Chiang Mai, Thailand.	naphthoquinone	Yellow	Antibacterial activity	Boonyapranai et al. (2008)
Isaria farinosa	Nilgris region of Western Ghats of Tamil Nadu, India	Anthraquinone.	Red	Textile dyeing	Velmurugan et.al. (2010b)
Monascus sp	Egypt	Monascorubrin Rubropuntatin Monascorubramine Rubropuntamine	Orange Red	Antibacterial activity Anticancer activity Antioxidant	Babitha et al. (2008), Babula et al. (2009), Moharram et al. (2012), Yang et al. (2014)

Table 1 Continued.

Fungi	Soil habitat	Pigment	Colour	Activity and Uses	Reference
Monascus sp.	Not specified	Ankaflavin Monascin	Yellow Yellow	Food colorant Pharmaceuticals	Juzlova et al. (1996), Mostafa & Abbady (2014)
Penicillium herquei	Not specified	Atronenetin	Yellow	Antioxidant activity	Takahashi & Carvalho (2010)
Penicillium oxalicum	Not specified	Anthraquinone	Red	Anticancer effect in food and pharmaceuticals Textile Dyeing	Dufosse, (2006), Atalla et al. (2011)
Penicillium purpurogenum	Brazil	Mitorubrinol	Red	cosmetics, pharmaceuticals, Textile industry	Santos-Ebinuma et al. (2013)
Penicillium sclerotiorum	Amazon soil, Brazil	Sclerotiorin	Yellow to orange	Antimicrobial activity	Lucas et al. (2007), Lucas et al. (2010); Celestino et al. (2014)
Penicillium striatisporum	Boat ramp on the Huon estuary, Australia	citromycetin, citromycin, (–)-2,3-dihydrocitromycetin	Yellow	Cytotoxic activity	Capon et al. (2007)
Trichoderma virens	Delhi, India	Viridol Virone	Yellow	Antifungal Activity, Textile dyeing	Mukherjee & Kenerley (2010), Sharma et al. (2012), Kamala et al. (2015)
Trichoderma viride	Institute of Home Economics premises, Delhi, India	Viridin	Yellow Green Brown	Antifungal activity, Food industry, Textile dyeing	Chitale et al. (2012), Neethu et al. (2012), Gupta et al. (2013)

Aromatic polyketides such as citromycin, citromycetin and di-hydro analogue (–)-2,3-dihydrocitromycetin were isolated from an Australian terrestrial isolate of *Penicillium striatisporum* (Capon et al. 2007). Sclerotiorin was isolated from *Penicillium sclerotiorum* (Curtin and Reilly, 1940) and several other fungi (Chidananda et al. 2006, Giridharan et al. 2012). *Penicillium sclerotiorum* 2AV2, *Penicillium sclerotiorum* 2AV6, *Penicillium citrinum* 2AV18, *Penicillium purpurogenum* 2BV41 and *Aspergillus calidoustus* 4BV13 inhabiting in the Amazon forest soil were identified as potential pigment producers. An intensely coloured yellow-orange pigment was produced by *Penicillium sclerotiorum* 2AV2, which was the first report of sclerotiorin pigment synthesis by the Amazon soil fungi (Celestino et al. 2014).

Approximately 40% of the soil fungi isolated from Agumbe and Koppa regions of the Western Ghats were reported to produce water-soluble pigments associated with the genera *Aspergillus*, *Curvularia*, *Fusarium* and *Trichoderma* (Mukunda et al. 2012). *Penicillium* spp.,

Fusarium spp., *Emericella* spp., *Isaria* spp., and *Monascus purpureus* isolated from Nilgiris soil sample produced yellow, reddish-brown, red, pink and red pigments, respectively (Velmurugan et al. 2010a). Fungal derived red coloured pigments were extensively studied by Hamano & Kilikian (2006). Red coloured anthraquinone pigment from *Isaria farinosa* exhibited high stability at 60°C and below 60°C. A range of colour shades was detected under different pH (Velmurugan et al. 2010b). A large number of pigment-producing fungi are observed in forest soils compared to other sources (Akilandeswari & Pradeep 2017). *Aspergillus terreus* KMBF1501 isolated from interior parts of Idukki district, Kerala, India produced an intense yellow coloured pigment. The high pigment production capacity of *Aspergillus terreus* KMBF1501 could be exploited for various industrial purposes (Akilandeswari & Pradeep 2017). *Aspergillus nidulans*, *Fusarium moniliforme*, *Penicillium purpurogenum* and *Phoma herbarum* were isolated from soil samples from Cairo – Alexandria agriculture road, Egypt. The extracellular pigment of *Penicillium purpurogenum* was observed to be active against some pathogenic microbes and possessed a potential role in the pharmaceutical drug industry (Geweely 2011).

In some opportunistically pathogenic fungi, virulence is ascribed due to the production of melanin or melanin like compounds (Nosanchuk & Casadevall 2003), playing no vital role in the growth and development of fungi. Melanin confers enhanced survival under desiccation, extreme temperatures, U.V radiation and in the presence of cell wall degrading enzymes (Cohen et al. 1991, Fogarty & Tobin 1996, Riley 1997). *Aspergillus bridgeri* ICTF-201 isolated from soil of *Eucalyptus* tree plantation in Lucknow, India, synthesized a blackish-brown pigment, which was confirmed as melanin by U.V, FTIR and EPR spectroscopic analyses (Kumar et al. 2011). Microorganisms generally produce melanin by either DHN (1,8-dihydroxynaphthalene) pathway or the DOPA (3,4-dihydroxyphenylalanine) pathway (Tran-Ly et al. 2020). The type of melanin produced by *Aspergillus bridgeri* ICTF-201 was identified as DHN melanin. The compound possessed a prominent antioxidant activity and employed as a naturally occurring antioxidant with applications in the cosmetic field (Kumar et al. 2011).

Indian Himalayan region is considered a biodiversity hotspot having unique climatic conditions. Himalayan soil acts as a repository of microorganisms important for biotechnological applications (Pandey et al. 2018a). Dhakar et al. (2014) reported *Penicillium* as the dominant genera in Himalayan soil. *Penicillium* sp. (GBPI_P155), inhabiting the high altitude soil of the Indian Himalayan region, produced orange coloured carotenoid pigment along with flavonoids and anthracene (Pandey et al. 2018b).

From various literatures, it is evident that soil fungi isolated from different habitats belong to the genera *Aspergillus, Fusarium, Isaria, Monascus, Paecilomyces, Penicillium* and *Phoma* (Celestino et al. 2014). The bulk of the pigments synthesized by these soil fungi are naphthoquinones, polyketides, flavins, quinones, anthraquinone and ankaflavin (Dufosse et al. 2005). The high stability and antimicrobial properties of these fungal derived pigments provide immense benefits to humankind.

Factors Influencing Pigment Production

From the point of pigment and biomass production, culture environment plays a pivotal role. Enhanced production of biomass and pigment could be achieved by manipulating media components, culture conditions such as temperature, pH, salt concentration, light, inoculum age and various culture configurations like agitation, static, aeration, solid or liquid media etc. (Kalra et al. 2020). Nitrogen source is very crucial for fungal biomass and pigment production (Pradeep et al. 2013). Nitrogen sources are involved in the expression and regulation of genes related to the production of these compounds (Chatterjee et al. 2009). Czapeck medium modified with nitrogen containing materials like peptone and yeast extract enhanced sclerotiorin production by six times (Celestino et al. 2014). Peptone is generally employed in various culture media, and many fungi possess the capability to utilize it during its metabolism, leading to increased production of pigments (Chatterjee et al. 2009). Diammonium phosphate, as an inorganic nitrogen source, enhanced pigment and biomass yield in *Isaria farinosa* (Velmurugan et al. 2010b). Inorganic

nitrogen in the form of sodium nitrate also produced the same effect (Akilandeswari & Pradeep 2017).

Sclerotiorin synthesis was accelerated by using rhamnose sugar instead of sucrose in the Czapeck medium (Celestino et al. 2014). In the case of *Monascus purpureus*, carbon source in the form of fructose increased the pigment formation, whereas for *Moascus ruber* a significant increase was achieved with lactose (Tseng et al. 2000, Costa & Vendruscolo 2017). For *Fusarium moniliforme*, the addition of glucose in the medium resulted in maximum pigment production (Pradeep & Pradeep 2013). Besides carbon and nitrogen source, microbial pigment production is greatly affected by trace elements (Anchanadevi. 2001). Modification of common PDB medium with Mg_2SO_4 accelerated pigment production capacity in *Aspergillus* sp. (Akilandeswari & Pradeep 2017). Similarly, maximum pigment production was noticed in *Isaria fariosa* at a concentration of 10 mM $CaCl_2$ (Velmurugan et al. 2010b).

Melanin synthesising enzymes like laccases and tyrosinases regulate the production of melanin (Tran-Ly et al. 2020). The addition of copper to the medium enhances melanin synthesis, as this metal acts as a cofactor for tyrosinases and laccases (Sendovski et al. 2011). Metals like nickel and iron can stimulate stress response in microbes and thereby increase the production of melanin (Gowri & Srivastava 1996).

Temperature and pH perform a crucial part in pigment production because physiological activities and metabolic processes of fungi depend on culture temperature and pH. Culture temperature and pH control growth, primary and secondary metabolite production and fermentation (Mendez et al. 2011). The ideal temperature for pigment production in many *Monascus* species ranges between 30 and 37°C (Babitha et al. 2007). Dufosse et al. (2005) reported that fungi preferred slightly acidic pH for better growth and activity. *Monascus purpureus* produces four different pigments such as rubropunctatin, monascorubramine, ankaflavin and monascin. A low pH (pH 4) favoured ankaflavin synthesis and growth; the other three pigments remained independent of pH (Chen & Johns 1993). In *Aspergillus terreus*, alkaline pH reduced pigment production. However, optimum pigment production was accomplished at pH 5 and a temperature of 27°C (Akilandeswari & Pradeep 2017).

Pigment generation in fungi is significantly affected by light. An increase in biomass and pigment yield was noted in *Emericella nidulans*, *Monascus purpureus*, *Fusarium verticillioides*, *Isaria farinosa* and *Penicillium purpurogenum* in response to total darkness (Velmurugan et al. 2010c). Moreover, incubation at the dark favoured maximum production of intracellular and extracellular red pigment in *Monascus purpureus* (Velmurugan et al. 2010c). The wavelength of light also had an impact on pigment synthesis. Exposure of *Monascus ruber* with red light yielded maximum red pigment, suggesting the role of photoreceptors in fungal physiology (Buhler et al. 2015). *Alternaria alternata* had an enhancement in red-brown pigment production upon exposure to blue light. Unlike *Monascus ruber*, red light had little effect on pigment formation in *Alternaria alternate* (Häggblom & Unestam 1979).

Extraction of Pigments

Extraction of extracellular and intracellular pigments from growth medium and mycelia is of utmost importance, as it can interfere with the qualitative and quantitative properties of pigments. The selection of an easy and cost-effective extraction protocol is challenging because of the effects from extraction conditions and the solvents, purity of the compounds and the extraction efficiency (Lebeau et al. 2017). Organic solvent extraction (soxhlet, homogenization, and shaking), centrifugation extraction, hydro-distillation, and steam distillation have been used as conventional extraction methods (Kalra et al. 2020). A diagrammatic representation of various pigment extraction techniques and characterization of the pigments from soil fungi is given in Fig. 1.

Extracellular Pigment Extraction

Extracellular pigments are usually water-soluble and hence do not need any organic solvent for its extraction. The pigmented compound is extracted from the fermented broth by an aqueous two-

phase system method (Iqbal et al. 2016). It is a liquid-liquid partition technique where different hydrophilic solutes separate into two immiscible aqueous phases as per their distinctive selectivity over various salt-salt, polymer-polymer or polymer-salt and solute blends developed in aforesaid two levels. Although this method is based on an aqueous system, it is considered a safe, green and economically feasible method (McQueen & Lai 2019).

Ventura et al. (2013) investigated the use of ionic liquid-based aqueous two-phase systems as extraction processes for the recovery of red colouring agents from the fermented broth of *Penicillium purpurogenum* DPUA 1275. Manipulation of some of the process conditions, like the use of quaternary ammonium with short alkyl chains, alkaline media, and short tie-line lengths (extraction point systems with lower concentrations of ionic liquid) resulted in better isolation of colouring agents from contaminants.

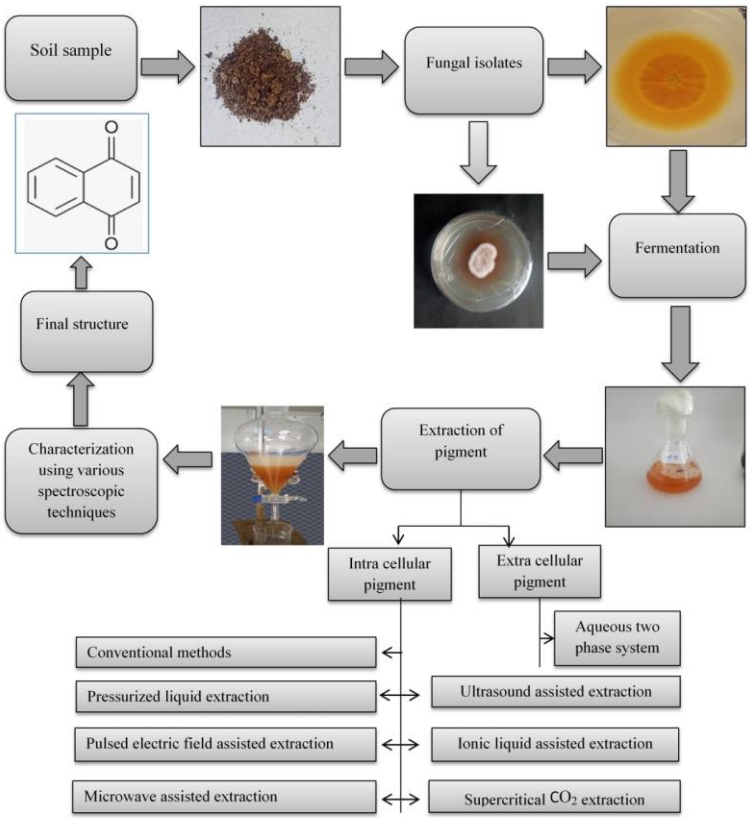


Fig. 1 – An overview of the pigment extraction and characterization from soil fungi.

Intracellular Pigment Extraction

Techniques such as ultrasound-assisted extraction (UAE) (Vilkhu et al. 2008, Cheung et al. 2012), pressurized liquid extraction (Lebeau et al. 2017), microwave-assisted extraction (MAE) (Vazquez-Delfin et al. 2014), pulsed electric field (PEF) assisted extraction (Goettel et al. 2013), supercritical CO_2 extraction (Cocks et al. 1995, Chaudhari 2013) and ionic liquid – assisted extraction (Ventura et al. 2013) are some green extraction techniques employed in the extraction of bioactive compounds from various natural resources.

Although the above-mentioned techniques have been successfully employed for pigment extraction from microalgae, plants and bacteria (Poojary et al. 2016), only a few attempts have been made by researchers to isolate pigments from soil fungi using the green extraction methods. The pressurized liquid extraction procedure was employed to extract polyketide red pigment from soil fungi, *Talaromyces* spp. (Lebeau et al. 2017). *Rhodotorula glutinis* is a carotenoid pigment-producing yeast commonly distributed in soil, air and phyllosphere (Aksu & Eren 2007). Recently,

Martinez et al. (2020a) employed a combination of ultrasound treatment and pressure to extract carotenoid pigment from *Rhodotorula glutinis* and found it to be effective over conventional extraction methods. As the innovative green extraction techniques are safe, eco-friendly and economically viable methods of pigment extraction, fungal pigment extraction using these novel techniques has emerged as a recent area of research (Kalra et al. 2020).

Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction is a process that has been designed recently for analysing food, biological samples and environmental samples (Carabias-Martínez et al. 2005). Lebeau et al. (2017) developed a six-step PLE as an advanced protocol for mycelial pigment extraction of a polyketide red pigment-producing organisms like *Talaromyces* spp., *Penicillium purpurogenum*, *P. rubisclerotium*, *Trichoderma atroviride* and *Fusarium oxysporum*, isolated out of the marine environment of the Reunion Island. Drawing out intracellular pigments was performed using lyophilized biomass, which was then subjected to PLE extraction sequence using different solvents selected according to the descending order of their polarity. Extraction is initiated with water after that 50% methanol, further 50% EtOH, >99.9% MeOH, and methanol: ethanol (1/1, v/v), finally >99.9% ethanol are used to deplete the mycelium. At the completion of PLE, the extract may be obtained within the collection bottle, which can be stored at –20°C (Lebeau et al. 2017). The efficient and optimum yield of pigment from *Talaromyces* species confirmed the use of PLE as an excellent method for fungal pigment extraction (Lebeau et al. 2017). PLE is developed as an advanced technique over solvent extraction techniques with less extraction time and minimum solvent requirements (Mustafa & Turner 2011). The extraction process is performed under high pressure and temperature conditions, which ensure efficient penetration of solvent into the sample as well as increased solubility and diffusion rate of metabolites (Richter et al. 1996).

Pulsed Electric Field (PEF) Assisted Extraction

It works on the idea of electropermeabilization. If we expose a sample of tissues to a short impulse of a strong electric field, it degrades the cell membrane and affects membrane permeability. Membrane permeability allows diffusion of solvents into the cell and extraction of secondary metabolites. Parameters like amplitude, exposure time, intensity, number and frequency of the electric waves determine the reversibility of the effect on membrane disintegration and membrane permeability (Kalra et al. 2020, Martinez 2020b).

Microwave-Assisted Extraction (MAE)

Electromagnetic frequencies graduating between 300 MHz and 300 GHz are considered microwave radiation. Based on the dipole moments, a sample in the solvent mixture is heated using microwave radiation energy (Li et al. 2013, Xiong et al. 2016). MAE requires less extraction time with minimum solvent, and hence it is considered as an advanced and efficient extraction method. Generally, high-value bioactive pigments are extracted using this technique, ensuring the extract quality (Pare et al. 1991). Nature of substrate, type of solvents, solid-liquid ratio, pressure, temperature, and particle size are the agents that regulate the efficiency of MAE extraction (Chupin et al. 2015).

Ultrasound – Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) relies on ultrasound pressure waves of high intensity for the extraction of compounds. The acoustic energy carried through an environment with the assistance of pressure waves can result in vibrational motion set forth by the molecules (Tiwari 2015). These waves generate localized pressure to rupture the tissue and thereby release intracellular substances into the solvent used. UAE has several advantages such as faster kinetics, relatively simple and cheap apparatus and applicability on a wide range of water-soluble bioactive compounds (Vilkhu et al. 2008).

Ionic liquid extraction (IL)

Ionic liquids are developed as a suitable solvent for the extraction of natural compounds. In a recent study, protic ionic liquids are used as an efficient cell lysing agent and extracted intracellular carotenoids from *Rhodotorula glutinis* (Mussagy et al. 2019). Ionic liquids can be used with water or organic solvents for the extraction of metabolites from biomass. Ionic liquid extraction can be used in combination with MAE and UAE because ILs can interact with the electromagnetic field (Ventura et al. 2017).

Supercritical Extraction (SFE)

Liquified carbon dioxide gas is utilized for isolating of biologically effective compounds (Khaw et al. 2017). This technique is developed recently by utilizing the property of specific gases as a supercritical fluid. Gases like carbon dioxide above their critical limit possess combined properties of liquid and gas, which can act as extracting fluid. The dissolving power of a supercritical fluid can be controlled by adjusting parameters like pressure and temperature of the extraction process (Zabot et al., 2012). As SPE allows adjustment in temperature, extraction of heat labile-bioactive compounds can be performed at low temperatures.

Dichloromethane (DCM) for Pigment Extraction from Fungal Agar Plates

Hinch & Robinson (2016) developed an alternative method for pigment extraction from agar plates. The agar plates were kept in a fume hood for drying mycelia. Dried mycelia were cut into small pieces and crushed. The plates were opened and kept in the fume hood to allow the mycelia to dry out. After drying, the mycelia were sliced into tiny bits of about 2 cm. and crushed. The mycelia were further taken in a round bottom flask, after crushing with 150 ml DCM and stirred for 30 minutes at 230 rpm. The contents were then filtered through Whatman filter paper to obtain the pigment.

Ethanol Extraction

One of the most common methods used for fungal pigment extraction is by using 95% v/v ethanol. The process consists of two stages, initially, 60% of the total solvent is added and is kept at 30° C for 30 minutes in a rotary shaker at 180 rpm. After 30 minutes, the ethanol mixture is centrifuged for 15 minutes at 3700-3800 rpm in order to remove any residual mycelia and centrifuged again for 5 minutes at 3790 rpm. The supernatant is collected and filtered through Whatman filter paper for further analysis (Velmurugan et al. 2010a, Sharma et al. 2012). Moharram et al. (2012) derived red dyestuff from *Monascus ruber* using 95% ethanol. The extraction process was completed in 24 hours by agitating at 10,000 rpm. The extract was filtered and dried using anhydrous sodium sulphate. Babitha et al. (2007) used 90% ethanol in the ratio of 5 ml per gram of fermented material followed by blending for one hour around 200 rpm.

Chloroform Extraction

Pandey et al. (2018b) extracted an orange pigment from *Penicillium* sp. using chloroform. An equal volume of chloroform was added to the culture broth after removing the mycelium and pigment was extracted in the organic phase after adequate blending. The chloroform separated part was air-dried and dissolved in minimal volume of chloroform for subsequent analysis.

Characterization of Pigments

Aromatic polyketide category like melanins, anthraquinones, quinones and flavins pigments are found commonly in fungi. (Dufosse et al. 2005, Dufosse 2006). Structure elucidation of fungal derived pigments was carried out generally by physical or spectroscopic methods. Advancements in the field of optics, atomic physics and nuclear magnetism have enabled the precise identification of pigments using optical spectra, NMR spectra, electron spin resonance spectra, etc. (Butnariu 2016). Different structural particularities of the compound can be revealed by U.V., VIS, I.R and Raman

spectroscopy (Andersen & Francis 2018). Chemical structure and related information of various pigments isolated from different soil fungi are provided in Table 2.

Table 2 Chemical structure of pigments isolated from soil fungi and their related information

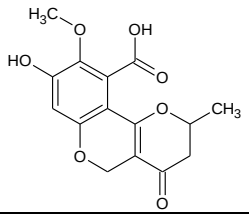
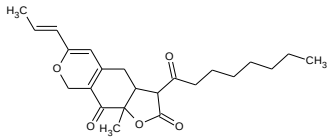
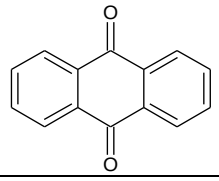
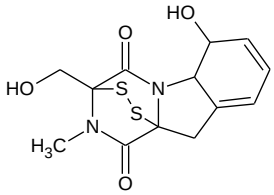
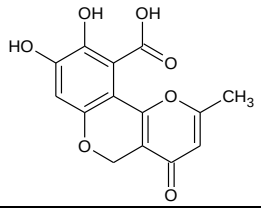
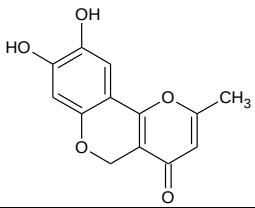
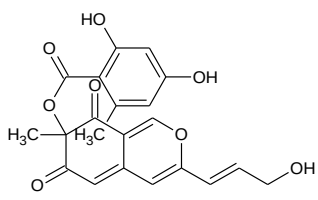
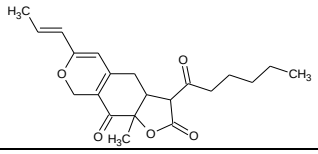
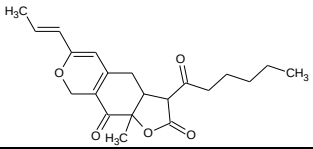
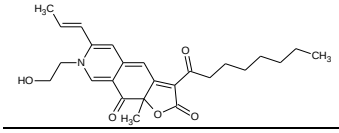
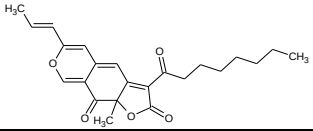
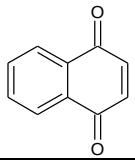
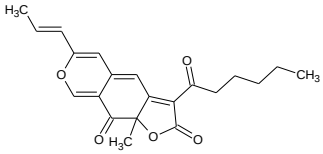
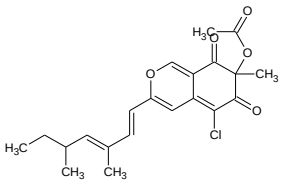
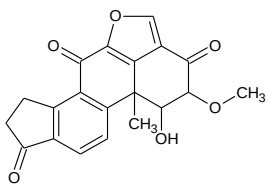
Chemical structure	Pigment	IUPAC name	Molecular formula	Molecular weight
	2,3-dihydrocitromycetin	(2R)-8-hydroxy-9-methoxy-2-methyl-4-oxo-3,5-dihydro-2H-pyrano[3,2-c]chromene-10-carboxylic acid	$C_{15}H_{14}O_7$	306.27 g/mol
	Ankaflavin	(3S,3aR,9aR)-9a-methyl-3-octanoyl-6-[(E)-prop-1-enyl]-3,3a,4,8-tetrahydrofuro[3,2-g]isochromene-2,9-dione	$C_{23}H_{30}O_5$	386.5 g/mol
	Anthraquinone	anthracene-9,10-dione	$C_{14}H_8O_2$	208.21 g/mol
	Aspergillin	(1*R*,7*S*,8*S*,11*R*)-7-hydroxy-11-(hydroxymethyl)-15-methyl-12,13-dithia-9,15-diazatetracyclo[9.2.2.$0^{1,9}$.$0^{3,8}$]pentadeca-3,5-diene-10,14-dione	$C_{13}H_{14}N_2O_4S_2$	326.4 g/mol
	Citromycetin	8,9-dihydroxy-2-methyl-4-oxo-5H-pyrano[3,2-c]chromene-10-carboxylic acid	$C_{14}H_{10}O_7$	290.22g/mol
	Citromycin	8,9-dihydroxy-2-methyl-5H-pyrano[3,2-c]chromen-4-one	$C_{13}H_{10}O_5$	246.21 g/mol
	Mitorubrinol	[(7R)-3-[(E)-3-hydroxyprop-1-enyl]-7-methyl-6,8-dioxoisochromen-7-yl] 2,4-dihydroxy-6-methylbenzoate	$C_{21}H_{18}O_8$	398.4 g/mol

Table 2 Continued.

Chemical structure	Pigment	IUPAC name	Molecular formula	Molecular weight
	Monascin	(3*S*,3*aR*,9*aR*)-3-hexanoyl-9*a*-methyl-6-[(*E*)-prop-1-enyl]-3,3*a*,4,8-tetrahydrofuro[3,2-g]isochromene-2,9-dione	$C_{21}H_{26}O_5$	358.4 g/mol
	Monascin	(3*S*,3*aR*,9*aR*)-3-hexanoyl-9*a*-methyl-6-[(*E*)-prop-1-enyl]-3,3*a*,4,8-tetrahydrofuro[3,2-g]isochromene-2,9-dione	$C_{21}H_{26}O_5$	358.4 g/mol
	Monascorubramine	7-(2-Hydroxyethyl)-monascorubramine	$C_{25}H_{31}NO_5$	425.5 g/mol
	Monascorubrin	(9*aR*)-9*a*-methyl-3-octanoyl-6-[(*E*)-prop-1-enyl]furo[3,2-g]isochromene-2,9-dione	$C_{23}H_{26}O_5$	382.4 g/mol
	Naphthaquinone	naphthalene-1,4-dione	$C_{10}H_6O_2$	158.15 g/mol
	Rubropunctatin	(9aR)-3-hexanoyl-9a-methyl-6-[(E)-prop-1-enyl]furo[3,2-g]isochromene-2,9-dione	$C_{21}H_{22}O_5$	354.4 g/mol
	Sclerotiorin	[(7R)-5-chloro-3-[(1E,3E,5S)-3,5-dimethylhepta-1,3-dienyl]-7-methyl-6,8-dioxoisochromen-7-yl] acetate	$C_{21}H_{23}ClO_5$	390.9 g/mol
	Viridin	(1*R*,17*S*,18*S*)-18-hydroxy-17-methoxy-1-methyl-13-oxapentacyclo[10.6.1.$0^{2,10}$.$0^{5,9}$.$0^{15,19}$]nonadeca-2(10),3,5(9),12(19),14-pentaene-6,11,16-trione	$C_{20}H_{16}O_6$	352.3 g/mol

The structure of a novel pigment from *Penicillium purpurogenum* was detected using U.V. and I.R. spectra. It indicated broad stretching O-H., C=C and C-H groups in the aromatic ring of the compound, suggesting the phenolic nature of the extracellular pigment (Geweely 2011). Nuclear magnetic resonance (NMR) techniques are considered the most advanced techniques employed in

structure elucidation of pigments, even in milligram quantities. The nature of the compound, number and disposition of hydrogen atoms as well as carbon atoms in a molecule can be identified by proton (^{1}H) and ^{13}C Nuclear Magnetic Resonance spectroscopic analysis (Anderson & Fossen 2003). More advanced mixed techniques like double electronic–nuclear resonance, gas chromatography coupled with mass spectrometry, along with elemental analysis made identification more precise and with ease (Hitaka et al. 2013). Recent characterization of natural pigments has done with the assistance of Raman and NIR (Near Infrared) Raman spectroscopic techniques (Li-Chan et al. 1996, Schrader et al. 1999).

Applications of fungal pigments

Soil fungi offer an array of pigments that could be utilised in various industries (Akilandeswari & Pradeep 2016). Fungal derived pigments offer a range of colour shades and possess antimicrobial, anticancerous and cytotoxic properties (Narsing Rao et al. 2017). Potent health hazards of synthetic colouring agents and their environmental concern diminished the usage of artificial colouring agents and increased demand for natural colouring compounds. Various applications of fungal pigments are depicted in Fig. 2.

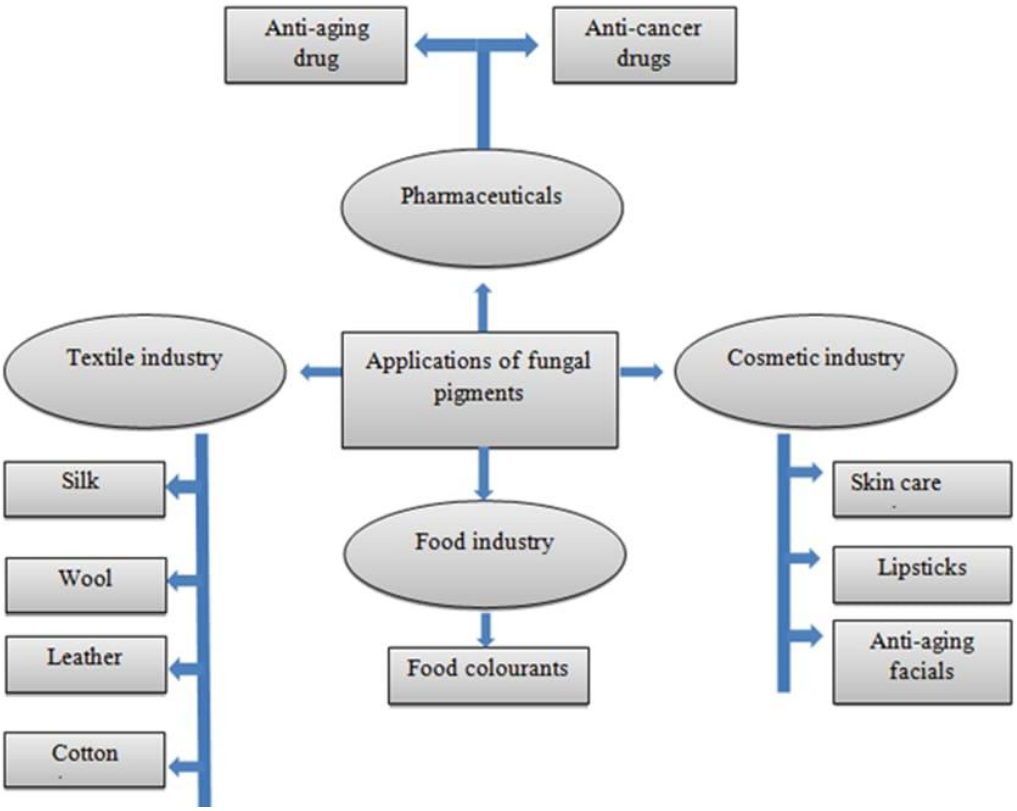


Fig. 2 – Applications of fungal pigments

Applications of Fungal Pigments as Food colouring agents

Humankind is ever fascinated by food additives in the form of colouring compounds. Considering the potential health hazards of synthetic colourants, several food-grade quality pigments are isolated from fungi (Dufosse 2006, Dharmaraj et al. 2009). Arpink red from *Penicillium oxalicum* var. *armeniaca* CCM 8242, of HAQN (hydroxyanthraquinoid) family serves as the first commercialized fungal pigment used in the food industry (Caro et al. 2012, Fouillaud et al. 2016). Genus *Monascus* is suitable for large scale production of monascus pigment (Fabre et al. 1993). In Asia, monascus pigment is widely used in red rice, red soya bean and red meat (Martínková et al. 1995, Hajjai et al. 1999, Patakova 2013, Vendruscolo et al. 2015). Yellow coloured monascin is extracted from *Monascus* sp. and is widely used in the food industry (Juzlova

et al. 1996). Many fungi can act as potential food-grade pigment producers, but simultaneous mycotoxin production must be evaluated for the sustainable use of natural colouring agents (Tan et al. 2018).

Applications of Fungal Pigments in Textile Industry

Textile industry mainly relies on artificial colouring agents to give colour to textile items like silk, wool and cotton. Natural pigments isolated from various fungi used as safer ones because of their biodegradable, less toxicity, and the great colour imparting nature. Because of these features, they are preferred over synthetic colouring agents and serves as a better substitute (Lagashetti et al. 2019). Fungal pigments are used in the textile sector to colour silk, wool, leather and cotton (Nagia & EL-Mohamedy 2007). Red or orange pigment-rich ethanolic extracts of *Monascus purpureus* C322 were used to dye raw wool (De Santis et al. 2005). Anthraquinone compounds such as 3-acetyl-2,8-dihydroxy-6-methoxy anthraquinone and 3-(1- hydroxyethyl)-2,8-dihydroxy-6-methoxy anthraquinone isolated from *Fusarium oxysporum* were used to dye wool fabric (Nagia & EL-Mohamedy 2007). Velmurugan et al. (2010a) coloured pre- tanned leather sample using fungal pigments extracted from soil fungi such as *Penicillium* spp., *Monascus purpureus*, *Fusarium* spp., *Emericella* spp. and *Isaria* spp. Natural colouring agents usually require mordants to overcome the drawbacks in terms of their narrow shade range and low fastness capability (Shahid et al. 2013). A novel yellow pigment obtained from *Aspergillus* sp. MBYP1 was found to be used effectively in cotton and silk without mordants (Pandiyarajan et al. 2018). The environmental factors and health concerns involved in the dyeing application overweigh the cost factor as compared to toxic synthetic dyes. Velmurugan et al. (2010a) suggested the use of anthraquinone based red pigment in the textile industry due to its high stability under sunlight, at various temperatures and various salt concentration treatment exposures.

Applications of Fungal Pigments as Antimicrobial Compounds

A large number of investigations revealed that pigments isolated from some potent species found in fungal genera like *Talaromyces*, *Aspergillus*, *Monascus*, *Penicillium*, *Trichoderma*, *Fusarium* and a yeast species *Rhodotorula glutinis* (commonly known as pink colour exhibiting yeast) showed a wide range of antimicrobial activities (Lagashetti et al. 2019). The green pigment produced by the Western Ghats soil fungi *Penicillium* sp. exhibited broad-spectrum antibacterial activity. On the other hand, *Aspergillus* sp. produced black coloured pigment possessing inhibition towards *Bacillus subtilis* and *Escherichia coli* (Saravanan & Radhakrishnan 2016). The extracellular pigment of *Penicillium purpurogenum* inhibits *Aspergillus fumigatus*, *Candida albicans*, *Epidermophyton floccosum*, *Microsporum canis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. This demonstrates its potential to be developed as antifungal and antibacterial agents (Geweely 2011). Sclerotiorin isolated from *Penicillium sclerotiorum* possessed antimicrobial activity (Chidananda & Sattur 2007, Lucas et al. 2007, Lucas et al. 2010).

Applications of Fungal Pigments as Antioxidants

Antioxidants are compounds that interact with free radicals, including the superoxide anion-radical and peroxides, thereby inhibiting or delaying cellular damage. Literature suggests the antioxidant property for fungal pigments like anthraquinones (Li et al. 2017) and melanin (El-Naggar & El-Ewasy 2017). Melanins readily interact with free radicals and other reactive species due to the presence of unpaired electrons in their molecules. Such property of melanin can be used in developing anticarcinogenic drugs (Shcherba et al. 2000). Blackish brown melanin pigment isolated from *Aspergillus bridgeri* exhibited promising anti-oxidant activity comparable to that of synthetic melanin (Kumar et al. 2011). Antioxidants enable protective function to DNA and many other biologically important compounds (Dunaway et al. 2018). Purified melanin pigments have shown significant free radical scavenging activity and confirmed that melanin is used in the

cosmetic industry for manufacturing photoprotective creams, which filter harmful UV radiations and protect skin from sun damage (Kumar et al. 2011).

Applications of Fungal Pigments as Pharmaceuticals

A surfeit of free radicals generated during oxidative metabolism in the human body is the major driving force in the process of ageing (Harman 2006). Fungal melanin can be developed as an anti-ageing drug due to its free radical scavenging activity and thereby enhance the efficiency of antioxidant enzymes (Lu et al. 2014). Being very sensitive to pH, melanin nanoparticles are considered a suitable nanocarrier drug delivery vehicle in treating intestine and colon diseases (Araujo et al. 2014). *Monascus* pigments like ankaflavin and monascin have proven antitumor activity towards cancer cell lines (Su et al. 2005, Akihisa et al. 2005).

Biotechnology in pigment production

Fungal pigments are secondary metabolites synthesised in response to their survival mechanisms. Major biosynthetic pathways underlying fungal pigment production are terpenoid synthetic pathway, polyketide synthetic pathway, nitrogen-containing metabolite pathway and shikimate pathway (Lin & Xu 2020). Understanding the biosynthetic pathway behind pigment production can open up new avenues in upscaling pigment production. In-depth knowledge of biological pathway is useful in developing the same product by alternative pathways (Viggiano et al. 2018). In *Penicillium chrysogenum* branched secondary metabolite pathway has been noted (Ali et al. 2013). Viggiano et al. (2018) studied the formation of yellow-tinted chrysogine pigment in *Penicillium chrysogenum* and reported a branched biosynthetic pathway for chrysogine production. Details of biosynthetic pathway facilitate the production of improved products using a modified form of intermediates involved in the pathway. Biotechnological approaches in the area of fungal pigment formation have made a breakthrough in this field. Efficient screening of mycotoxin producing strains and gene manipulation studies resulted in enhanced pigment production without coproduction of mycotoxin (Mapari et al. 2005). Present improvements in respect of metabolomics also helped in the metabolic profiling with special emphasis on the identification of functional groups responsible for colour production (Archer 2000). Another approach named chemoinformatics provided details of novel pigments based on chromophore similarity with known pigments (Elyashberg et al. 2002).

Furthermore, metabolic profiling, genome mining and sequence determination assisted in the detection of genes significantly correlated with pigment biosynthesis (Sankari et al. 2018). Advances in metabolic engineering offered constructive techniques to study the manifestation of gene clusters, that otherwise not expressed *in vitro* (Nielsen & Nielsen 2017). Identification of such gene clusters is useful in genetic engineering for large scale or commercial production of desired pigment using heterologous host (Pfeifer & Khosla 2001). Chen et al. (2017) performed gene knockout of MrPigF gene to study monascin and ankaflavin pigment formation in *Monascus ruber* M7. The experiment provided a route map dealing with the biosynthesis of MonAzPs. Thus, various tools in molecular biology can be claimed for establishing the molecular background of pigment production and provide insights into the complex biosynthetic pathway networks associated with pigment production.

Limitation and Challenges

Fungal strains offer a plethora of hues with applications in the textile industry, food industry and pharmaceuticals. However, several disadvantages limit the use of such natural pigments. Co-production of citrinin, a mycotoxin that reduces the use of many fungal pigments in the food industry as its consumption may cause food poisoning (Mapari et al. 2005). However, issues related to mycotoxin production can be resolved by screening and selecting harmless strains, the development of genetically modified or mutant fungal strains and altered culture conditions (Mapari et al. 2005). The requirement of large capital investment and public acceptance of the developed colours impose another problem (Dufosse 2016). In the European Union, monascus

pigments are not authorized for their commercial use in the food industry (Mapari et al. 2010). A cost-effective fermentation technique for the massive production of fungal pigments must be developed for the successful replacement of artificial colours with natural colouring agents.

Commercial value of inherent pigments is proportional to their stability and shelf life in response to variations in temperature, light, pH and moisture content (Kalra et al. 2020). In this regard, researchers have developed new approaches such as nanoformulation and microencapsulation (Mehrad et al. 2018). Matsuo et al. (2018) designed two categories of nanoparticles called poly (lactic-co-glycolic) acid (PLGA) and hydroxypropyl cellulose (HPC) to encapsulate monascus pigments as a means to increase the photostability of pigment. Photobleaching studies have indicated that PLGA nanoparticles are advantageous over HPC based nanoformulations to improve pigment stability. Pigment extraction and purification pose another problem. Selection of a suitable method for extraction of pigment is inevitable, as it can hamper the quality and quantity of the pigment.

Conclusion

Increasing interest in microbial bioactivities has spurred research related to fungal metabolites. Soil fungi isolated from various habitats offer a vast array of of pigments possessing biological activities, which could be used in pharmaceuticals, foodstuffs and the textile industry. Safety concerns associated with the usage of synthetic colouring compounds encouraged fungal pigments as a suitable substitute in the food and textile industry. Awareness of health hazards associated with synthetic dyes has stimulated the search for natural pigments. Owing to the season independent availability, cheap cost of production, easy processing and a broad range of colours, soil fungi can be considered as a potential candidate for pigment production with various applications in several industries. However, further studies are required for the large scale production of fungal derived pigments. As several potent pigment-producing strains like *Monascus* species are reported for the simultaneous production of mycotoxins which narrow down the usage of pigments. Hence, it is necessary to promote further research by focusing on the isolation of potent fungal strains, which can produce stable colourants with little or no mycotoxin production.

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