

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Production of Melanin Pigment by Fungi and Its Biotechnological Applications

Sandra R. Pombeiro-Sponchiado, Gabriela S. Sousa,
Jazmina C. R. Andrade, Helen F. Lisboa and
Rita C. R. Gonçalves

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/67375>

Abstract

Production of the microbial pigments is one of the emerging fields of research due to a growing interest of the industry for safer products, easily degradable and eco-friendly. Fungi constitute a valuable source of pigments because they are capable of producing high yields of the substance in the cheap culture medium, making the bioprocess economically viable on the industrial scale. Some fungal species produce a dark-brown pigment, known as melanin, by oxidative polymerization of phenolic compounds, such as glutaminyl-3,4-dihydroxybenzene (GDHB) or catechol or 1,8-dihydroxynaphthalene (DHN) or 3,4-dihydroxyphenylalanine (DOPA). This pigment has been reported to act as “fungal armor” due to its ability to protect fungi from adverse conditions, neutralizing oxidants generated in response to stress. Apart from the scavenging activity, melanin exhibits other biological activities, including thermoregulatory, radio- and photoprotective, antimicrobial, antiviral, cytotoxic, anti-inflammatory, and immunomodulatory. Studies have shown that the media composition and cultivation conditions affect the pigment production in fungi and the manipulation of these parameters can result in an increase in pigment yield for large-scale pigment production. This chapter presents a comprehensive discussion of the research on fungal melanin, including the recently discovered biological activities and the potential use of this pigment for various biotechnological applications in the fields of biomedicine, dermocosmetics, materials science, and nanotechnology.

Keywords: fungi, pigment, melanin, biological activity, industrial applications

1. Introduction

Considering the harmful effects of synthetic dyes on human health and to the environment, developmental process for obtaining pigments from natural sources has become significant worldwide. Microbial pigments have gained attention owing to a growing interest of the industry in safer products, easily degradable, eco-friendly and do not cause harmful effects. The pigment production from microorganisms is considered more advantageous because it is a more efficient and cost-effective process than chemical synthesis of pigments. Microorganisms are also more feasible sources of pigments in comparison to pigments extracted from plants and animals because they do not have seasonal constraints, do not compete for limited farming land with actual foods, and can be produced easily in the cheap culture medium with high yields [1–6]. Besides, the microorganisms produce an extraordinary range of pigments that include several chemical classes such as carotenoids, melanins, flavins, phenazines, quinones, monascins, violacein, or indigo, as shown in **Table 1**.

Pigment	Microorganism
Indigoidine (blue-green)	<i>Streptomyces aureofaciens</i> CCM 323, <i>Corynebacterium insidiosum</i>
Carotenoid (orange)	<i>Gemmatimonas aurantiaca</i> T-27
Melanin (black-brown)	<i>Kluyveromyces marxianus</i> , <i>Streptomyces chibanensis</i> , <i>Cryptococcus neoformans</i> , <i>Aspergillus</i> sp., <i>Wangiella dermatitidis</i> , <i>Sporothrix schenckii</i> , and <i>Burkholderia cepacia</i>
Prodigiosin (red)	<i>Serratia marcescens</i> , <i>Rugamonas rubra</i> , <i>Streptoverticillium rsubireticuli</i> , <i>Serratia rubidaea</i> , <i>Vibrio psychroerythrus</i> , <i>Alteromonas rubra</i> , and <i>Vibrio gazogenes</i>
Zeaxanthin (yellow)	<i>Staphylococcus aureus</i> , <i>Vibrio psychroerythrus</i> , <i>Streptomyces</i> sp., and <i>Hahella chejuensis</i>
Canthaxanthin (orange)	<i>Monascus roseus</i> , <i>Bradyrhizobium</i> sp.
Xanthomonadin (yellow)	<i>Xanthomonas oryzae</i>
Astaxanthin (red)	<i>Phaffia rhodozyma</i> , <i>Haematococcus pluvialis</i>
Violacein (purple)	<i>Janthinobacterium lividum</i>
Anthraquinone (red)	<i>Paecilomyces farinosus</i>
Halorhodopsin and rhodopsin (pink)	<i>Halobacterium halobium</i>
Rosy pink	<i>Lamprocystis roseopersicina</i>
Violet/purple	<i>Thiocystis violacea</i> , <i>Thiodictyon elegans</i>
Rosy peach	<i>Thiocapsa roseopersicina</i>
Orange brown	<i>Allochromatium vinosum</i>
Pink/purple violet	<i>Allochromatium warmingii</i>

Table 1. Pigments produced by different microorganisms. Adapted from Ref. [3].

Among microbial species, fungi represent an economically significant source of these compounds because they can act as microbial cell factories producing high yields of metabolites with great diverse chemical structures combined with ease of large-scale cultivation [7–9].

As shown in **Table 1**, some fungal species produce a dark-brown pigment, known as melanin. In general, this pigment is located in the outermost layer of the cell wall associated with chitin (referred as cell wall-bound melanin), but in some fungi, melanin can also be found outside the fungal cell, usually in the form of granules in culture fluids [10].

Fungal melanins are negatively charged, hydrophobic pigments of high molecular weight formed by oxidative polymerization from phenolic and/or indolic compounds, such as glutaminy-3,4-dihydroxybenzene (GDHB) or catechol or 1,8-dihydroxynaphthalene (DHN) or 3,4-dihydroxyphenylalanine (DOPA). Most Ascomycota fungi synthesize DHN-melanin from the polyketide synthase pathway, whereas few species are able to produce melanin through L-DOPA, in a pathway that resembles mammalian melanin biosynthesis [11–13].

The melanin pigment is not essential for fungal development, but it has been reported to act as “fungal armor” due to its ability to protect the microorganisms from harmful environmental conditions. In vitro studies have shown that melanized fungi are more resistant to UV light-induced and oxidant-mediated damages, temperature extremes, hydrolytic enzymes, heavy metal toxicity, and antimicrobial drugs than those nonmelanized [10, 14–17]. Recent studies have shown that in industrial and roadside areas, there is an increase in the proportion of dark melanin-containing fungi, as *Cladosporium* and *Alternaria*, which were more resistant to contamination by heavy metals and unsaturated hydrocarbons. Radionuclide contamination also led to a change in fungal communities, with an increased proportion of melanized fungi. For example, melanized fungal species as *Cladosporium* spp., *Alternaria alternata*, *Aureobasidium pullulans*, and *Hormoconis resinae* were found to colonize the walls of the damaged reactor at Chernobyl where they are exposed to a high constant radiation field [18, 19].

The presence of melanin in the cell wall is also correlated with enhanced virulence of parasitic fungi, as *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and *Exophiala (Wangiella) dermatitidis* [17, 20, 21]. This pigment protects the conidia against digestion by proteases and hydrolases secreted by competitive microorganisms or against bactericidal and fungicidal proteins of animal origin, such as defensins, magainins, or protegrins [22]. This effect was observed for *Cryptococcus neoformans*, whose in vitro melanization has been associated with resistance against host effector cells, oxidants, microbicidal peptides, and amphotericin B [23–25], and in *Wangiella (Exophiala) dermatitidis*, when the polyketide synthase gene WdPKS1 associated on melanin production was disrupted, this strain has become more susceptible to voriconazole and amphotericin B [26]. Others studies suggest that melanin contributes to fungal pathogenesis because this pigment alters the host defense response mechanisms, decreases phagocytosis, and reduces the toxicity of microbicidal peptides, reactive oxygen species, and antifungal drugs as well as to play a significant role in fungal cell wall mechanical strength [27, 28].

Although the molecular structure of fungal melanin remains enigmatic, significant progress has been made in understanding particular aspects of its macro- and microstructure. These advances allow to elucidate the molecular mechanisms of the various biological functions of

melanin [22]. Studies have shown that the effect of melanin enhancing the survival of fungi under adverse conditions can be mainly due to its powerful free radical scavenger properties, acting as a “sponge” for other free radicals generated by the fungus in response to environmental stress [20, 29, 30]. Apart from this scavenging ability, melanin exhibits other biological activities, including thermoregulatory, photoprotective, antimicrobial, antiviral, cytotoxic, anti-inflammatory, radioprotective, and immunomodulatory [13, 17, 18, 31–34].

Since melanin has characteristics of functional materials and bioorganic, a growing number of researchers see this pigment with great interest, taking advantage of their properties for numerous biotechnological applications in cosmetics, pharmaceutical, electronic, and food processing industries [12, 19, 35].

The purpose of this chapter involves a comprehensive discussion of the research on fungal melanin, including the recently discovered biological activities and the potential use of this pigment for several biotechnological applications. Additionally, we discussed the ways to explore the metabolic potential of the pigment-producing fungi by manipulation of cultivation conditions to improve performance of the process, increasing yields, and reducing cost, for large-scale production.

2. Factors influencing the melanin production

Microbial pigment production is now one of the emerging fields of research due to its potential for various industrial applications, as foodstuff, cosmetics, pharmaceutical, and textile manufacturing processes. However, it is known that for the success of microbial fermentation processes, it is necessary to choose the correct productive culture strain and to determine the appropriate cultivation conditions [4, 8, 36].

An ideal pigment-producing microorganism should be capable of using a wide range of C and N sources; must be tolerant to pH, temperature, and minerals concentration; and must give reasonable pigments yield. The nontoxic and nonpathogenic natures, coupled with easy separation from cell biomass, are also preferred qualities. The potential of using filamentous fungi as pigment sources is due to their extraordinary metabolic versatility because they can be cultivated over a wide range of temperatures (10–50°C), pH (2–11), salinity (0–34%), and water activity (0.6–1) and under oligotrophic or nutrient-rich conditions. They can grow in different culture systems (submerged and solid), and fermentation protocols have been established for large-scale industrial processes. In addition, these organisms can be genetically modified to increase productivity and quality of the produced pigments [37, 38].

In order to improve performance and reduce the cost of pigments produced by microbial fermentation, it is essential to identify the nutritional and physical factors that have a greater influence on the cell growth and metabolite biosynthesis [4, 6, 39, 40].

Several studies have shown that the composition of the growth medium, nature and concentration of carbon and nitrogen sources, minerals, vitamins, temperature, pH, the presence of

oxygen and aeration, light, stress, and irradiation, among others, affect the growth and pigment production in fungi and that the manipulation of the culture conditions can result in enhanced pigment production [41–47].

Experimental evidences indicate that the growth temperature influences the performance of the pigment production process, but this effect depends on the type of organism. *Pseudomonas* requires 35–36°C for its growth and pigment production, while in *Monascus purpureus*, maximum pigment production was observed at 30°C with a reduction of the yield at 37°C [48]. Another study in *Monascus* sp. *J101* reported that the yield of pigment at 25°C was ten times higher than at 30°C, probably due to long growing (120 hours) and lower viscosity of the broth at 25°C compared to 30°C [49]. Studies developed in our laboratory, using a melanin-overproducing mutant (MEL1) from *Aspergillus nidulans* fungus, showed that the higher production of pigment occurred at incubation temperature of 28°C compared to 37°C [50].

Researches support that the pH of the medium also affects the growth of fungi and type of pigment produced. In species of *Monascus*, the pH influences the yield and quality of the produced pigment, with the highest red pigment excretion and production at alkaline pH [51, 52]. Studies on wood-inhabiting fungi indicate that pH of the substrate potentially plays an important role in fungal melanin formation. Fungi *Trametes versicolor* and *Xylaria polymorpha* tested on wood substrates produced maximum pigmentation at the pH range 4.5–5.0, except for *Scytalidium cuboideum*, which produce maximum intensity of red pigment at pH 6 and blue pigment at pH 8 [53]. In our study with the hypermelanized mutant (MEL1) from *A. nidulans*, we observed an increase in the production of pigment when the initial pH of the culture was at 6.8 compared to pH 8.0 [50]. Metabolically, the effects of pH and temperature on fungal pigment production is associated with changes in protein activity, so that the culture conditions may control certain activities such as cell growth, production of primary and secondary metabolites, fermentation, and oxidation processes of the cell [54].

The influence of light on intra- and extracellular pigment production was studied in five pigment-producing fungi: *M. purpureus*, *Isaria farinosa*, *Emericella nidulans*, *Fusarium verticillioides*, and *Penicillium purpurogenum* [55]. These authors concluded that the cultivation in the total absence of light increased biomass and production of extracellular and intracellular pigments in all fungi. The fungi grown under red light have no effect, and green or yellow light resulted in worsening effect in all the fungi, thus postulating the existence of photoreceptors responsive to dark and light in all the fungi. In a similar study, [56] noted that the production of pigment by *Monascus* species also was favored when the fungus was grown in the dark.

Some studies report that the pigment synthesis requires proper aeration probably related to the oxygen dependency of some enzymatic reactions responsible for the production of pigment. In *Monascus ruber*, it was observed that the highest levels of pigments production were obtained at an aeration rate of 0.05 L min⁻¹, which appeared to be clearly sufficient for providing the fungus with oxygen and removing carbon dioxide [57]. In our studies, it was noted that no melanin pigment production takes place during stationary cultivation of hypermelanized mutant (MEL1) from *A. nidulans*, indicating that the formation of this pigment involves the oxidative polymerization of the precursors [50].

Carbon and nitrogen are necessary for cellular metabolism, and these sources are related to the formation of biomass, the type produced pigment, and the yield of the desired substance. These nutrients may regulate the expression of genes of interest and activate important metabolic pathways for the production of pigments [45, 58, 59]. In general, glucose, an excellent carbon source for growth, interferes with the formation of many secondary metabolites, including pigments. For example, the pigment production by *Penicillium* sp. was evaluated in the presence of 10 different carbon sources, and the maximum mycelial growth was obtained with fructose, whereas the maximum pigment production was obtained with soluble starch [60]. This result shows that the increased biomass does not necessarily result in increased pigment production because pigments produced by fungi are secondary metabolites whose production usually occurs at the late growth phase (idiophase) of these microorganisms [61]. The pigment production capability of fungal species belonging to the genera *Penicillium*, *Aspergillus*, *Epicoccum*, *Lecanicillium*, and *Fusarium* was evaluated in different culture media, and the results showed that the complex media, as potato dextrose (PD) and malt extract (ME), favored increased pigment production [47]. According to the authors, these media contain nutrients that can regulate the expression of genes of interest and activate metabolic pathways important for the production of pigments.

Studies have demonstrated that the promoting or repressing effect of a nitrogen source on pigment production is strain dependent. It has been reported that various types of peptone, used as a nitrogen source, are able to promote an increase in the production of pigments in many species of fungi [55, 59, 62, 63]. However, *M. purpureus* was not able to grow in media containing peptone, and a maximum yield of the pigment was achieved when the media were supplemented with yeast extract (1%) and monosodium glutamate (5%) as nitrogen source [41]. In *M. ruber*, the use of glutamic acid as a nitrogen source showed promising results, either as stimulating the accumulation of extracellular pigments or contributing to increase the efficiency of the pigment production process [45]. The production of high amounts of extracellular melanin by the fungus *Gliocephalotrichum simplex* was obtained in cultures supplemented with tyrosine (2.5%) and peptone (1%) [64].

The optimization of medium composition is an important strategy to increase pigment production because some sources of carbon and nitrogen can be more easily assimilated and promote higher yields of the desired product. During the optimization experiments to enhance the production of melanin by *Auricularia auricula*, it was observed that soluble starch, tyrosine, peptone, CaCO_3 , and K_2HPO_4 had positive effects, while glucose, $(\text{NH}_4)_2\text{SO}_4$, MgSO_4 , CuSO_4 , and FeSO_4 negatively impacted melanin production [46]. In other study with *A. auricula*, it was observed that yeast extract, tyrosine, and lactose have significant effects on pigment production and the optimization of medium resulted in 2.14-fold higher melanin concentration than that of the unoptimized medium [65].

Since the substrates for the production of pigment strongly influence the cost of the bioprocess, there is a need to select cheap and efficient substrates to make the process economically viable on the industrial scale. Large amounts of agro-industrial residues generated from diverse economic activities have attracted strong industry interest on the utilization of these residues as inexpensive substrates to support the growth of microorganisms in bioprocesses.

This strategy may represent an added value to the industry and also helps in solving pollution problems, reducing or preventing their disposal in the environment [1, 66, 67].

Various studies have reported the successful utilization of agro-industrial residues for the production of fungal pigments. The use of corn cob powder as a substrate for production of pigments by *M. purpureus* resulted in greater pigment production [68] than other substrates, as jackfruit seed [69], corn steep liquor [70], and grape waste [71]. In the black yeast *Hortaea werneckii*, it was observed that rice bran acts as the cheapest source for increased production of melanin by than wheat bran and coconut cake [72]. Wheat bran extract, L-tyrosine, and CuSO_4 represent the best combination of medium components to obtain the maximum melanin yield from the fungus *A. auricula* in submerged culture [73]. A study conducted in our laboratory evaluated the use of corn steep liquor, sugarcane bagasse, and molasses as nutritional source on pigment production by melanin-overproducing mutant (MEL1) from *A. nidulans*. We observed that, in the presence of 0.2% corn steep liquor, an increase in the pigment production occurred, while a high yield of biomass was obtained at a concentration of 2%. The supplementation of medium with molasses and sugar cane bagasse hydrolysate did not have a positive effect on pigment production but promoted an increase in the fungal growth. These results indicate that corn steep liquor contains substances that stimulate the synthesis of pigment and it represents a low-cost fermentation medium for large-scale production of the pigment melanin by MEL1 mutant for future industrial applications [74].

3. Pathways of melanin biosynthesis

Various techniques, including electron paramagnetic resonance [75], X-ray diffraction [76], infrared, ultraviolet and visible spectroscopy [77], and nuclear magnetic resonance [78], have been used to elucidate the melanin structure from different organisms. These studies have shown that fungi can produce different types of melanins by oxidative polymerization of phenolic or indolic compounds [11, 27].

Melanin in cell walls of *Basidiomycotina* is derived from phenolic precursors, as glutaminy-3,4-dihydroxybenzene (GDHB) or catechol. In the parasitic fungus *Ustilago maydis*, polymerization of catechol dimers with the formation of fibrils of melanin was shown [79]. The precursor of melanin in *Agaricus bisporus* and other *Basidiomycetes* is a metabolite of the shikimic acid pathway- γ -glutaminy-4-hydroxybenzene oxidized under the action of peroxidase and/or phenolase into γ -glutaminy-3,4-benzoquinone, followed by its polymerization [80]. *C. neoformans*, a pathogenic basidiomycetous yeast, is known to synthesize DOPA-melanin when o-diphenolic compounds, such as 3,4-dihydroxyphenylalanine, are present in the culture medium. This fungus may use a wide array of substrates, such as D- and L-dopamine [81], homogentisic acid [82], catecholamines, and other phenolic compounds [83], maximizing its ability to produce melanin. Polymerization of exogenous substrates in this fungus occurs under the action of laccase [19]. However, it is important to emphasize that different properties are observed for melanins derived from different substrates. Comparison of the catecholamines L-dopa, methyl-dopa, epinephrine, and norepinephrine shows differences in term of

color, yield, and thickness of the cell wall melanin layer. It was also observed that the pigments vary in the strength of the stable free radical signal detectable by EPR [13, 83].

In the Ascomycota fungi, melanin pigment is generally synthesized from the pentaketide pathway in which 1,8-dihydroxynaphthalene (DHN) is the immediate precursor of the polymer, as described by Bell and Wheeler [11] based on genetic and biochemical evidence obtained from *Verticillium dahliae* and *W. dermatitidis* [84, 85]. **Figure 1** shows a general model for fungal dihydroxynaphthalene (DHN)-melanin biosynthesis. In this pathway, the polyketide synthase (PKS) converts malonyl-CoA to 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN), which undergoes several reduction and dehydration reactions to produce scytalone, 1,3,8-trihydroxynaphthalene (THN), and vermeline. A further dehydration step leads to the intermediate 1,8-dihydroxynaphthalene (DHN), which is polymerized to DHN-melanin, possibly by a laccase enzyme [10, 13, 27].

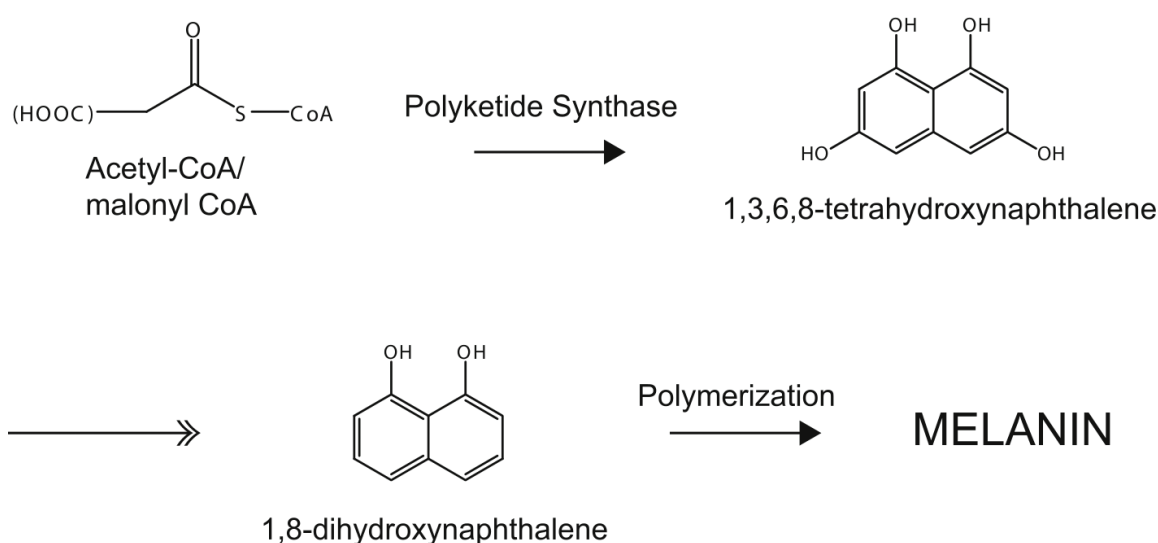


Figure 1. The biosynthetic pathway of fungal dihydroxynaphthalene(DHN)-melanin. Scheme adapted from Ref. [13].

However, some species of this class, including *Cladosporium resinae*, *Epicoccum nigrum*, *Hendersonula toruloidea*, *Eurotium echinulatum*, *Humicola grisea*, and *Hypoxylon archeri*, do not produce this type of pigment [11, 28, 86–88]. In the genus *Aspergillus*, DHN-melanin has not been identified in some members, as *A. nidulans* and *A. niger*. Bull [89] identified dopachrome (indole 5,6-quinone 2-carboxylic acid) and melanochrome (indole 5,6-quinone), which are intermediates in the DOPA-melanin pathway, in *A. nidulans* mutants defective in the production of melanin. Other studies confirmed the indolic nature of the melanin produced by *A. nidulans* [11, 90]. In *A. nidulans* strains, one tyrosinase was identified as the enzyme responsible for the production of melanin pigment, based on its substrate specificity (DOPA substrate) and susceptibility to inhibitors [91, 92]. In a recent study, our group characterized the pigment produced by *A. nidulans* mutants as DOPA-melanin according to the results obtained with specific inhibitors of DHN- and DOPA-melanin pathways [93].

The production of DOPA-melanin has also been investigated in other fungi such as *Neurospora crassa* [94], *Podospora anserina* [95], *A. nidulans* [91], *A. oryzae*, and *C. neoformans* [96]. A biosynthesis pathway for fungal DOPA-melanin, proposed by [11], is shown in **Figure 2**, which strongly resembles the pathway found in mammalian cells, though some of the details may differ.

In this pathway, there are two possible starting molecules, L-dopa and tyrosine. If L-dopa is the precursor molecule, it is oxidized to dopaquinone by laccase. If tyrosine is the precursor, it is first converted to L-dopa and then dopaquinone. The same enzyme, tyrosinase, carries out both steps. Dopaquinone, a highly reactive intermediate, forms leucodopachrome, which is then oxidized to dopachrome. Hydroxylation (and decarboxylation) yields dihydroxyindoles, which can polymerize spontaneously to form DOPA-melanin [10, 27, 97].

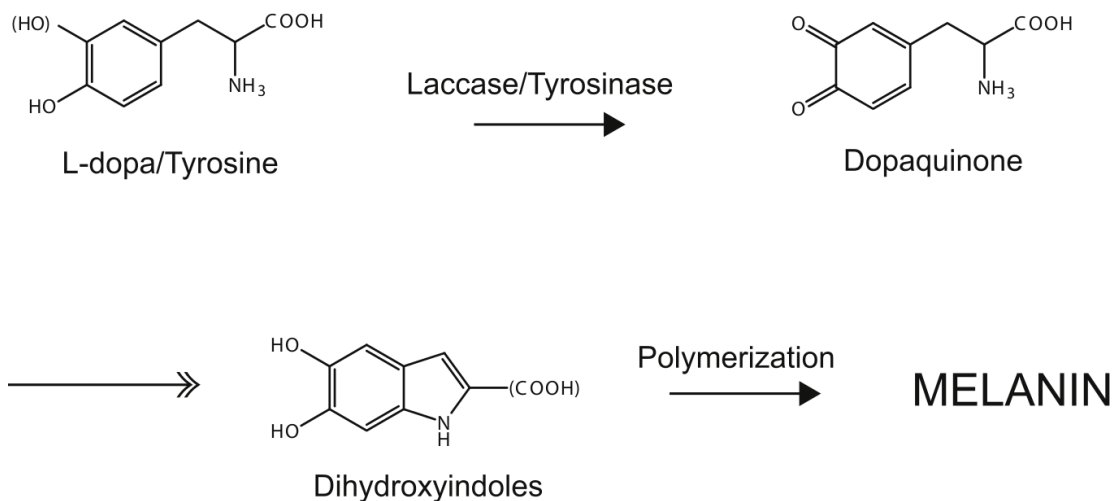


Figure 2. The biosynthetic pathway of the dihydroxyphenylalanine (DOPA)-melanin in fungi. Scheme adapted from Ref. [13].

Some fungi have more than one biosynthetic pathway of melanins. For example, *Aspergillus fumigatus* synthesizes DHN-melanin [98] and also produces a second type of melanin, piemelanins, from homogentisic acid by the tyrosine degradation pathway that protects the cell wall of hyphae from ROS, and gray-green DHN-melanins determine the structural integrity of the cell wall of conidia and their adhesive properties [99]. In *Agaricus bisporus*, melanins are formed from DOPA by tyrosinase and from γ -glutaminy-4-hydroxybenzene by peroxidase and phenolase [100].

The extracellular fungal melanin, which is found in culture fluids usually in the form of granules, can be formed from some culture components, which are autoxidized or are oxidized by phenoloxidases released from the fungus during autolysis [10, 11, 27].

4. Biological activities of melanin

Despite the difference in their origins, melanin pigments have a number of common characteristics that allow them to fulfill their protective function. Several biological functions of

melanins are closely associated to their chemical composition and structure. The presence of unpaired electrons in the melanin structure is responsible for various properties, including antioxidant, semiconductor, optical, electronic, and radio- and photoprotective [19].

The effect of melanin enhancing the survival of fungi under adverse conditions is mainly due to its function as an extracellular redox buffer, which can neutralize oxidants generated by the fungus in response to environmental stress [19]. It has been reported that melanin contributes for virulence of *C. neoformans*, protecting the pathogen against free radicals generated immunologically [29]. In *W. dermatitidis* and *A. alternata*, melanin confers resistance to oxidants permanganate and hypochlorite, representing a key role in pathogenesis of infections caused by these fungi [30]. Studies have shown that melanin of zoopathogenic and phytopathogenic fungi is essential for their parasitizing, due to its antioxidant properties [101].

Melanin pigment extracted from several fungal species has shown the ability to scavenge free radicals (reactive nitrogen and oxygen species), becoming a potential natural antioxidant. Melanins produced by *Exophiala pisciphila* and *Aspergillus bridgeri* ICTF-201 exhibited a significant DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity comparable with that of synthetic melanin, indicating its antioxidant potential [102, 103]. Melanin produced by *Schizophyllum commune* showed high free radical scavenging activity in a dose-dependent manner, when the melanin concentration was increased from 10 to 50 μg , the scavenging activity was also increased from 87% to 96%, similar to those obtained using ascorbic acid (standard compound used to measure free radical scavenging activity) [34]. Melanin pigment of *Fonsecaea pedrosoi* has antioxidant potential by reducing Fe(III) to Fe(II), ensuring the balance of its redox chemical microenvironment and minimizing the effect of oxidation of fundamental structures on fungal growth [104]. Similar results were also observed for melanin from *Ophiocordyceps sinensis*, which proved to be an effective DPPH radical scavenger and a strong ferrous iron chelator [105]. The chelating power of fungal melanin can be explained by various functional groups present in the structure of this pigment, which provide an array of multiple nonequivalent binding sites for metal ions [14, 22].

It has been reported that substances acting as antioxidants protect cells from ROS-mediated DNA damage, which can result in mutation and subsequent carcinogenesis. The excess free radicals may attack cellular constituents, as the cell membrane, nucleic acid, protein, enzymes, and other biomolecules, by peroxidation, resulting in the severe damage of cell functions and subsequent serious deleterious effects on the organism [106]. It has been reported that melanin protects melanocytes and keratinocytes from the induction of DNA strand broken by hydrogen peroxide, indicating that this pigment also has an important antioxidant role in the skin [107]. Studies in our laboratory showed that melanin extracted from hyperpigment-productive mutant (MEL1) of *A. nidulans* has the ability to scavenge the biological oxidants, as HOCl, and may be a promising material in cosmetic formulations to protect the skin against possible oxidative damage [31].

There is experimental evidence that fungal melanin may also act as an anti-aging drug, due to its action in reducing the generation of free radicals, clearing away the free radicals produced in excess, and enhancing the activities of antioxidant enzymes. Studies have shown that one of the major causes of aging is the surplus free radicals produced during

the oxidative metabolism in the human body [108]. It was demonstrated that the melanin produced by fungus *Lachnum singerianum* YM296 significantly inhibited the formation of lipid peroxidation products and slowed down the aging process, elevating the levels of superoxide dismutase, glutathione peroxidase, and catalase and decreasing the level of malondialdehyde in mice liver and brain homogenate and serum, suggesting that this pigment could be used as a new anti-aging drug [109].

Researches have also shown that some fungal melanin exhibits immunomodulatory activity through the inhibition of pro-inflammatory cytokine production in T lymphocytes and monocytes, as well as fibroblasts and endothelial cells [12, 110, 111]. During an inflammatory response, cells of the innate and acquired immune systems release a variety of mediators, such as nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukins (IL), and the reactive nitrogen and oxygen species, which are implicated in the pathogenesis of a number of inflammatory diseases [112]. [113] reported that treatment of macrophages activated in vitro with melanin from the fungus *F. pedrosoi* inhibited the production of nitric oxide and Th1 cytokines. The study performed by [114] showed that the expression of inducible nitric oxide synthase gene decreased and lower levels of cytokines, such as IL-12 and TNF- α , were observed when activated macrophages were incubated with melanized cells of the *Fonsecaea monophora* fungus. Our studies demonstrated that melanin extracted from a highly melanized mutant (MEL1) of *A. nidulans* inhibited NO production in LPS-stimulated macrophages, with a maximum response of 82% inhibition, and also showed a dose-dependent inhibitory effect on TNF- α production, reaching an inhibition of 51.86% at a melanin concentration of 100 $\mu\text{g/mL}$. These results suggest that melanin from *A. nidulans* has potential as an anti-inflammatory agent and may be used in the future for development of new drugs with therapeutic utility [32].

Some studies have proposed that fungal melanin exhibits anti-radiation activity in vivo and in vivo and then could be explored as a probable radioprotector [16, 115]. Since melanin has a stable free radical population, it is thought that the radioprotective properties of this pigment result from a combination of physical shielding and quenching of cytotoxic free radicals generated by radiation [18]. [116] showed that *Lachnum* extracellular melanin (LEM404) had strong anti-ultraviolet radiation activity because the survival rates of *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* under UV radiation were significantly increased after in vitro addition of LEM404. Compared with the control groups, the antioxidant defense systems, such as superoxide dismutase and glutathione peroxidase activities, were improved significantly in mice of experiment groups, and the reactive oxygen species detected by malondialdehyde content were decreased significantly. These results confirmed that fungal melanin could be used as component of photoprotective creams mainly for its free radical scavenging rather than its light absorption properties. The probable mechanisms of radioprotection by melanin appear to be modulated in pro-survival pathways, immune system, and prevention of oxidative stress. It was reported that melanin isolated from the fungus *G. simplex* reduced the radiation-induced overproduction of pro-inflammatory cytokines (IL-6 and TNF- α), which might help in the recovery from radiation injury by preventing the aggravation of inflammation and oxidative stress [33]. This study confirmed the possible use of melanin-coated nanoparticles for protecting against radiotoxicity during radioimmunotherapy [117].

Recent studies have demonstrated that, in addition to the ability of transferring electrons arising under the action of radiation, melanin also possesses ionic conductivity due to its ability to transform any type of radiation energy not only into heat but also use it for the maintenance of redox processes in cells [118]. It was assumed that melanin pigments, participating in redox reactions, are able to perceive the energy of radiation (UV, visible light, and radiation) and convert it into useful reducing power for metabolic processes. This hypothesis is supported by the discovery of melanized fungi in soils contaminated by radioactive nuclides and areas around the damaged Chernobyl nuclear reactors, which not only survive high radiation levels but also have enhanced growth upon exposure [16, 19, 119, 120]. Owing to its semiconductor property, melanin becomes a promising material for organic bioelectronic devices like transistors, sensors, and batteries [121].

Fungal melanins also exhibit growth inhibitory effect against various microorganisms. The extracellular melanin isolated from *S. commune* showed significant antibacterial activity against *E. coli*, *Proteus* sp., *Klebsiella pneumonia*, and *Pseudomonas fluorescens* and antifungal activity against dermatophytic fungi, *Trichophyton simii*, and *T. rubrum* [34]. The *A. auricula* melanin displayed inhibitory activity on biofilm formation of the three bacterial strains, *E. coli* K-12, *Pseudomonas aeruginosa* PAO1, and *P. fluorescens* P-3, and there was a proportional reduction in biofilm biomass with the increase in pigment concentration. Confocal laser scanning microscopy (CLSM) analyses showed that the three strains formed thick and compact biofilms when grown in the absence of pigment, but the presence of *A. auricula* melanin resulted in thinner and looser cell aggregations on surfaces instead of normal biofilm architecture. This study suggested that *A. auricular* melanin inhibits quorum-sensing (QS)-regulated biofilm formation in all strains tested without interfering with their growth [122]. Silver nanoparticles incorporated *Yarrowia lipolytica* melanin exhibited antimicrobial activity against the pathogen *Salmonella paratyphi*, and they were also effective at disrupting biofilms on polystyrene as well as glass surfaces [123]. These nanoparticles displayed excellent antifungal properties toward an *Aspergillus* sp. isolated from a wall surface, suggesting the application of these nanoparticles as effective paint additives. The melanin-silver nanostructures with broad-spectrum antimicrobial activity against food pathogens also have potential applicability in food processing and food packaging industries [124].

The anti-cell proliferation effect of fungal melanin in tumoral cell lines has already been demonstrated. [34] reported that the extracellular melanin produced by the fungus *S. commune* was effective against human epidermoid larynx carcinoma cell line (HEP-2) in a concentration-dependent manner, indicating its potential application in cancer chemoprevention and chemotherapy.

The evaluation of the effect of fungal melanin on non-tumor cells is also interesting because it may serve as alternative to acute in vivo toxicity testing, avoiding the indiscriminate use of animals. The melanin produced by *A. bridgeri* was evaluated in vitro cytotoxicity assay using cell lines TE 355.Sk derived from normal human skin fibroblasts and HEK-293 derived from human embryonic kidney cells, and no cytotoxicity was observed against the two cell lines [103]. In our studies, the toxicity of the melanin from *A. nidulans* was also evaluated due to its potential practical application as antioxidant and anti-inflammatory agent. The results showed that the viability of mouse macrophages remained greater than 90% when these cells

were treated with a high melanin concentration (100 $\mu\text{g}/\text{mL}$), indicating that this pigment has low cytotoxicity [32]. We also showed that the toxicity of *A. nidulans* melanin on mouse fibroblast McCoy cell line, after metabolic activation with hepatic S9 microsomal fraction, was much lesser ($\text{CI}_{50} = 413.4 \pm 3.1 \mu\text{g}/\text{mL}$) than known cytotoxic agents such as cyclophosphamide ($\text{CI}_{50} = 15 \pm 1.2 \mu\text{g}/\text{mL}$). In this study, we demonstrated that this melanin pigment did not induce gene mutations in different strains of *Salmonella typhimurium* used in the Ames assay. Based on these results, we suggest that the melanin produced by *A. nidulans* does not cause significant damage to the cellular components and might be used in the future for development of new therapeutic drugs [32].

5. Biotechnological applications of melanin

With the current knowledge about physical and chemical properties and the broad spectrum of biological activities, fungal melanins have attracted growing interest for their potential use in the fields of biomedicine, dermocosmetics, nanotechnology, and materials science.

5.1. Bioelectronic applications

In recent years, the electronics industry has been driven to develop materials and components that are cheaper and more environmentally friendly. As melanin has characteristics of functional materials and bioorganic, a growing number of researchers in the fields of materials science and organic electronics see the melanin with great interest, taking advantage of their properties for applications in organic electronic devices. Melanins present interesting optoelectronic properties, such as high optical absorption in the UV-Vis range, good transmission electronic, and ionic conductivity appreciably, pointing this biomaterial as a promising active component in organic electronic devices with low environmental impact [118, 121, 125–127].

Among the physical properties of melanin, the electrical conductivity is one of the most interesting to investigate in the perspective of technological application. The electrical conductivity properties of this biopolymer are similar to those of amorphous semiconductor solids, and then it can be considered an organic semiconductor, which is largely available and biocompatible and, consequently, cheaper and easier to process with respect to inorganic semiconductors, as silicon germanium. In particular, it can be considered a promising material for sensors and photovoltaic devices, due to broadband spectral absorbance and charge transport properties [128].

The technical literature describes the integration of organic semiconducting polymers as melanin in silicon electronic devices in view of the possibility of achieving multifunctional systems that combine electrical and optical properties of semiconductors, the structural versatility and mechanical characteristics of materials, and processing polymeric [129]. The production of devices based on thin film melanin exhibited electrical conductivity comparable to that of amorphous silicon [130]. In this study, melanin films showed excellent thermal stability and adhere well to glass substrates and silicon, indicating the possibility of using this technique for the production of films from synthetic melanin. Other groups have published

various device architectures with applications such as memory (metal-insulator-semiconductor geometries) [131], batteries [132], and biomimetic interfaces [133].

Deposition of homogeneous melanin layers for optoelectronics application is an issue of considerable technological relevance. Synthetic melanin thin films deposited by spray-coating presented features ascribed to an amorphous semiconducting material [134]. They also showed that further improvement of conductivity together with an increased absorption in the NIR region, by doping the synthetic melanin macromolecule, could make this material a good candidate for optical sensing applications. It has been reported that the iron-melanin coating markedly enhances the catalytic activity of the gold nanoparticles (AuNPs) for both the hydrogen peroxide electroreduction and hydrogen evolution reaction [135]. This strategy may be used to improve nanomaterials with potential applications as efficient catalysts and electrocatalysts. Studies have shown that synthetic melanin-like nanoparticles complexed with paramagnetic Fe^{3+} ions have potential as a highly efficient and nontoxic contrast agent for magnetic resonance imaging instead of Gd^{3+} -based contrast agents, which can cause nephrotoxicity [136].

The optical and electronic properties of melanin have attracted the attention of researchers for the production of continuous thin films from conventional synthetic melanin, which have been used for a number of different device configurations, including chemi-sensors, next-generation solar cells, and a range of other detectors [126, 130, 134]. Potential also exists to use melanin films as an effective radiation sensitizer that could greatly improve the spectral range and efficiency of superconducting transition-edge bolometers [137].

The metal chelation properties of melanin offer interesting possibilities for melanin-based metal ion sensing. A piezoelectric sensor system capable of real-time detection of metal ions was constructed by cross-linking melanin onto the gold electrode of quartz crystal microbalance (QCM) and showed high sensitivity and selectivity to metal ions particularly for $\text{Hg}(\text{II})$ [138].

Melanin has many other interesting properties, such as ultraviolet absorption, which has been utilized to prepare optical lenses or filters. Studies have shown that it is possible to use melanin as an ultraviolet, visible and near-infrared absorbing pigment in ophthalmic devices, protective eyewear, windows, packaging material, umbrellas, canopies, and other similar media suitable for providing protection from radiation [139, 140]. The incorporation of the melanin in solid plastic films of polyvinyl alcohol (PVA-melanin film system) to be used in conjunction with other plastics to make laminated sheets or lenses, including sunglasses, ski goggles, ophthalmic prescription lenses, helmets, windows, light filters for artificial lighting, and other light filters that protect people from potentially damaging UV and high-energy visible light has also been reported [141].

5.2. Medical applications

Despite its high biocompatibility, the use of melanin as a novel biomaterial in pharmaceutical and biomedical applications reported in literature is still scarce. A study performed with melanin nanoparticles as biocompatible drug nanocarriers, using metronidazole (antibiotic

drug), showed that melanin could be a very interesting nanocarrier drug release device because it strongly responds to pH, being a very interesting feature for the treatment of intestine and colon diseases, which would greatly benefit with pH targeting [142]. Another study showed that systemic melanin-covered nanoparticle (MN) administration reduced hematologic toxicity in mice treated with radiation and that these structures provide efficient protection to bone marrow against radiotoxicity during radioimmunotherapy and in some cases external beam radiation therapy, permitting the administration to tumors of significantly higher doses [117].

Melanin has also been used to treat various types of malignant cancer tumors, disorders of the immune system including AIDS, diseases of blood origin and disorders due to the disturbances in cell homeostasis, and complex and hardly curable mental disorders (schizophrenia, epilepsy) involving nervous and other regulatory systems. A study on the use of melanin for the treatment of Parkinson's disease, an amelioration in the monkeys' overall functional ability and secondary motor manifestations by the administration of an effective amount of melanin in monkeys treated with MPTP (1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine), a toxin that causes a neurodegenerative disease, was observed. This study demonstrated that toxin-induced Parkinson's disease could be prevented in the melanin-treated animals because the administered melanin causes chelation or scavenging of toxins, such as MPTP, thus preventing a neurodegenerative disease, such as Parkinson's disease. The results of this study also showed that melanin administration to aid the recovery of neurons in a mammal having neuron injury suggests that melanin can be used to treat Alzheimer's disease [143].

Owing to their ability to increase the permeability of the blood-brain barrier, the melanin is also useful as carriers for other therapeutic agents, which must reach brain tissue to produce their therapeutic responses [144]. Two examples of such therapeutic agents that will cross the blood-brain barrier when linked to melanin are boron and nerve growth factor. According to the same authors, the melanin is also an effective vehicle for the transport of boron to cancerous sites in the body, mainly when the cancerous cells to be treated are located in the brain, because this pigment binds boron very strongly. The melanin can also function as a carrier for nerve growth factor due to the ability to get nerve growth factor across the blood-brain barrier, and this is the major advantage over conventional therapy.

In recent years, efforts have been focused on investigating the potential use of this pigment as active material in tissue repair engineering. Bettinger et al. [145] reported that thin films of melanin were found to enhance Schwann cell growth and neurite extension in rat pheochromocytoma cells (PC12 cells) compared to collagen films *in vitro*. Melanin films also induced an inflammation response that was comparable to silicone implants *in vivo*, and the implants were significantly resorbed after 8 weeks. These results showed that melanin thin films have great potential in the reconstruction of tissues, being biodegradable, and possess inflammatory response comparable to silicone. Another study of the biocompatibility of melanin thin films demonstrated that the melanin film effectively supports the growth of undifferentiated stem cells and their differentiation into neuronal precursors and neurons [146]. They related that high-quality melanin thin films display appealing features,

such as reversible conductivity by controlled hydration—dehydration steps—excellent biocompatibility with stem cells, and water-resistant adhesion, for bioelectronic applications, e.g., in organic electrochemical transistors (OECTs), which can translate cellular activity into electrical signals [125, 147]. It has also been reported that melanin thin films possess highly desirable physical and biological properties that make them ideal for organic bioelectronic devices [130].

In cosmetic industry, there are great interests in the melanin, especially to protect against the noxious effects of UV radiation by incorporation in skin photoprotection formulations [35, 148]. The protective action of melanin is related to its high efficiency to absorb and scatter photons, particularly the higher-energy photons from the UV and blue part of the solar spectrum. Very likely, melanin photoprotection is also due to its ability to quench excited states of certain molecules and scavenge ROS that may be generated in pigmented cells [126]. Development of methods for producing melanin soluble in aqueous cosmetic buffers at physiological pH and temperature may make possible the use of this pigment as ingredients of face and hand creams, lotions, antiaging ointments, or foundation makeups, acting as a screen and antioxidant for the protection against photoinduced skin damages [149]. Other dermo-cosmetic applications of melanins include the use of the pigment for hair dyeing and the development of novel strategies for hair recoloration [150].

Since melanin has a stable free radical population, it is thought that the radioprotective properties of this pigment result from a combination of physical shielding and quenching of cytotoxic free radicals generated by radiation [18]. Some studies suggest the possible use of melanin-coated nanoparticles in medicine, mainly for protecting patients against the harmful effects of gamma rays during radioimmunotherapy [34, 151]. Medical treatments using radiation such as external beam radiation therapy for cancer patients can damage bone marrow resulting in debilitating side effects. In experimental models, melanin can successfully shield bone marrow from such side effects. Mice treated with melanin-coated nanoparticles have higher white blood cell and platelet counts than control mice after radiation treatment [117]. It has been reported the use of melanin, a biopolymer with good biocompatibility and biodegradability, intrinsic photo-acoustic properties, binding ability to drugs, and chelating property to radioactive metal ions, as an efficient endogenous nanosystem for imaging-guided chemotherapy [152]. According to the authors, melanin nanoparticles could successfully enter into the tumor and act as an efficient drug-delivery system, thereby greatly increasing the safe utility of the drugs for tumor treatment and significantly lowering the dosage used and its side effects.

A valuable biotechnological approach to the melanin-mediated synthesis of silver nanostructures with broad-spectrum antimicrobial activity has been developed. Silver nanostructures synthesized with melanin derived from *Y. lipolytica* displayed excellent antifungal activity against an *Aspergillus* sp. isolated from a wall surface, indicating its potential application as effective paint additives [123]. The melanin-mediated nanostructures with broad-spectrum antimicrobial activity against food pathogens may be considered suitable for many practical food packaging applications because they can effectively inhibit the growth of pathogens and increase the shelf life of packed food products [124].

5.3. Environmental applications

The chemical structure of melanin presents many oxygen-containing groups, including carboxyl, phenolic and alcoholic hydroxyl, carbonyl, and methoxy groups, which have the ability to bind to a broad spectrum of substances [153]. In literature, studies have confirmed that fungal melanin acts as metal chelators, enhancing the biomass-metal interaction and consequently its biosorption capacity [14]. Study conducted by [154] showed that a melanin-rich strain of the fungus *Cladosporium cladosporioides* biosorbed 2.5- to 4-fold more Ni, Cu, Zn, Cd, and Pb ion than non-melanin *Penicillium digitatum*. These authors also studied the culture of *C. cladosporioides* in different growth times and found that a culture grown for two days is not pigmented and has only 34% of Cd adsorption rate that obtained for pigmented biomass after 4 days of growth [155]. Another study reported that melanized fungus *Armillaria* adsorb high concentrations of cations from the surrounding environment; some ions (Al, Zn, Fe, Cu, and Pb) were 50–100 times more concentrated on rhizomorphs than in soil [156]. The results obtained in our laboratory using a melanin-overproducing mutant (MEL1) from *A. nidulans* fungus [31, 93] showed that biosorption capacity for neodymium and lanthanum varied with stage of growth of this mutant; the biomass obtained after 72 hours of growth exhibited a 75% increase compared to the biomass of 48 hours. This result is related to melanin production during growth of the MEL1 mutant, since the biomass 48 hours is slightly pigmented, while the 72 hours biomass is dark due to the increased production of pigment [157]. Therefore, the pigmented biomass of the MEL1 mutant may be considered as a promising biosorbents for removal/recovery of the rare earth elements from wastewater due to the presence of the melanin increase significantly metal complexing capacity, improving the efficiency of biosorption process [157].

Some melanized fungi have shown to be good candidates for bioremediation of contaminated sites, due to the ability of fungal melanin to bind to heavy metals and radionuclides in contaminated sites. Experimental evidence shows that the accumulation of ^{90}Sr by conidia or mycelium by a range of microfungi species is greater in pigmented than in unpigmented species [158]. [159] In a study on the uptake efficiency of the radiocesium (^{137}Cs) and radiocobalt (^{60}Co) in melanized and nonmelanized fungi, it was observed that 60% of both radionuclides were uptaken by melanin of *A. alternata* and *Aspergillus pulverulents*. These results can be explained by melanin or other natural pigments present in the cell wall of these fungi that can act as the radiation receptor and/or as an energy transporter for metabolism. Other studies have demonstrated the potential application of the melanized fungi for the removal of radionuclides and heavy metals from aqueous solutions, providing an alternative means to affect cleanup of industrial effluent [16, 120, 160–164]. It has been reported that fungal melanin arranged in nanoparticles protects against extremely high levels of ionizing radiation and suggests that the protective efficacy of this pigment is a function of its chemical structure, the presence of stable free radical, and spatial arrangement [18]. According to the authors, these nanoshells have the potential use for environmental bioremediation, for example, to prevent the spread of radioactive contamination to ground water because the melanin is expected to encapsulate the radioactive particles and thereby reduce their spread. In this way, melanin nanoshells may be used to contain radiation from radioactive waste and biomedical radioactive materials.

6. Conclusion

Melanin possesses physicochemical properties and biological activities that make it a suitable biomaterial for a wide range of applications in cosmetic, pharmaceutical, electronic, and food processing industries. In addition, this pigment has a considerable interest biotechnological because it can be produced on a large scale with low cost, making its use for future practical applications economically advantageous. However, it is necessary to expand the knowledge about the structure-property-function relationships for the development of melanin-based technology. In the context, we hope that the information in this book will be useful and will encourage a greater number of researches on fungal melanin, which might be useful to deploy innovative and sustainable solutions for human health and the environment.

Author details

Sandra R. Pombeiro-Sponchiado^{1*}, Gabriela S. Sousa¹, Jazmina C. R. Andrade¹, Helen F. Lisboa² and Rita C. R. Gonçalves³

*Address all correspondence to: sponchia@iq.unesp.br

1 Department of Biochemistry and Technology Chemistry, Institute of Chemistry, UNESP, São Paulo State University, Araraquara, São Paulo, Brazil

2 Institute of Natural and Exact Sciences, UFMT, Mato Grosso Federal University, Rondonópolis, Mato Grosso, Brazil

3 Department of Pharmaceutical Sciences, UFES, Espírito Santo Federal University, Vitória, Espírito Santo, Brazil

References

- [1] Lopes FC, Tichota DM, Pereira JQ, Segalin J, De Oliveira Rios A, Brandelli A. Pigment production by filamentous fungi on agro-industrial byproducts: An eco-friendly alternative. *Appl Biochem Biotechnol.* 2013;171(3):616–625.
- [2] Demain AL. Importance of microbial natural products and the need to revitalize their discovery. *J Ind Microbiol Biotechnol.* 2014;41(2):185–201.
- [3] Kirti K, Amita S, Priti S, Kumar AM, Jyoti S. Colorful world of microbes: Carotenoids and their applications. *Adv Biol.* 2014;2014(1):1–13.
- [4] Kumar A, Vishwakarma HS, Singh J, Kumar M. Microbial pigments: Production and their applications in various industries. *Int J Pharm Chem Biol Sci.* 2015;5(1):203–212.

- [5] Panesar R, Kaur S, Panesar PS. Production of microbial pigments utilizing agro-industrial waste: A review. *Curr Opin Food Sci* 2015;1:70–76. Available from: <http://dx.doi.org/10.1016/j.cofs.2014.12.002>
- [6] Akilandeswari P, Pradeep BV. Exploration of industrially important pigments from soil fungi. *Appl Microbiol Biotechnol*. 2016;100(4):1631–1643.
- [7] Duran N, Teixeira MF, De Conti R, Esposito E Ecological-friendly pigments fungi. *Critic Rev Food Sci Nutr*. 2002;42(1):53–66.
- [8] Dufossé L, Fouillaud M, Caro Y, Mapari SAS, Sutthiwong N. Filamentous fungi are large-scale producers of pigments and colorants for the food industry. *Curr Opin Biotechnol*. 2014;26:56–61.
- [9] Chambergo FS, Valencia EY. Fungal biodiversity to biotechnology. *Appl Microbiol Biotechnol*. 2016;100(6):2567–2577.
- [10] Butler MJ, Day AW. Fungal melanins: A review. *Can J Microbiol*. 1998;44(12):1115–1136.
- [11] Bell AA, Wheeler MH. Biosynthesis and functions of fungal melanins. *Annu Rev Phytopathol*. 1986;24:411–451.
- [12] Plonka PM, Grabacka M. Melanin synthesis in microorganisms – biotechnological and medical aspects. *Acta Biochim Pol*. 2006;53(3):429–443.
- [13] Eisenman HC, Casadevall A. Synthesis and assembly of fungal melanin. *Appl Microbiol Biotechnol*. 2012;93(3):931–940.
- [14] Fogarty RV, Tobin JM. Fungal melanins and their interaction with metals. *Enzyme Microb Technol*. 1996;19(June 1995):317.
- [15] García-Rivera J, Casadevall A. Melanization of *Cryptococcus neoformans* reduces its susceptibility to the antimicrobial effects of silver nitrate. *Med Mycol*. 2001;39(October 2000):353–357.
- [16] Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, et al.. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. *PLoS One*. 2007;2(5):1–13.
- [17] Gómez BL, Nosanchuk JD. Melanin and fungi. *Curr Opin Infect Dis*. 2003;16(2):91–96.
- [18] Dadachova E, Bryan RA, Howell RC, Schweitzer AD, Aisen P, Nosanchuk JD, et al.. The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. *Pigment Cell Melanoma Res*. 2008;21(2):192–199.
- [19] Gessler NN, Egorova AS, Belozerskaya TA. Melanin pigments of fungi under extreme environmental conditions (Review). *Appl Biochem Microbiol*. 2014;50(2):105–113.
- [20] Schnitzler N, Peltroche-Llacsahuanga H, Bestier N, Zündorf J, Lütticken R, Haase G. Effect of melanin and carotenoids of *Exophiala (Wangiella) dermatitidis* on phagocytosis, oxidative burst, and killing by human neutrophils. *Infect Immun*. 1999;67(1):94–101.

- [21] Romero-Martinez R, Wheeler M, Guerrero-Plata A, Rico G, Torres-Guerrero H. Biosynthesis and functions of melanin in *Sporothrix schenckii*. *Infect Immun*. 2000;68(6):3696–3703.
- [22] Nosanchuk JD, Stark RE, Casadevall A. Fungal melanin: What do we know about structure? *Front Microbiol*. 2015;6:1–7.
- [23] Doering TL, Nosanchuk JD, Roberts WK, Casadevall A. Melanin as a potential cryptococcal defence against microbicidal proteins. *Med Mycol*. 1999;37(3):175–181.
- [24] Casadevall A, Rosas AL, Nosanchuk JD. Melanin and virulence in *Cryptococcus neoformans*. *Curr Opin Microbiol*. 2000;3(4):354–358.
- [25] Rosas AL, Casadevall A. Melanization decreases the susceptibility of *Cryptococcus neoformans* to enzymatic degradation. *Mycopathologia*. 2001;151(2):53–56.
- [26] Paolo WF, Dadachova E, Mandal P, Casadevall A, Szaniszló PJ, Nosanchuk JD. Effects of disrupting the polyketide synthase gene WdPKS1 in *Wangiella [Exophiala] dermatitidis* on melanin production and resistance to killing by antifungal compounds, enzymatic degradation, and extremes in temperature. *BMC Microbiol*. 2006;6:55.
- [27] Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA. Biosynthesis of fungal melanins and their importance for human pathogenic fungi. *Fungal Genet Biol*. 2003;38(2):143–158.
- [28] Nosanchuk JD, Casadevall A. The contribution of melanin to microbial pathogenesis. *Cell Microbiol*. 2003;5(4):203–223.
- [29] Wang Y, Casadevall A. Susceptibility of melanized and nonmelanized *Cryptococcus neoformans* to nitrogen- and oxygen-derived oxidants. *Infect Immun*. 1994;62(7):3004–3007.
- [30] Jacobson ES, Hove E, Emery HS. Antioxidant function of melanin in black fungi. *Infect Immun*. 1995;63(12):4944–4945.
- [31] Goncalves CRR, Pombeiro-Sponchiado SR. Antioxidant activity of the melanin pigment extracted from *Aspergillus nidulans*. *Biol Pharm Bull*. 2005;28(6):1129–1131.
- [32] Gonçalves R de CR, Kitagawa RR, Raddi MSG, Carlos IZ, Pombeiro-Sponchiado SR. Inhibition of nitric oxide and tumour necrosis factor- α production in peritoneal macrophages by *Aspergillus nidulans* melanin. *Biol Pharm Bull*. 2013;36(12):1915–1920.
- [33] Kunwar A, Adhikary B, Jayakumar S, Barik A, Chattopadhyay S, Raghukumar S, et al.. Melanin, a promising radioprotector: Mechanisms of actions in a mice model. *Toxicol Appl Pharmacol*. 2012;264(2):202–211.
- [34] Arun G, Eyini M, Gunasekaran P. Characterization and biological activities of extracellular melanin produced by *Schizophyllum commune* (Fries). *Indian J Exp Biol*. 2015;53(6):380–387.
- [35] d'Ischia M, Wakamatsu K, Cicoira F, Di Mauro E, Garcia-Borron JC, Commo S, et al.. Melanins and melanogenesis: From pigment cells to human health and technological applications. *Pigment Cell Melanoma Res*. 2015;28(5):520–544.

- [36] Demain AL, Adrio JL. Strain improvement for production of pharmaceuticals and other microbial metabolites by fermentation. *Nat Compd Drugs*. 2008;1:251–289.
- [37] Babitha S. Microbial Pigments. In: Nigam PSN, Pandey A, editors. *Biotechnology for agro-industrial residues utilization: utilization of agro-residues*. Netherlands: Springer; 2009. p. 147–162.
- [38] Meyer V, Wu B, Ram AFJ. *Aspergillus* as a multi-purpose cell factory: Current status and perspectives. *Biotechnol Lett*. 2011;33(3):469–476.
- [39] Mapari SAS, Nielsen KF, Larsen TO, Frisvad JC, Meyer AS, Thrane U. Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. *Curr Opin Biotechnol*. 2005;16(2):231–238.
- [40] Mapari SA, Meyer AS, Thrane U, Frisvad JC. Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. *Microb Cell Fact*. 2009;8:24.
- [41] Dikshit R, Tallapragada P. *Monascus purpureus*: A potential source for natural pigment production. *J Microbiol Biotechnol Res*. 2011;1(4):164–174.
- [42] Marova I, Carnecka M, Halienova A, Certik M, Dvorakova T, Haronikova A. Use of several waste substrates for carotenoid-rich yeast biomass production. *J Environ Manage*. 2012;95(Suppl.):S338–S342.
- [43] Sharmila G, Nidhi B, Muthukumar C. Sequential statistical optimization of red pigment production by *Monascus purpureus* (MTCC 369) using potato powder. *Ind Crops Prod*. 2013;44:158–164.
- [44] Prajapati VS, Soni N, Trivedi UB, Patel KC. An enhancement of red pigment production by submerged culture of *Monascus purpureus* MTCC 410 employing statistical methodology. *Biocatal Agric Biotechnol*. 2014;3(2):140–145.
- [45] Hajjaj H, Goma G, François JM. Effect of the cultivation mode on red pigments production from *Monascus ruber*. *Int J Food Sci Technol*. 2015;50(8):1–6.
- [46] Zhang M, Xiao G, Thring RW, Chen W, Zhou H, Yang H. Production and characterization of melanin by submerged culture of culinary and medicinal fungi *Auricularia auricula*. *Appl Biochem Biotechnol*. 2015;176(1):253–266.
- [47] da Costa Souza PN, Grigoletto TLB, de Moraes LAB, Abreu LM, Guimarães LHS, Santos CR, et al.. Production and chemical characterization of pigments in filamentous fungi. *Microbiology*. 2015;162:12–22.
- [48] Joshi VK, Attri D, BaJa A, Bhushan S. Microbial pigments. *Indian J Biotechnol*. 2003;2(Aug):362–369.
- [49] Ahn J, Jung J, Hyung W, Haam S, Shin C. Enhancement of *Monascus* pigment production by the culture of *Monascus* sp. J101 at low temperature. *Biotechnol Prog*. 2006;22(1):338–340.

- [50] Lisboa HCF. Influence of culture conditions on the production of melanin pigment by *Aspergillus fungus* [thesis]. Araraquara: Sao Paulo State University; 2003.
- [51] Orozco SFB, Kilikian BV. Effect of pH on citrinin and red pigments production by *Monascus purpureus* CCT3802. *World J Microbiol Biotechnol.* 2008;24(2):263–268.
- [52] Kang B, Zhang X, Wu Z, Wang Z, Park S. Production of citrinin-free *Monascus* pigments by submerged culture at low pH. *Enzyme Microb Technol.* 2014;55:50–57.
- [53] Tudor D, Robinson SC, Cooper PA. The influence of pH on pigment formation by lignicolous fungi. *Int Biodeterior Biodegrad.* 2013;80:22–28.
- [54] Méndez A, Pérez C, Montañéz JC, Martínez G, Aguilar CN. Red pigment production by *Penicillium purpurogenum* GH2 is influenced by pH and temperature. *J Zhejiang Univ Sci B.* 2011;12(12):961–968.
- [55] Velmurugan P, Lee YH, Venil CK, Lakshmanaperumalsamy P, Chae JC, Oh BT. Effect of light on growth, intracellular and extracellular pigment production by five pigment-producing filamentous fungi in synthetic medium. *J Biosci Bioeng.* 2010;109(4):346–350.
- [56] Zhou Z, Yin Z, Hu X. Corn cob hydrolysate, an efficient substrate for *Monascus* pigment production through submerged fermentation. *Biotechnol Appl Biochem.* 2014;61(6):716–723.
- [57] Said FM, Chisti Y, Brooks J. The effects of forced aeration and initial moisture level on red pigment and biomass production by *Monascus ruber* in packed bed solid state fermentation. *Int J Environ Sci Dev.* 2010;1(1):1–4.
- [58] Ruiz B, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, et al.. Production of microbial secondary metabolites: Regulation by the carbon source. *Crit Rev Microbiol.* 2010;36(2):146–167.
- [59] Pradeep FS, Begam MS, Palaniswamy M, Pradeep BV. Influence of culture media on growth and pigment production by *Fusarium moniliforme* KUMBF1201 isolated from paddy field soil. *World Appl Sci J.* 2013;22(1):70–77.
- [60] Gunasekaran S, Poorniammal R. Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. *Afr J Biotechnol.* 2008;7(12):1894–1898.
- [61] nee'Nigam PS. Production of bioactive secondary metabolites. In: Nigam PSN, Pandey A, editors. *Biotechnology for agro-industrial residues utilization: utilization of agro-residues.* Netherlands: Springer; 2009. p. 129-145.
- [62] Quereshi S, Pandey AK, Singh J. Optimization of fermentation conditions for red pigment production from *Phoma herbarum* (FGCC# 54) under submerged cultivation. *J Phytol.* 2010;2(9).
- [63] Celestino JR, de Carvalho LE, da Paz Lima, M, Lima AM, Ogusku MM, de Souza JVB. Bioprospecting of Amazon soil fungi with the potential for pigment production. *Process Biochem.* 2014;49(4):569–575.

- [64] Jalmi P, Bodke P, Wahidullah S, Raghukumar S. The fungus *Gliocephalotrichum simplex* as a source of abundant, extracellular melanin for biotechnological applications. *World J Microbiol Biotechnol.* 2012;28(2):505–512.
- [65] Sun S, Zhang X, Chen W, Zhang L, Zhu H. Production of natural edible melanin by *Auricularia auricula* and its physicochemical properties. *Food Chem.* 2016;196:486–492.
- [66] Pandey A, Soccol CR, Mitchell D. New developments in solid state fermentation: I-bioprocesses and products. *Process Biochem.* 2000;35(10):1153–1169.
- [67] Singhania RR, Soccol CR, Pandey A. Application of tropical agro-industrial residues as substrate for solid-state fermentation processes. In: Pandey A, Soccol CR, Larroche C, editors. *Current developments in solid-state fermentation.* New York: Springer; 2008. p. 412–442.
- [68] Velmurugan P, Hur H, Balachandar V, Kamala-Kannan S, Lee KJ, Lee SM, Chae JC, Oh BT. *Monascus* pigment production by solid-state fermentation with corn cob substrate. *J Biosci Bioeng.* 2011;112(6):590–594.
- [69] Babitha S, Soccol CR, Pandey A. Jackfruit seed—a novel substrate for the production of *Monascus* pigments through solid-state fermentation. *Food Technol Biotechnol.* 2006;44(4):465–471.
- [70] Hamano PS, Kilikian BV. Production of red pigments by *Monascus ruber* in culture media containing corn steep liquor. *Brazilian J Chem Eng.* 2006;23(4):443–449.
- [71] Silveira ST, Daroit DJ, Brandelli A. Pigment production by *Monascus purpureus* in grape Waste using factorial design. *LWT – Food Sci Technol.* 2008;41(1):170–174.
- [72] Rani MHS, Ramesh T, Subramanian J, Kalaiselvam M. Production and characterization of melanin pigment from halophilic black yeast *Hortaea werneckii*. *Int J Pharma Res Rev.* 2013;2(8):9–17.
- [73] Zou Y, Tian M. Fermentative production of melanin by *Auricularia auricula*. *J Food Process Preserv.* 2016;0:0–5.
- [74] Pretti TS. Optimization of the culture conditions of *Aspergillus nidulans* fungus for the production of melanin using agroindustrial residues [thesis]. Araraquara: Sao Paulo State University, 2009.
- [75] Enochs WS, Nilges MJ, Swartz HM. A standardized test for the identification and characterization of melanins using electron paramagnetic resonance (EPR) spectroscopy. *Pigment Cell Res.* 1993;6(2):91–99.
- [76] Crippa R, Horak V, Prota G, Svoronos P, Wolfram L. Chemistry of melanins alkaloids. *Chem Pharmacol.* 1990;36:253–323.
- [77] Wilczok T, Bilińska B, Buszman E, Kopera M. Spectroscopic studies of chemically modified synthetic melanins. *Arch Biochem Biophys.* 1984;231(2):257–262.

- [78] Duff GA, Roberts JE, Foster N. Analysis of the structure of synthetic and natural melanins by solid-phase NMR. *Biochemistry*. 1988;27(18):7112–7116.
- [79] Piattelli M, Fattorusso E, Nicolaus RA, Magno S. The structure of melanins and melanogenesis—V: Ustilagomelanin. *Tetrahedron*. 1965;21(11):3229–3236.
- [80] Stüssi H, Rast DM. The biosynthesis and possible function of γ -glutaminy-4-hydroxybenzene in *Agaricus bisporus*. *Phytochemistry*. 1981;20(10):2347–2352.
- [81] Eisenman HC, Mues M, Weber SE, Frases S, Chaskes S, Gerfen G, Casadevall A. *Cryptococcus neoformans* laccase catalyses melanin synthesis from both D- and L-DOPA. *Microbiology*. 2007;153(12):3954–3962.
- [82] Frases S, Salazar A, Dadachova E, Casadevall A. *Cryptococcus neoformans* can utilize the bacterial melanin precursor homogentisic acid for fungal melanogenesis. *Appl Environ Microbiol*. 2007;73(2):615–621.
- [83] Garcia-Rivera J, Eisenman HC, Nosanchuk JD, Aisen P, Zaragoza O, Moadel T, et al.. Comparative analysis of *Cryptococcus neoformans* acid-resistant particles generated from pigmented cells grown in different laccase substrates. *Fungal Genet Biol*. 2005;42(12):989–998.
- [84] Geis PA, Wheeler MH, Szaniszló PJ. Pentaketide metabolites of melanin synthesis in the dematiaceous fungus *Wangiella dermatitidis*. *Arch Microbiol*. 1984;137(4):324–328.
- [85] Wheeler MH, Stipanovic RD. Melanin biosynthesis and the metabolism of flavioline and 2-hydroxyjuglone in *Wangiella dermatitidis*. *Arch Microbiol*. 1985;142(3):234–241.
- [86] Saiz-Jimenez C. Microbial melanins in stone monuments. *Sci Total Environ*. 1995;167(1):273–286.
- [87] Henson JM, Butler MJ, Day AW. The dark side of the mycelium: Melanins of phytopathogenic fungi. *Annu Rev Phytopathol*. 1999;37(1):447–471.
- [88] Wu Y, Shan L, Yang S, Ma A. Identification and antioxidant activity of melanin isolated from *Hypoxylon archeri*, a companion fungus of *Tremella fuciformis*. *J Basic Microbiol*. 2008;48(3):217–221.
- [89] Bull AT. Chemical composition of wild-type and mutant *Aspergillus nidulans* cell walls. The nature of polysaccharide and melanin constituents. *J Gen Microbiol*. 1970;63(1):75–94.
- [90] Rowley BI, Pirt SJ. Melanin production by *Aspergillus nidulans* in batch and chemostat cultures. *Microbiology*. 1972;72(3):553–563.
- [91] Bull AT, Carter BLA. The isolation of tyrosinase from *Aspergillus nidulans*, its kinetic and molecular properties and some consideration of its activity in vivo. *Microbiology*. 1973;75(1):61–73.
- [92] Martinelli SD, Bainbridge BW. Phenoloxidases of *Aspergillus nidulans*. *Trans Br Mycol Soc*. 1974;63(2):361–370.

- [93] Gonçalves RCR, Lisboa HCF, Pombeiro-Sponchiado SR. Characterization of melanin pigment produced by *Aspergillus nidulans*. *World J Microbiol Biotechnol.* 2012;28(4):1467–1474.
- [94] Fling M, Horowitz NH, Heinemann SF. The isolation and properties of crystalline tyrosinase from *Neurospora*. *J Biol Chem.* 1963;238(6):2045–2053.
- [95] Esser K. Phenoloxidases in the ascomycete *Podospora anserina*. I. The identification of laccase and tyrosinase in the wild strain. *Arch Mikrobiol.* 1963;46:217–226.
- [96] Williamson PR, Wakamatsu K, Ito S. Melanin biosynthesis in *Cryptococcus neoformans*. *J Bacteriol.* 1998;180(6):1570–1572.
- [97] Prota G. *Melanins and Melanogenesis*. Academic Press, San Diego, CA; 1992.
- [98] Tsai HF, Fujii I, Watanabe A, Wheeler MH, Chang YC, Yasuoka Y, Ebizuka Y, Kwon-Chung KJ. Pentaketide melanin biosynthesis in *Aspergillus fumigatus* requires chain-length shortening of a heptaketide precursor. *J Biol Chem.* 2001;276(31):29292–29298.
- [99] Schmalzer-Ripcke J, Sugareva V, Gebhardt P, Winkler R, Kniemeyer O, Heinekamp T, Brakhage AA. Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*. *Appl Environ Microbiol.* 2009;75(2):493–503.
- [100] Jolivet S, Arpin N, Wichers HJ, Pellon G. *Agaricus bisporus* browning: A review. *Mycol Res.* 1998;102(12):1459–1483.
- [101] Gessler NN, Aver'yanov AA, Belozerskaya TA. Reactive oxygen species in regulation of fungal development. *Biochemistry.* 2007;72(10):1091–1109.
- [102] Zhan F, He Y, Zu Y, Li T, Zhao Z. Characterization of melanin isolated from a dark septate endophyte (DSE), *Exophiala pisciphila*, *World J Microbiol Biotechnol.* 2011;27(10):2483–2489.
- [103] Kumar CG, Mongolla P, Pombala S, Kamle A, Joseph J. Physicochemical characterization and antioxidant activity of melanin from a novel strain of *Aspergillus bridgeri* ICTF-201. *Lett Appl Microbiol.* 2011;53(3):350–358.
- [104] Cunha MML, Franzen AJ, Seabra SH, Herbst MH, Vugman NV, Borba LP, Souza W, Rozental S. Melanin in *Fonsecaea pedrosoi*: A trap for oxidative radicals. *BMC Microbiol.* 2010;10(1):1–9.
- [105] Dong C, Yao Y. Isolation, characterization of melanin derived from *Ophiocordyceps sinensis*, an entomogenous fungus endemic to the Tibetan Plateau. *J Biosci Bioeng.* 2012;113(4):474–479.
- [106] Lopaczynski W, Zeisel SH. Antioxidants, programmed cell death, and cancer. *Nutr Res.* 2001;21(1):295–307.
- [107] Hoogduijn MJ, Cemeli E, Anderson D, Wood JM, Thody AJ. Melanin protects against Ho-induced DNA strand breaks through its ability to bind Ca. *Br J Dermatol.* 2003;148(4):867.

- [108] Harman D. Free radical theory of aging: An update. *Ann NY Acad Sci.* 2006;1067(1):10–21.
- [109] Lu Y, Ye M, Song S, Li L, Shaikh F, Li J. Isolation, purification, and anti-aging activity of melanin from *Lachnum singerianum*. *Appl Biochem Biotechnol.* 2014;174(2):762–771.
- [110] Mohagheghpour N, Waleh N, Garger SJ, Dousman L, Grill LK, Tusé D. Synthetic melanin suppresses production of proinflammatory cytokines. *Cell Immunol.* 2000;199(1):25–36.
- [111] Mednick AJ, Nosanchuk JD, Casadevall A. Melanization of *Cryptococcus neoformans* affects lung inflammatory responses during cryptococcal infection. *Infect Immun.* 2005;73(4):2012–2019.
- [112] Cruvinel WDM, Mesquita Júnior D, Araújo JAP, Catelan TTT, Souza AWS, Silva NPD, Andrade LEC. Immune system: Part I. Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. *Revista brasileira de reumatologia.* 2010; 50(4):434–447.
- [113] Bocca AL, Brito PP, Figueiredo F, Tosta CE. Inhibition of nitric oxide production by macrophages in chromoblastomycosis: A role for *Fonsecaea pedrosoi* melanin. *Mycopathologia.* 2006;161(4):195–203.
- [114] Zhang J, Wang L, Xi L, Huang H, Hu Y, Li X, Huang X, Lu S, Sun J. Melanin in a meristematic mutant of *Fonsecaea monophora* inhibits the production of nitric oxide and Th1 cytokines of murine macrophages. *Mycopathologia.* 2013;175(5–6):515–522.
- [115] Schweitzer AD, Howell RC, Jiang Z, Bryan RA, Gerfen G, Chen CC, Mah D, Cahill S, Casadevall A, Dadachova E. Physico-chemical evaluation of rationally designed melanins as novel nature-inspired radioprotectors. *PLoS One.* 2009;4(9):1–8.
- [116] Ye M, Guo G, Lu Y, Song S, Wang HY, Yang L. Purification, structure and anti-radiation activity of melanin from *Lachnum* YM404. *Int J Biol Macromol.* 2014;63(1):170–176.
- [117] Schweitzer AD, Revskaya E, Chu P, Pazo V, Friedman M, Nosanchuk JD, Cahill S, Frases S, Casadevall A, Dadachova E. Melanin-covered nanoparticles for protection of bone marrow during radiation therapy of cancer. *Int J Radiat Oncol Biol Phys.* 2010;78(5):1494–1502.
- [118] Mostert AB, Powell BJ, Pratt FL, Hanson GR, Sarna T, Gentle IR, Meredith P. Role of semiconductivity and ion transport in the electrical conduction of melanin. *Proc Natl Acad Sci.* 2012;109(23):8943–8947.
- [119] Mironenko NV, Alekhina IA, Zhdanova NN, Bulat SA. Intraspecific variation in gamma-radiation resistance and genomic structure in the filamentous fungus *Alternaria alternata*: A case study of strains inhabiting Chernobyl Reactor No. 4. *Ecotoxicol Environ Saf.* 2000;45(2):177–187.
- [120] Dighton J, Tugay T, Zhdanova N. Fungi and ionizing radiation from radionuclides. *FEMS Microbiol Lett.* 2008;281(2):109–120.
- [121] Mostert AB, Powell BJ, Gentle IR, Meredith P. On the origin of electrical conductivity in the bio-electronic material melanin. *Appl Phys Lett.* 2012;100(9).

- [122] Bin L, Wei L, Xiaohong C, Mei J, Mingsheng D. In vitro antibiofilm activity of the melanin from *Auricularia auricula*, an edible jelly mushroom. *Ann Microbiol.* 2012;62(4):1523–1530.
- [123] Apte M, Girme G, Bankar A, Ravikumar A, Zinjarde S. 3,4-dihydroxy-L-phenylalanine-derived melanin from *Yarrowia lipolytica* mediates the synthesis of silver and gold nanostructures. *J Nanobiotechnol.* 2013;3–11.
- [124] Kiran GS, Dhasayan A, Lipton AN, Selvin J, Arasu MV, Al-Dhabi NA. Melanin-templated rapid synthesis of silver nanostructures. *J Nanobiotechnol.* 2014;12(1):18.
- [125] Goncalves PJ, Baffa O, Graeff CFO, Gonçalves PJ, Filho OB. Effects of hydrogen on the electronic properties of synthetic melanin. *J Appl Phys.* 2006;99(10):104701.
- [126] Meredith P, Sarna T. The physical and chemical properties of eumelanin. *Pigment Cell Res.* 2006;19(6):572–594.
- [127] Ambrico M, Ambrico PF, Ligonzo T, Cardone A, Cicco SR, d'Ischia M, Farinola GM. From commercial tyrosine polymers to a tailored polydopamine platform: Concepts, issues and challenges en route to melanin-based bioelectronics. *J Mater Chem C.* 2015;3(25):6413–6423.
- [128] Ligonzo T, Ambrico M, Augelli V, Perna G, Schiavulli L, Tamma MA, et al.. Electrical and optical properties of natural and synthetic melanin biopolymer. *J Non Cryst Solids.* 2009;355(22–23):1221–1226.
- [129] Ambrico M, Ambrico PF, Cardone A, Ligonzo T, Cicco SR, Di Mundo R, et al.. Melanin layer on silicon: An attractive structure for a possible exploitation in bio-polymer based metal-insulator-silicon devices. *Adv Mater.* 2011;23(29):3332–3336.
- [130] Bothma JP, de Boor J, Divakar U, Schwenn PE, Meredith P. Device-quality electrically conducting melanin thin films. *Adv Mater.* 2008;20(18):3539–3542.
- [131] Ambrico M, Ambrico PF, Ligonzo T, Cardone A, Cicco SR, Lavizzera A, et al.. Memory-like behavior as a feature of electrical signal transmission in melanin-like bio-polymers. *Appl Phys Lett.* 2012;100(25).
- [132] Kim YJ, Wu W, Chun SE, Whitacre JF, Bettinger CJ. Biologically derived melanin electrodes in aqueous sodium-ion energy storage devices. *Proc Natl Acad Sci.* 2013;110(52):20912–20917.
- [133] Ambrico M, Ambrico PF, Cardone A, Cicco SR, Palumbo F, Ligonzo T, Mundo RD, Petta V, Augelli V, Favia P, Farinola GM. Melanin-like polymer layered on a nanotextured silicon surface for a hybrid biomimetic interface. *J Mater Chem C.* 2014;2(3):573–582.
- [134] Morresi L, Ficcadenti M, Pinto N, Murri R, Cuccioloni M, Angeletti M, et al.. Optical and electrical behavior of synthetic melanin thin films spray-coated. *Energy Procedia.* 2010;2(1):177–182.

- [135] Orive AG, Grumelli D, Vericat C, Ramallo-López JM, Giovanetti L, Benitez G, et al.. "Naked" gold nanoparticles supported on HOPG: Melanin functionalization and catalytic activity. *Nanoscale*. 2011;3(4):1708.
- [136] Ju KY, Lee JW, Im GH, Lee S, Pyo J, Park SB, Lee JH, Lee JK. Bio-inspired, melanin-like nanoparticles as a highly efficient contrast agent for T1-weighted magnetic resonance imaging. *Biomacromolecules*. 2013;14(10):3491–3497.
- [137] Seppä H. Superconducting transition-edge bolometer in a resistive and in an inductive mode. *IEEE Trans Appl Supercond*. 2001;11(1):759–761.
- [138] Huang GS, Wang M Te, Su CW, Chen YS, Hong MY. Picogram detection of metal ions by melanin-sensitized piezoelectric sensor. *Biosens Bioelectron*. 2007;23(3):319–325.
- [139] Gallas JM. Optical lens system incorporating melanin as an absorbing pigment for protection against electromagnetic radiation. Patent No. 4,698,374, 1987.
- [140] Gallas JM. Medium incorporating melanin as an absorbing pigment for protection against electromagnetic radiation. Patent No. 5,047,447, 1991.
- [141] Gallas J, Eisner M. Melanin polyvinyl alcohol plastic laminates for optical applications. Patent No. 7,029,758, 2006.
- [142] Araújo M, Viveiros R, Correia TR, Correia IJ, Bonifácio VDB, Casimiro T, et al.. Natural melanin: A potential pH-responsive drug release device. *Int J Pharm*. 2014;469(1):140–145.
- [143] Berliner DL, Erwin RL, McGee DR. Therapeutic uses of melanin. Patent No. 5,776,968, 1998.
- [144] Berliner DL, Erwin RL, McGee DR. Methods of treating Parkinson's disease using melanin. Patent No. 5,210,076, 1993.
- [145] Bettinger CJ, Bruggeman JP, Misra A, Borenstein JT, Langer R. Biocompatibility of biodegradable semiconducting melanin films for nerve tissue engineering. *Biomaterials*. 2009;30(17):3050–3057.
- [146] Pezzella A, Barra M, Musto A, Navarra A, Alfè M, Manini P, et al.. Stem cell-compatible eumelanin biointerface fabricated by chemically controlled solid state polymerization. *Mater Horiz*. 2015;2(2):212–220.
- [147] Rivnay J, Owens RM, Malliaras GG. The rise of organic bioelectronics. *Chemistry of Materials*. 2013;26(1):679–685.
- [148] Solano F. Melanins: Skin pigments and much more—types, structural models, biological functions, and formation routes. *New J Sci*. 2014; 2014:1–28.
- [149] Pawelek JM, Platt JT. Cosmetic melanins. Patent No. 5,744,125, 1998.
- [150] D'Ischia M, Wakamatsu K, Napolitano A, Briganti S, Garcia-Borrón JC, Kovacs D, et al.. Melanins and melanogenesis: Methods, standards, protocols. *Pigment Cell Melanoma Res*. 2013;26(5):616–633.

- [151] Kunwar A, Adhikary B, Jayakumar S, Barik A, Chattopadhyay S, Raghukumar S, Priyadarsini KI. Melanin, a promising radioprotector: Mechanisms of actions in a mice model. *Toxicol Appl Pharmacol.* 2012;264(2):202–211.
- [152] Zhang R, Fan Q, Yang M, Cheng K, Lu X, Zhang L, et al.. Engineering melanin nanoparticles as an efficient drug-delivery system for imaging-guided chemotherapy. *Adv Mater.* 2015;27(34):5063–5069.
- [153] Riley PA. Melanin. *Int J Biochem Cell Biol.* 1997;29(Ii):1235–1239.
- [154] Siegel SM. Fungal biosorption: A comparative study of metal uptake by penicillium and cladosporil'h. *Met Speciat Sep Recover.* 1987;1:339.
- [155] Siegel SM, Galun M, Siegel BZ. Filamentous fungi as metal biosorbents: A review. *Water Air Soil Pollut.* 1990;53(3–4):335–344.
- [156] Rizzo DM, Blanchette RA, Palmer MA. Biosorption of metal ions by *Armillaria rhizomorpha*. *Can J Bot.* 1992;70(8):1515–1520.
- [157] Caporalin CB. Comparison of biosorption of rare earth metals by melanized biomass of *Aspergillus nidulans* fungus in free and immobilized forms [thesis]. Araraquara: Sao Paulo State University, 2011.
- [158] Zhdanova NN, Vasilevskaya AI, Sadovnikov YS, Artyschkova LA. Dynamics of micro-mycete complexes from soils contaminated with radionuclides. *Mikol i Fitopatol.* 1990;24(6):504–512.
- [159] Mahmoud YA. Uptake of radionuclides by some fungi. *Mycobiology.* 2004;32(3):110–114.
- [160] Singleton I, Tobin JM. Fungal interactions with metals and radionuclides for environmental bioremediation. In: Frankland JC, Magan N, Gadd GM, editors. *Fungi and Environmental Change*. Cambridge: Cambridge University Press; 1996. p. 282–298.
- [161] Steiner M, Linkov I, Yoshida S. The role of fungi in the transfer and cycling of radionuclides in forest ecosystems. *J Environ Radioact.* 2002;58(2):217–241.
- [162] Zhdanova NN, Tugay T, Dighton J, Zheltonozhsky, V, Mcdermott P. Ionizing radiation attracts soil fungi. *Mycol Res.* 2004;108(9):1089–1096.
- [163] Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh UB, Sahu A, et al.. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustain.* 2015;7(2):2189–2212.
- [164] Shakya M, Sharma P, Meryem SS, Mahmood Q, Kumar A. Heavy metal removal from industrial wastewater using fungi: Uptake mechanism and biochemical aspects. *J Environ Eng.* 2015; 142(9):C6015001.

