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REVIEW

Melanin of fungi: from classification to application

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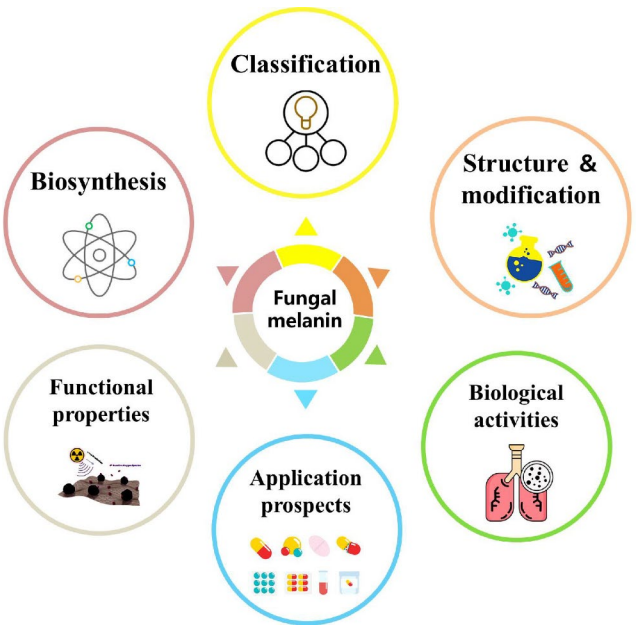
Abstract
Melanin is a secondary metabolite composed of complex heterogeneous polymers. Fungal melanin is considered to be a sustainable and biodegradable natural pigment and has a variety of functional properties and biological activities. On one hand, due to its own specific properties it can play the role of antioxidant, anti-radiation, adsorption, and photoprotection. On the other hand, it has good biological activities such as hepatoprotective effect, hypolipidemic effect and anti-cancer. Therefore, it is widely used in various fields of daily life, including dyeing, food, biomedical and commercial industry. It is conducive to environmental protection and human health. However, the insolubility of fungal melanin in water, acids and organic solvents has been an obstacle to its commercial applications. Thus, the chemical modification methods of fungal melanin are summarized to increase its solubility and expand the application fields. Although fungal melanin has been used in many industries, as the structure and function of fungal melanin and modified melanin are further studied, more functional properties and bioactivities are expected to be discovered for a wide range of applications in the future.

Graphic abstract



Keywords Application · Bioactivity · Chemical modification · Fungi · Melanin

Extended author information available on the last page of the article

Table 1 Precursors, synthetic pathways and elements of different types in fungal melanin

Type	Precursor (s)	Synthetic pathway	Elements	Source (s)	Color (s)	Reference (s)
Eumelanin	Tyrosine	L-DOPA	C, H, O, N	*Auricularia auricula Cryptococcus neoformans*	Brown, black	Riley 1997; Singh et al. 2021; Camacho et al. 2019
Pheomelanin	5-S-cys-DOPA	L-DOPA	C, H, O, N, S	*Auricularia auricula*	Black, red, brown	Strycker et al. 2021; Zou et al. 2015
DHN-melanin	Acetyl CoA, Malonyl CoA	DHN	C, H, O	*Aspergillus terreus Auricularia auricula Aspergillus fumigatus Phoma* sp.	Yellow, brown, black	Strycker et al. 2021; Zhou et al. 2022a, b; Surendirakumar et al. 2022; Henson et al. 1999; Prados-Rosales et al. 2015; Geib et al. 2016
Pyomelanin	Homogentisic acid	Homogentisate	C, H, O	*Penicillium chrysogenum Sporothrix schenckii Aspergillus fumigatus*	Brown-black	Kalra et al. 2020; Perez-Cuesta et al. 2020; Vasanthakumar et al. 2015; Almeida-Paes et al. 2017
GHB-melanin	PAP	GHB	C, H, O, N	*Agaricus bisporus*	Black, brown-black	Pierce and Rast 1995

Introduction

The word "melanin" originated from the Greek word "melanos" and was named by the Swedish chemist Berzelius in 1840 (Riley 1997). Melanin is defined as a pigment of diverse structure and origin derived from the oxidation and polymerization of tyrosine in animals or phenolic compounds in lower organisms (d'Ischia et al. 2013). It is usually black or dark brown and is widely found in animals, plants, and microorganisms. Fungi can provide an excellent source to obtain natural pigments (Chatragadda and Dufossé 2021). The production of melanin by fungi has attracted the interest of many researchers since the 19th century (Kalra et al. 2020), and the studies of fungal melanin are still a hot spot.

As a secondary metabolite of fungi, melanin gives fungi special advantages to increase their resistance in harsh or extreme environments, which is also known as "fungal armor" (Suryanarayanan 2004). Compared to synthetic melanin, fungal melanin is not only eco-friendly and biodegradable, but also exhibits a series of excellent functional characteristics and biological activities. There are certain health benefits to human body by ingesting exogenous fungal melanin. Besides, the chemical modified fungal melanin is found to become more soluble and biologically active, which could further facilitate its applications. Thus, the development and application of natural fungal melanin are of great significance to the environment and human health.

In recent years there has been an increasing number of studies on fungal melanin, such as functional properties (Eisenman et al. 2020), biological activities (Hou et al. 2021), and chemical modification (Xu et al. 2020a, b). Therefore, a comprehensive review is necessary to understand the importance and current research progress of fungal melanin. In this paper,, the structure, classification, biosynthesis, and special functions of fungal melanin are introduced. Some chemically modified methods to optimize the properties of fungal melanin are reviewed. Then possible future directions for the application of fungal melanin are proposed. Finally, this article gives an overview of the current challenges in fungal melanin research and suggests prospects for future studies.

Classification of fungal melanin

According to precursor substances of synthetic pathways, fungal melanin can be classified into eumelanin, 1,8-dihydroxynaphthalene (DHN) melanin, pyomelanin, pheomelanin, and 4-Glutaminylhydroxybenzene (GHB) melanin (Table 1).

Eumelanin is a substance that is synthesized by the action of tyrosinase through the L-3,4-dihydroxyphenylalanine (L-DOPA) pathway using tyrosine as a precursor (Bayram 2022). DHN-melanin is generated by the oxidation or polymerization of 1,8-dihydroxynaphthalene (di-DHN) or 1,3,6,8-tetrahydroxynaphthalene (THN) using acetyl CoA or malonyl CoA as precursors, and produces melanin of various colors (Singh et al. 2021). Pyomelanin is a multimer composed of 2-acetyl-p-benzoquinone as the basic unit, formed by the homogentisate pathway. Homogentisic acid as the precursor substance, is catabolized to produce fumarate and acetoacetate, while acetoacetate is accumulated, oxidized, and polymerized to produce pyomelanin (Xiao et al. 2018). A precursor substance of pheomelanin is 5-S-cys-DOPA synthesized through the L-DOPA pathway (Strycker et al. 2021). In the subsequent reactions with the participation of cysteine or glutathione, dopaquinone (DAQ) forms cysteine dopachrome in the presence of cysteine, which eventually polymerizes to generate pheomelanin (Singh et al. 2021). GHB-melanin is also known as 4-aminophenol

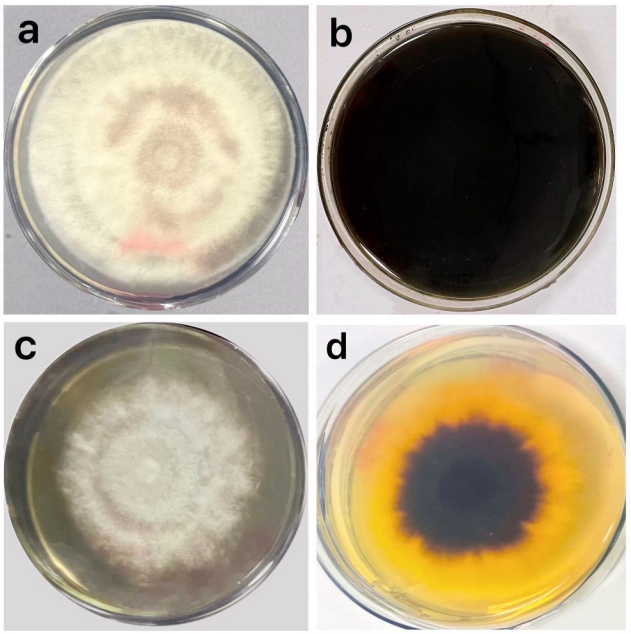


Fig. 1 Melanin production of *Annulohypoxylon stygium* under different nitrogen sources (25°C, 14 d)
a Front side of the medium with peptone as the nitrogen source. b Back side of the medium with peptone as the nitrogen source. c Front side of the medium with ammonium sulfate as the nitrogen source. d Back side of the medium with ammonium sulfate as the nitrogen source

(PAP)-melanin. As the basic unit, 2-hydroxy-p-alkylidene-benzoquinone (2-HpIBQ) is synthesized by the combination of PAP and glutamate to form 4-glutamyl hydroxybenzene, which is then polymerized to form GHB-melanin by the action of tyrosinase (Pierce and Rast 1995).

Moreover, fungal melanin can also be classified as cell wall melanin, cytoplasmic melanin and extracellular melanin depending on its location in the cell (DeSouza et al. 2018). Cell wall melanin is a portion of the cell wall that is usually recognizable as a distinct, fairly well-defined outer layer, and a few melanin are associated with the protofibrillar matrix that extends outward from many fungal cell walls (Nosanchuk et al. 2015). Extracellular melanin is present outside the fungal cell and is distinct from the cell wall-bound melanin. It can be formed in two ways: one is that phenol oxidase released by the fungus into the culture medium can oxidize some culture components to melanin; the other is that the phenolic compounds secreted into the medium may slowly and automatically oxidize to form melanin (Langfelder et al. 2003). Other fungal melanin is formed in the cytoplasm and deposited in the cell wall or excreted as extracellular polymers. Like the melanin of *Cryptococcus neoformans* locates in the cell wall (Chrissian et al. 2020), the melanin of *Termitomyces albuminosus* also locates in the cell wall or septum (DeSouza et al. 2018), while the melanin of *Magnaporthe oryzae* adherent cells is

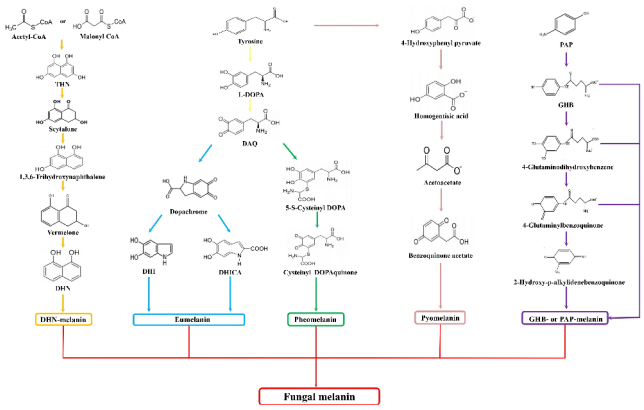


Fig. 2 Synthetic pathways of fungal melanin

present in the cell wall close to the cytoplasmic membrane, and the melanin of *Saccharomyces cerevisiae* is deposited on the surface in the form of particles (Cordero and Casadevall 2020).

Biosynthesis of fungal melanin

Synthetic pathways of fungal melanin

The synthesis of fungal melanin can be influenced by a variety of factors, such as inhibitor, carbon source and nitrogen source (Luo et al. 2019). For example, the use of wheat bran extract as the carbon source could significantly increase the production of *Auricularia auricula* melanin (Zou et al. 2017). In our previous study, *Annulohypoxylon stygium* could produce more melanin in the medium with peptone as the nitrogen source than that with ammonium sulfate as the nitrogen source (Fig. 1). Most fungi synthesize melanin through two main synthetic pathways: the DHN and the L-DOPA pathway. However, there are also additional pathways utilized by other fungi. For example, *Agaricus bisporus* can produce GHB-melanin through the GHB pathway (Wu et al. 2016; Lin and Xu 2020). The process of fungal melanin synthesis is illustrated in Fig. 2. Generally, the melanin synthesis pathways and even types can be determined using synthesis inhibitors (Lim et al. 2022).

Fungal melanin is primarily synthesized through the DHN pathway. In the DHN pathway, the precursor molecules acetyl CoA and malonyl CoA are produced internally. The formation of THN is catalyzed by polyketide synthase firstly. THN is synthesized and then reductively dehydrated to produce intermediates such as cycloalkanone and 1,3,6-trihydroxynaphthalene, leading to the formation of melanin from the final polymerization product DHN (Cao et al. 2021; Maranduca et al. 2019). The inhibitors of DHN-melanin are 5-methyl-1,2,4-triazole[3,4]benzothiazole (tricyclazole) and pyroquilon. Tricyclazole reduces

melanin biosynthesis by inhibiting the two-step dehydrogenation reaction of THN reduction to scytalone and 1,3,6-trihydroxynaphthalene reduction to vermelone during DHN-melanin production (Koehler et al. 2021). Pyroquilon inhibits tetrahydroxynaphthalene reductase in the DHN-melanin biosynthetic pathway and interferes with melanin generation. Moreover, carpropamid and fenoxanil inhibit 1,3,6-Trihydroxynaphthalene reductase as a single dehydratase in the synthesis pathway, thus preventing the formation of melanin (Lim et al. 2022).

The other major pathway for melanin synthesis is the L-DOPA pathway, which is similar to the mammalian pathway. Depending on the precursor molecule, levodopa or tyrosine is converted to produce DAQ. The tyrosinase catalyzes the process and acts as the key rate-limiting enzyme for its reaction (Maranduca et al. 2019; Vitiello et al. 2019). Then DAQ undergoes a series of reactions to form eumelanin and pheomelanin respectively. Inhibition of melanin production by the L-DOPA pathway involves inhibiting tyrosinase activity, and inhibitors of tyrosinase can be classified into four types: competitive, non-competitive, uncompetitive, and mixed (Cordero and Casadevall 2020). Utilizing kojic acid derived from microorganisms to chelate Cu^{2+} at the active site of tyrosinase and to prevent the formation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) from dopachrome, the activity of the rate-limiting enzyme tyrosinase is restricted, preventing the production of DOPA-melanin. Moreover, azelaic acid reduces melanin growth by binding to amino and carboxyl groups, then prevent tyrosinase from acting on the binding site (Manap et al. 2021). Furthermore, compounds that inhibit tyrosinase are some resveratrol derivatives and analogues, indole derivatives, hydroxycinnamic acid derivatives, and chalcone (Pillaiyar et al. 2018; Kumari et al. 2018), which are all able to effectively reduce DOPA-melanin formation.

Synthetic regulation of fungal melanin

A complex series of enzymatic reactions regulate the melanin synthesis process, which are regulated by multiple genes and signaling pathways. Melanin formation is regulated by the following major signaling pathways: PI3K/Akt, MAPK, Wnt/β-catenin, and NO (Pillaiyar et al. 2017; D'Mello et al. 2016). But there may be other pathways involved in melanin synthesis.

The key enzymes of the DHN pathway and DOPA pathway for fungal melanin synthesis are polyketide synthase (PKS) and laccase/tyrosinase (LAC/TYR). Fungal melanin synthesis is mainly regulated by the PKS genes, which are controlled by transcription factor Cmr1 (Jia et al. 2021). In fungi, genes encoding PKS are typically grouped into gene clusters in which genes with similar functions co-regulate each other. Among these clusters are the genes encoding enzymes, including PKS, oxidases and reductases, and transcription factors (Zhou et al. 2022a, b).

PKS facilitates the biosynthesis of melanin by regulating DHN-melanin synthesis. CmrA is a transcriptional regulator encoding a DHN-melanin synthesis pathway gene in *Alternaria alternata* (Fernandes et al. 2021). Pmr1 and Pmr2 have been identified as transcription factors in the filamentous fungus *Pestalotiopsis microspora* that regulate melanin biosynthesis (Zhou et al. 2022a, b). The DHN-melanin pathway is encoded by a cluster including six genes (*abr1*, *abr2*, *ayg1*, *arp1*, *arp2*, and *pksP*/*alb1* genes) in *Aspergillus fumigatus* (Perez-Cuesta et al. 2020; Yu et al. 2015) demonstrated that the polyketide synthase gene *PKS1* is responsible for the synthesis of melanin in the endophytic fungus *Pestalotiopsis microspore*. Ma et al. (2017) used a knockout approach to verify the function of *StLAC2* in *Setosphaeria turcica* and found that it is an important catalytic gene for the synthesis of DHN-melanin. In *Botrytis cinerea*, transcription factors regulating DHN melanogenesis include *Bcscd1*, *Bcsmr1*, *Bcztf1/2*, *Bcltf1/2*, *BcVEA*, *BcVELB*, and *BcVEL1* (Zhou et al. 2022a, b; Cohrs et al. 2016; Schumacher 2016; Schumacher et al. 2014).

The biosynthesis of DOPA-melanin is catalyzed by tyrosinase and laccase. Tyrosinase, the major enzyme involved in the synthesis of melanin, is tissue-specific and controlled by many factors. Laccase catalyzes the production of melanin from levodopa in the L-DOPA pathway, which performs a key role in the synthesis process. Laccase CNLAC1 is identified as catalase for the initial step of the DOPA-melanin synthetic pathway in *C. neoformans* (Williamson 1994). *C. neoformans* produce brown eumelanin by laccase (*Lac1* and *Lac2*). Researchers found that *C. neoformans* significantly reduced *LAC1* induction and melanin synthesis via the cAMP/PKA pathway, as well as that four key transcription factors, Bzp4, Hob1, Usv101, and Mbs1, had a common effect on *Lac1* (Lee et al. 2019; Liu et al. 2022) found that melanin synthesis in *A. stygium* was not only regulated by laccase, but also the alternative oxidase was involved in melanin biosynthesis.

Pyomelanin synthesis in *A. fumigatus* is associated with the activation of the L-tyrosine/L-phenylalanine degradation pathway that includes six genes *hppD*, *hmgX*, *hmgA*, *fahA*, *maiA* and *hmgR* (Perez-Cuesta et al. 2020; Vasanthakumar et al. 2015) demonstrated that the presence of tyrosine promoted the transcription of *hmgA* which stimulated a high expression of the tyrosine degradation pathway, thereby promoting the production of pyomelanin.

Fungal melanin synthesis is regulated by a set of genes and signaling pathways that are different, probably due to the different types of fungi. The genes regulating melanin

synthesis are not completely discovered, the signaling pathways and the synthesis regulation mechanism need further studies.

Structure and chemical modification methods of fungal melanin

Melanin is a complex class of compounds that is complicated to separate and identify, because it is often combined with proteins, polysaccharides, lipids and other macromolecules (Cao et al. 2021). Melanin is thought to be a high molecular weight amorphous polymer, and these polymers form graphite-like planar sheets that aggregate in a layered way to form colloidal particles. The diameter of these melanin particles can reach hundreds of nanometers (Cordero and Casadevall 2020; Toledo et al. 2017) speculated that the melanin chemical formula may be $[C_{18}(OR)_3H_7O_4N_2]_n$. The study of nuclear magnetic resonance (NMR) revealed that fungal melanin was composed of a network of regions composed of aliphatic and aromatic structures (Pralea et al. 2019), and through X-ray diffraction it was determined that fungal melanin was amorphous and heterogeneous in structure (Bayram 2022). With the techniques for studying the structure of fungal melanin becoming increasingly advanced, researches on melanin structure are essential.

Chemical modification methods

At present, the common extraction methods for fungal melanin include alkali solubilization and acid precipitation (Wu et al. 2008a), ultrasonic-assisted extraction (Lu et al. 2020), microwave-assisted extraction (Lu et al. 2014), enzymatic extraction (Chen et al. 2021), and ionic liquid extraction (Ghadge et al. 2022). Most types of extracted fungal melanin are insoluble in water, acids, or organic solvents due to their specific structure, but has higher solubility in alkaline solutions (Singh et al. 2021). Therefore, the industrial applications are limited, for example, the difficulty of dissolving in oral preparations. The solubility of fungal melanin becomes obstacle of expanding the application (El-Naggar and Saber 2022). Consequently, researchers are committed to studying the modification method of melanin in order to solve the problem of insolubility.

Amino acid modification

There are several amino acids are used to modify melanin to prepare amino acid-melanin derivatives to improve the solubility, antioxidation and other physicochemical properties of melanin, such as arginine, glycine, lysine, aspartic acid, tryptophan, threonine and histidine. The method for amino acid modification is to dissolve different amino acids in distilled water, centrifuge the supernatant and dilute it, then measure the absorbance value at 500 nm and select the amino acid with the highest absorbance for subsequent experiments. Ye et al. (2019) found after modification of *Lachnum singerianum* YM296 melanin with different amino acids that histidine-melanin had the highest water solubility up to 47.7 mg/L. In contrast, Li et al. (2019) found that *Lachnum* YM156 melanin modified with arginine had higher water solubility and biological activity, and that the modified melanin exhibited better radiation resistance characteristic. Likewise, *Ganoderma lucidum* melanin modified with arginine demonstrated an increase in solubility and antioxidant activity, and enhanced inhibition of pancreatic lipase activity (Xu et al. 2020a, b).

Carboxymethylation modification

Melanin mixes with sodium hydroxide and isopropanol in an ice bath, and then a certain proportion of sodium hydroxide, isopropanol, and chloroacetic acid are added. Then after complete reaction in a water bath at 60°C for 2 h, cooled to room temperature and adjusted to neutral with glacial acetic acid. Carboxymethylated modified melanin is obtained after dialysis (Ye et al. 2011; Zong et al. 2017) demonstrated that the modification of *Lachnum* YM205 melanin by means of carboxymethylation could greatly increase the water solubility of melanin, but its solubility was lower than that of amino acid-modified melanin. As well, the preparation of carboxymethylated derivatives from *Lachnum* YM205 melanin presented higher water solubility, better antioxidant properties, and reduced lipid peroxidation and enhanced immune regulation in mice (Li et al. 2017).

D-Glucosamine modification

Melanin is added with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, triethylamine, and hydroxybenzotriazole under an atmosphere of nitrogen, then anhydrous N,N-dimethylformamide is added and stirred for a period of time until the temperature rises to room temperature. After adding $C_6H_{13}NO_5$-HCl and triethylamine, the reaction is continued with stirring. The modified melanin is obtained by dialysis with dialysis membrane for 48 h after the reaction is completed (Song et al. 2016a, b). Because of the inherent antioxidant properties and free radical scavenging activity of glucosamine, melanin modification with D-glucosamine leads to more biologically active melanin

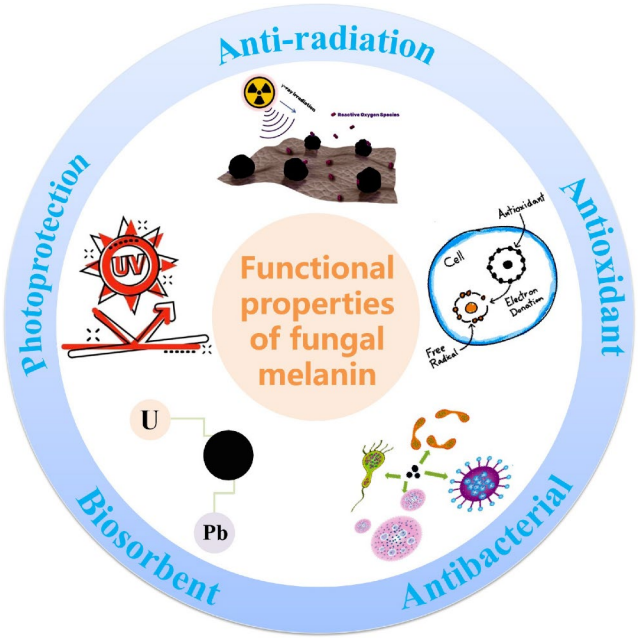


Fig. 3 Functional properties of fungal melanin

derivatives. Song et al. (2016a, b) obtained D-glucosamine melanin derivatives from *Lachnum* YM226 melanin and found that not only the water solubility was largely higher, but also the antioxidant activity and anti-inflammatory level were significantly increased as well as the obvious protective effect against alcoholic liver injury.

Sulfation modification

Pyridine and sulfur trioxide-pyridine are mixed to a three-necked vial and stirred with an electromagnetic heating stirrer. After adding melanin, the mixture is stirred at a constant temperature before cooling and adjusting to neutral with sodium hydroxide. Tap water and distilled water are dialyzed separately for two days and freeze-dried to obtain the sulfated melanin (Li et al. 2016) modified *Lachnum* YM205 melanin by carboxymethylation and sulfation methods, revealing an increase in solubility and biological activity, this might be owing to the introduction of new groups causing much higher antioxidant activity and water solubility. However, the water solubility of the melanin derivatives prepared by sulfation is relatively low compared to the carboxymethylated modified melanin.

The inconsistent conclusions drawn from the modification of different fungal melanin may be explained by differences in structure. The melanin modified methods improve the problem of complete insolubility in water which is of great significance to expanding the fields of melanin application.

Functional properties of fungal melanin

The specific structure of fungal melanin gives it functional properties, including anti-radiation, anti-oxidation, photoprotection, biosorbent, and antibacterial effect (Fig. 3). These properties make it one of the most valuable pigments for application.

Anti-radiation

Because fungal melanin has unique properties and is able to absorb visible light, ultraviolet light, resistance to γ-ray, x-ray, nuclear radiation and other kinds of radiation, it plays a key role in protecting the organism and cells from radiation damage (Eisenman et al. 2020). Pacelli et al. (2017) investigated the effects of densely ionized deuterium and sparsely ionized X-rays in two fungi, *C. neoformans* and *Cryomyces antarcticus*, revealing that melanin protects the fungi from high doses of deuterons under physiological conditions. This resistance to ionizing radiation is determined by the chemical composition, free radical quenching and spherical spatial arrangement of melanin (Dadachova and Casadevall 2008). Fungal melanin can interact with high-energy electromagnetic radiation and convert it into electrical and chemical energy, which promotes the utilization of energy. This property provides more survival advantages compared to nonmelanotic organisms in extreme environments (Casadevall et al. 2017). Moreover, fungal melanin can enhance the activity of catalase and SOD enzymes to prevent the production of reactive oxygen species under radiation conditions and avoid the impact on cells (Kothamasi et al. 2019).

Anti-oxidation

Fungal melanin is a powerful antioxidant due to its unique electrochemical properties, which allow it to act as an electron donor and electron acceptor. The antioxidant properties of fungal melanin are attributed to the addition of free radicals, probably because melanin has unpaired electrons that make it to interact with peroxyl radicals (Oh et al. 2021a). It can also protect cells by scavenging hydrogen peroxide, hydroxyl radicals and superoxide anions (Cordero and Casadevall 2020). And it can enhance virulence by interfering with host defense factors, including neutralizing the oxidative burst of phagocytes (Cordero and Casadevall 2017). More, DHN-melanin is resistant to oxidation by higher concentrations of potassium permanganate and hypochlorite (Jacobson et al. 1995). According to the research, melanin plays a protective role against fungi. Because fungal melanin can act as an unpaired electron trap for NO, protecting

the fungus from oxidation by direct interaction with NO (Cunha et al. 2010). *Hypoxylon archeri* melanin has a better ability to protect 80.95% 5-thio-2-nitrobenzoic acid (TNB) from oxidative damage by H_2O_2 than synthetic melanin. It also has a better effect on scavenging hydrogen peroxide oxygen radicals (Wu et al. 2008a), and promotes the production of other fungal polyphenol oxidases (Wu et al. 2008b).

Photoprotection

Fungal melanin exhibits broadband absorbance in all UV-visible ranges and is capable of displaying good photoprotection. It can prevent subsequent photodamage and minimize adverse influence on cells. Photoprotection is more effective in eumelanin because it is more abundant in DHICA than in 5,6-dihydroxyindole (DHI), DHICA can provide more photoprotection than DHI, and the DHICA/DHI ratio can also regulate its properties and light absorption capacity (Solano 2016). But because of differences in structures, not all types of melanin are photoprotective. It is generally believed that eumelanin is photoprotective, while pheomelanin is phototoxic as a dangerous photosensitizer (Ito et al. 2018).

Biosorbent

Adsorption of organic substances Influenced by its chemical structure, melanin can be allowed to adsorb aromatic compounds, including toluene, ethylbenzene and styrene. The hydrophobicity and negatively charged properties of fungal melanin allow it to bind widely varying substances, making it useful in industry for removing volatile organic compounds and lowering economic costs (Eisenman and Casadevall 2012). Fungal melanin is acid-tolerant and dry, it also can be utilized as a biosorbent for soil remediation in extreme environments (Cordero et al. 2017).

Adsorption of metal ions Melanin contains carboxyl, phenolic, hydroxyl and amine functional groups, which offer many potential binding sites or biosorption sites for metal ions. It can enable to combine metal ions, like heavy metals Cu, Pb, Zn and Cr in contaminated soil, to decrease metal toxicity and remediate soil (Gadd and de Rome 1988; Sarna et al. 2022; Ban et al. 2012). Fungi containing melanin biosorbed 2.5–4 times more Ni, Cu, Zn, Cd, and Pb than fungi without melanin (Fogarty and Tobin 1996). Fungal melanin has the ability to adsorb metal ions which makes it ecologically crucial in wastewater treatment and soil contamination remediation, and it can be employed to remove toxic or heavy metal ions from polluted water and to recycle precious metal ions from solution.

Adsorption of radioactive material Radioactive substances have serious negative impact on the ecological environment and even produce irresistible harm to human body, so there is an urgent need for some green and economic technologies to solve this problem. Fungal melanin is negatively charged and hydrophobic, allowing it to bind to a wide range of substances. It strengthens the ability to adsorb uranium and helps decontaminate uranium-contaminated areas, with fungal melanin having a better ability to adsorb uranium than commercial resins (Coelho et al. 2020). Meanwhile, melanin synthesized by different precursors showed adsorption of radionuclides ^{111}In, ^{213}Bi, ^{225}Ac (Howell et al. 2008).

Antibacterial effect.

A. auricular melanin has high biofilm formation inhibition rate against *Escherichia coli* K-12, *Pseudomonas aeruginosa* PAO1 and *P. fluorescens* P-3, this property provides its antibacterial effect (Li et al. 2012). The melanin in the mycelium of *Lachnum* YM30 can destroy the integrity of the bacterial cells, and has remarkable inhibition effect on Gram-negative bacteria such as *Escherichia coli*, *Vibrio parahaemolyticus* and Gram-positive bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus* (Xu et al. 2017). Melanin from endophytic fungi *Phoma* sp. RDSE17 melanin has a significant inhibitory effect on some Gram-positive and Gram-negative bacteria. At the melanin concentration of 100 µg/mL, the maximum zone of inhibition was up to 14.7 mm against *Bacillus subtilis* and 18 mm against *Ustilaginoidea virens* (Surendirakumar et al. 2022).

Biological activities of fungal melanin

Lowering blood lipids

In obese mice, *Sphacelotheca reiliana* melanin has been shown to decrease fat content and reduce the probability of getting fatty liver, in addition to lowering blood lipids significantly (Lu et al. 2020). It was observed that mice fed with water-soluble melanin extracted from *Inonotus obliquus* exhibited enhanced expression of fatty acid oxidation genes, demonstrating that melanin promotes a beneficial metabolism of lipids (Lee and Hyun 2014). Melanin extracted from *Lachnum* YM226 was effective in reducing the body weight of hyperlipidemic mice, improving the lipid status and increasing the activity of antioxidant enzymes to lower the blood lipid level (Shi et al. 2018a, b).

Hepatoprotection and anti-tumor

Lachnum YM226 melanin can protect the body of kidney tissue by inhibiting glomerular atrophy, which effectively prevented liver and kidney damage (Shi et al. 2018a). In addition, the extracted melanin showed a significant inhibitory effect on tumor growth in H22 tumor-bearing mice. And the anti-tumor function of arginine-modified melanin is better, probably because melanin improves immune system and induces apoptosis in mice (Shi et al. 2018b). In mice with alcoholic liver injury, *A. auricular* melanin has a significant effect on the reduction of cell viability induced by alcohol. It is possible that melanin can raise the antioxidant capacity of mice to improve the oxidative stress response caused by alcohol (Hou et al. 2021). Melanin obtained from *Lachnum* YM30 increases inflammatory factors in mice, reduces inflammatory stress to protect the liver, and has a noticeable therapeutic effect on mice with acute liver injury induced by lipopolysaccharide/D-galactosamine (LPS/D-GalN) (Xu et al. 2020a, b).

Lowering blood sugar

Melanin extracted from *S. reiliana* is an excellent hypoglycemic substance that significantly suppresses the activity of α-glycosidase and protein tyrosine phosphatase-1B (PTP-1B), and has great application in the field of future medicine (Lu et al. 2020). Water-soluble melanin complexes derived from *I. obliquus* improved insulin sensitivity in high-fat-fed obese mice and effectively lowered blood sugar, making it a potential candidate for anti-diabetic drugs (Lee and Hyun 2014).

Other biological activities

The fungus *Nadsoniella nigra* melanin prevents esophageal damage by increasing peroxide dismutase activity and decreasing catalase activity, which can be treated for burn-induced oxidative stress (Chornenka et al. 2018). Melanin from *Lachnum* sp dramatically facilitates Pb excretion in mice by inhibiting lipid peroxidation and enhancing superoxide dismutase and glutathione peroxidase activities for Pb detoxification (Zong et al. 2017). The melanin of *A. auricular*, in addition to its protective effect on mice with alcoholic liver injury, can also adjust the intestinal microbial disorder in mice with alcohol intake, increase the number of intestinal microorganisms *Akkermansia* and *Bifidobacterium* and improve lipid metabolism in the liver (Lin et al. 2021). Feeding iron-deficient anemic mice with *Lachnum* YM226 melanin-iron compounds was discovered to be highly effective in relieving the symptoms of anemia, raising the activities of superoxide dismutase, catalase and glutathione peroxidase and alleviating the symptoms of immune disorders in mice. Thus melanin can be utilized as a versatile and high-efficiency iron supplement (Song et al. 2016a, b).

Application prospects of fungal melanin

Applications in the dyeing industry

The use of synthetic pigments is not only harmful to the human body but also to the environment. But fungal melanin is the sustainable and biodegradable pigment. Therefore, it is one of the most promising pigments to be developed in the dyeing industry. The use of it in textiles protects the skin from damage due to its photoprotective, antioxidant, and anti-UV properties (Venil et al. 2020). Further, the development of eco-friendly inks that take full advantage of the biodegradability of fungal melanin has potential application. Hair dyes, and cosmetics with natural melanin are already on sale in the market, but the market for fungal melanin products is likely to be larger than that for natural melanin products (Panzella et al. 2018).

Applications in packaging materials

In recent years, the production of composite chemical materials derived from fungal melanin has become increasingly prominent in the packaging industry. This is owing to its many advantages, such as environmental benefits, antibacterial effect, and antioxidant properties. Bioactive films prepared from carvacrol and fungal melanin exhibit good antioxidant and antibacterial properties which are future alternatives to plastic films for green packaging and have great potential in food packaging (Łopusiewicz et al. 2021). At appropriate concentrations, melanin can also be applied as a modifier in polylactic acid composite packaging films, enhancing the mechanical and barrier properties, as well as enhancing the antioxidant capacity of the film (Łopusiewicz et al. 2018). Moreover, fungal melanin binds well to the surface of a variety of materials to form functional materials, including metals, polymeric materials, ceramics, biological surfaces, and mineral complexes, thus it can act as a coating agent for material surface modifications. Because the special physicochemical properties of fungal melanin, functional surfaces with charge-dependent sorption, antibacterial and free radical scavenging activities are formed. It has the potential to develop as a functional material for packaging (Jeon et al. 2016). Melanin derived from black knot fungus is a green and economical source of allomelanin

(present in fungal cell walls and synthesized from nitrogen-free precursors), which is an excellent choice as an anti-UV radiator and antioxidant for cosmetics and packaging materials (Toledo et al. 2017; Singla et al. 2021).

Applications in anti-radiation products

Fungi melanin has special anti-radiation property, it can be used to develop radiation-protective eye wear, sunglasses and sunscreen. There is already a market for sunscreens and sunscreen cosmetics that incorporate natural fungal melanin (Panzella et al. 2018). In the aerospace field, using fungal melanin as a composite material can protect space equipment from the hazards of space radiation. It can also be applied to protect humans in space and patients receiving radiation treatments (Cordero 2017). Besides, melanin in black edible mushrooms can be developed into low-cost oral radioprotectors for patients, thus minimizing the damaging influence of radiation on human health (Revskaya et al. 2012). It is also photoprotective and antioxidant, which makes it suitable for use in pesticide products to reduce degradation of effectiveness. For example, *Bacillus thuringiensis* has been used as a biopesticide in the world, but the insecticidal activity is relatively unstable and rapidly loses under field conditions due to UV radiation. Fungal melanin is capable of absorbing radiation and considered to be a promising photoprotectant for *B. thuringiensis*-based biopesticides (Sansinenea and Ortiz 2015).

Applications in environmental protection

Fungal melanin is considered a potential biosorbent for the effective removal of toxic metal ions from wastewater and soil because of its metal binding and stability. The fungal melanin extracted from *Armillaria mellea* was bound to polymeric nanofiber membranes to obtain a stable and highly porous filtration system that removed more than 90% of toxic metal ions from single-component solutions (Tran-Ly et al. 2020). The metal ion adsorption capacity of fungal melanin increased with higher pH and exhibited better adsorption capacity at pH > 4 (Oh et al. 2021b). It has been demonstrated allomelanin has higher porosity and can be used as a toxin adsorbent for environmental protection (McCallum et al. 2021). As a potential biosorbent, it can also adsorb radioactive elements for the protection of both the natural environment and human health. Therefore, these membranes with melanin compound can effectively filter to remove heavy metals from wastewater and achieve the purpose of industrial wastewater purification.

Applications in the food industry

Food-grade melanin is generally derived from edible and medicinal fungi. Melanin can be used as a functional ingredient in food and has some other effects, such as color enhancement and shelf-life extension. Fungal melanin can enhance the functional value of food by exerting biological activities, for example, *A. auricular* melanin is a widely used food color additive. It is commonly used in preparing yogurt to enhance its prebiotic properties or to improve the antioxidant and free radical scavenging properties of food (Pak et al. 2021). In order to improve the diversity of food culture, fungal melanin may be used as a food additive to develop a variety of novel food products, such as black vermicelli, black soybean curd skin, and black caviar (Mesías and Delgado-Andrade 2017). Fungal melanin also has lowered blood cholesterol, lowered blood sugar, and antitumor effects, which can be applied to functional foods. It is expected that more use of melanin extracted from edible and medicinal fungi as food additives will improve human health.

Applications in the biomedical industry

Fungal melanin can scavenge free radicals and perform better antioxidant and anti-inflammatory effect, which means it can be useful in the development of medicines and healthcare, and provide a new method for enhancing antioxidant therapy (Qi et al. 2019). Besides, it used as a prebiotic can effectively promote the activation of *Bifidobacterium bifidum*, contributing a new method to increase the production of *B. bifidum* in the pharmaceutical industry (Burmasova et al. 2019). In order to enhance human immunity, the efficacy ingredients of fungal melanin are being extracted to create multi-functional capsules, nutritional health tablets, oral liquid, etc. As a biochemical functional material, fungi melanin is a powerful antimicrobial agent that is widely used in pathology and biomedical fields. Moreover, the ability of fungal melanin to improve immune function, induce apoptosis, and inhibit blood angiogenesis makes it an effective anticancer treatment component (Marcovici et al. 2022). Because of its natural biocompatibility, melanin has a multifunctional role in nanomedicine. Due to the π-conjugated structure on the surface of melanin, it can be used for drug delivery by binding to various aromatic structures (Cuzzubbo and Carpentier 2021). Fungal melanin will make it a better candidate in imaging because of its biocompatibility and metal-ions chelation ability, such as fluorescence imaging, photoacoustic imaging, and magnetic resonance imaging (Wang et al. 2020). The antioxidative and light-absorbing properties of fungal melanin make it ideal for

use in antioxidative and photothermal therapies (Mavridi-Printezi et al. 2020).

Conclusion and outlook

The pursuit of safe, non-toxic and economical natural pigments has become one of the issues that must be solved nowadays, and the natural ingredients sought by consumers have ignited more enthusiasm among scholars to study natural pigments (Fonseca et al. 2022). Fungal melanin as one of the natural pigments has become the focus of industrial interest. This review confirms that fungal melanin has a promising future. Therefore, the development and utilization of functional properties and biological activities in fungal melanin will have an important effect on the future industrial application. Compared to synthetic one, fungal melanin has better environmental sustainability, functional properties, biodegradability and biological activities, implying that it has higher value for future applications in food, medicine and industry.

However, there are still some problems that need to be solved in the research process. The precise structure of fungal melanin needs to be explored deeply. Because the traditional methods of melanin extraction no longer meet the existing demand at present, more simple, economical and eco-friendly extraction methods are required for the future development and application of fungal melanin. For example, the use of ionic liquids to extract melanin has the benefit of increasing not only the extraction rates, but also the ability to reuse the solvents (Ghadge et al. 2022). Additionally, to elucidate the molecular mechanism of melanin formation in fungi, the signaling pathways and genes involved in melanin synthesis and metabolism need to be further explored. These researches will be helpful for modifying genetically engineered bacteria to achieve higher yields. Moreover, based on the research of melanin chemical modification methods, it is necessary to optimize the methods of modification and to study whether other modification methods can maximize the problem of insolubility. It is worth exploring more functional properties and biological functions of melanin in edible and medicinal fungi in the future, which may benefit human health and the environment. Fungal melanin has shown great social benefits in medicine, aerospace, food, and industry, it deserves further research to provide more advantages for future life.

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Declarations

Disclaimer The views stated here are ours and ours only. We apologize if we failed to mention other significative researches of fungal melanin and if our interpretation of the data is not accepted by other researchers.

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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