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The Role of Melanin in the Biology and Ecology of Nematophagous Fungi

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Abstract

Melanin is a heteropolymer formed by the polymerization of phenolic and indolic compounds. It occurs in organisms across all biological kingdoms and has a range different of functions, thus indicating its important evolutionary role. The presence of melanin offers several protective advantages, including against ultraviolet radiation, traumatic damage, oxidative stress, extreme temperatures, and pressure. For many species of fungi, melanin also participates directly in the process of virulence and pathogenicity. These organisms can synthesize melanin in two main ways: using a substrate of endogenous origin, involving 1,8-dihydroxynaphthalene (DHN); alternatively, in an exogenous manner with the addition of L-3, 4-dihydroxyphenylalanine (L-DOPA or levodopa). As melanin is an amorphous and complex substance, its study requires expensive and inaccessible technologies and analyses are often difficult to perform with conventional biochemical techniques. As such, details about its chemical structure are not yet fully understood, particularly for nematophagous fungi that remain poorly studied. Thus, this review presents an overview of the different types of melanin, with an emphasis on fungi, and discusses the role of melanin in the biology and ecology of nematophagous fungi.

Keywords Melanin · Fungi · Nematophagous fungi · Melanosomes · Tyrosinase · Laccase · Polyketide synthase

Introduction

Melanin is a heterogeneous, amorphous, and highly resistant pigmentary substance that occurs universally across life forms. Evidence of the substance in preserved dinosaur fossils, on the feathers of prehistoric birds, and in plants, marine cephalopods, bacteria, and fungi indicate that the substance has occurred naturally since the beginning of life on Earth (Pralea et al. 2019; Solano 2014; Wogelius et al. 2011).

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Melanins are coloring pigments that vary in tone from light to dark. They are formed by the polymerization of phenolic and indolic compounds into intermediates and quinones that are synthesized in specialized structures called melanosomes and then deposited on the cell wall of eukaryotic cells (Takano et al. 1997). Thus, the different types of melanin present in representatives of almost all large modern taxa suggest the unquestionable evolutionary importance of melanogenesis across the different kingdoms (Plonka and Grabacka 2006).

Often fundamental in protecting the organisms in which they are found, the production of melanin by fungi can directly contribute to an increase in virulence of pathogens for humans, domestic and farm animals, as well as food crops (Eisenman and Casadevall 2012). As such, several studies have been published in the last several decades demonstrating that virulence tends to be multifactorial since it is intrinsically linked to a combination of biological characteristics of the fungus, including the size of reproductive and propagation structures, thermotolerance, oxidative stress, high rate of development, and nutritional versatility, as well as the immunological status of its host (Abad et al. 2010).

The presence of melanin in the fungal cell wall can help species survive in a range of extreme or severe environments including those with drastic changes in temperature, such as polar regions and deserts, and inside domestic machinery where they are subjected to heat and detergents (Zalar et al. 2011), or even in harsh environments such as in nuclear reactors contaminated with radiation (Dadachova et al. 2008; Rosa et al. 2010).

Despite its importance and ubiquity, fundamental questions remain about the melanin pigment. One such detail is its chemical structure, which can be explained by the simple fact that melanin is practically insoluble in organic solvents and, therefore, cannot be studied by conventional biochemical methods. In addition, it is grouped into substances of structurally complex, diverse, and still undefined categories (Eisenman and Casadevall 2012).

Among the diversity of fungi existing in nature, nematophagous fungi, those with the ability to trap and prey on nematodes, are of particular interest given their potential use in biocontrol (Larsen 2006). *Monacrosporium haptotilum* and *Arthrobotrys oligospora*, both nematophagous fungi, express tyrosinase (Meerupati et al. 2013). When compared to other proteins found in the mycelium in *M. haptotilum*, tyrosinase has been shown to positively regulate the nematode trapping structure (Andersson et al. 2013).

In fungi, tyrosinase is a cytosolic enzyme involved in melanogenesis, with considerable heterogeneity from other enzymes (Bell and Wheeler 1986). Its molecular weight can vary widely, and it is generally associated with spore formation and stability, defense mechanisms, increased virulence, and tissue regeneration after traumatic damage. In addition, tyrosinase plays a fundamental role in pigmentation in some species of fungi and is an enzyme involved in the synthesis and maintenance of melanin (Halaouli et al. 2006).

As it is a polymer with unique characteristics, melanin has attracted significant interest across a range of industries worldwide (Lopes 2013; Suwannarach et al. 2019). Further research on its synthesis, structure, location and biological role in the survival of melanized fungi can help to understand how this macromolecule acts in biological processes and ecological interactions in the constant struggle for survival (Solano 2014).

Due to the scarcity of studies on the melanogenic process in nematophagous fungi, this review concentrates on the theme of fungal melanogenesis and succinctly presents key examples of this critical biological phenomenon. Furthermore, this review seeks to characterize and demonstrate for the first time melanin in nematophagous fungi, providing a new understanding of melanogenesis in these organisms.

Melanin: Structure and Formation

Melanins are enigmatic pigments that, in evolutionary terms, may have appeared early in the development of life on Earth. They have been identified in 160 million-year-old primitive dinosaurs and cephalopods (Glass et al. 2012) and are found in all biological kingdoms (Solano 2014).

These pigments provide organisms with a variety of functions, including an increase in virulence and pathogenicity in microorganisms, increased resistance to antibiotics and antifungals, protection against ultraviolet and nuclear radiation, and thermoregulation, in addition to responding positively against anthropogenic pollutants (Casadevall et al. 2012; Pralea et al. 2019; Shindler et al. 2019; Tian et al. 2003; Treseder and Lennon 2015; Zhdanova et al. 2000).

The term melanin comes from the Greek *melanos*, meaning dark, and was first used in 1840 by the Swedish chemist Berzelius, who used the term to describe a dark-colored pigment extracted from the membrane of the eyes of some animals (D'ischia et al. 2013). Inspired by these discoveries, the Swiss Bruno Bloch observed that melanin granules were deposited in the cytoplasm of cells, usually located in the epidermal basement membrane. He called these specialized cells melanoblasts, precursor cells of melanocytes, and provided evidence that the catalyst effect existing in the oxidation of DOPA-melanin was due to an enzyme, which he called DOPA-oxidase (Nordlund et al. 1989). Until then, dihydroxyphenylalanine (DOPA-melanin) had never been described in mammals and there was insufficient scientific understanding to relate the melanin molecule to a possible enzyme, as observed and isolated by Bloch in the early twentieth century (Simon et al. 2009).

Early work by Nicolaus (1968) defined melanin as dark pigments found mostly in the skin, irises, and hair of animals and humans, which are classified as eumelanins and phaeomelanins (Plonka and Grabacka 2006). Allomelanins are a type of non-animal pigment resulting from the oxidation of phenols, containing predominantly Hydrogen, Carbon, and Oxygen, that are synthesized by algae, bacteria, plants, and some species of fungi (Kejzar et al. 2013; Solano 2014). In addition, neuromelanins are present only in nerve cells in the brains of mammals and birds (Magarelli et al. 2010) (Table 1).

In general, melanins are insoluble, hydrophobic phenolic compounds with a high molecular weight. Their structural basis is formed by indolic compounds represented covalently in paired structures and joined by Van der Waals interactions (Chatterjee et al. 2012; Figueiredo-Carvalho et al. 2014; Lee et al. 2019).

In terms of structure, melanins make up a group of complex pigments that are relatively diverse and undefined;

Table 1 Melanin types according to their formation, color, occurrence, function, and references

Melanin types	Formation	Color	Occurrence	Function	References
Eumelanin	Polymerization by tyrosine oxidation through the action of tyrosinase in DOPAand dopaquinone for the formation of DHI and DHICA	Black, brown	Marine cephalopods, fish skin, human hair, mammal hair, bird feather, fungi. Reptiles, and bacteria	Protect against radiation and toxic heavy metals; thermoregulation; camouflage; sexual selection; resistance to abrasion; expression of virulence traits	Meunier et al. 2011; Bonser 1995; Hoekstra et al. 2006; Clusella Trullas et al. 2007; Berzelius 1840; Pavan et al. 2020; Shalaby et al. 2019; Kollias and Baqer 1988; Bashkatov et al. 2006.
Pheomelanin	Cysteinization produces dopaquinone in cysteinyldopa or glutathionedopa and benzothiazine derivatives	Yellow, red, brown	Reptiles. mammal hair, bird feathers, fungi, and hair in humans	Protect against radiation and toxic heavy metals; thermoregulation; sexual selection; camouflage; expression of virulence traits	Bashkatov et al. 2006; Freitas et al. 2019; Roulim et al. 2013.
Allomelanin	Input from acetyl-CoA or malonyl-CoA for formation of 1,3,6,8-THN, catalyzed by a PKS which produces intermediaries, such as scytalone, 1,3,8-trihydroxynaphthalene, and vermilion, leading to polymerization subsequent of DHN-melanin	Light brown, dark brown, black	Plant, algae, fungi	Photoprotection; free radical stabilizers; ion balance in the soil	Turick et al. 2002; Plonka and Grabacka 2006; Moses et al. 2006. Kejzar et al. 2013.
Neuromelanin	Accumulation of oxidized catecholamines in dopaquinone and DA-quinone that react with cysteine to form 5-S-Cys-DOPA and 5-S-Cys-DA, producing PTCA and PDCA residues that are mixtures of eumelanin and pheomelanin	Black	Different types of neurons present in the anterior regions of the brain, the motor cortex, cerebellum, and substantia nigra	Protect cells against toxic quinones produced from catecholamines, neuromelanin-induced mitochondrial dysfunction, and apoptosis in Parkinson's disease	Smythies 1996; Magarelli et al. 2010; Double et al. 2011.

there is still no molecular regularity that can concretely represent them or any common way to structurally classify them, be it primary, secondary, tertiary, or quaternary. Furthermore, there is no evidence of crystallinity that can distinguish them, since most of the proposed definitions are flawed due to difficulty in differentiating compounds with vast diversity in composition, color, size, occurrence, and function (Pralea et al. 2019; Solano 2014; Treseder and Lennon 2015). Thus, some structural models have been proposed for commercial melanin that classify the substance as a heteropolymer arranged in a pile of oligomers of four to seven monomers (Duff et al. 1988). However, this model is described based on X-ray diffraction analysis and may be inadequate when compared to the natural polymer, since the intrinsic structural details of the melanin that are widely distributed across the different biological kingdoms may vary (D'ischia et al. 2013).

Although the structural basis has been studied through electronic paramagnetic resonance (EPR), understanding of the structure is still limited by the lack of existing information. However, the structure is well defined in terms of possible covalent bonds to proteins, lipids, and carbohydrates (Chatterjee et al. 2012).

Eumelanins

Eumelanins are macromolecules that are black or brown. They are produced through the oxidative polymerization of tyrosine due to the action of the tyrosinase enzyme in dihydroxyphenylalanine (DOPA) and dopaquinone (Sarna and Plonka 2005). This reaction includes a series of cyclization, which leads to the formation of intermediate indole monomers known as dopachrome. Subsequently, these monomers undergo decarboxylation to form the compound 5,6-dihydroxyindole (DHI). They can also be catalyzed by tyrosinase-related proteins (TRP2 and TRP1) to produce Levodopachrome and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and finally oxidized to form eumelanin (Solano 2014).

Eumelanins have been identified in a range of organisms including: marine cephalopods (Palumbo and Yeh 1994); humans, a fact that can be easily seen by hair color in juveniles due to the abundance of pigment (Robbins 2012); the exocuticle of insects (Nappi and Sugumaran 1993); and some groups of bacteria, such as *Streptomyces antibioticus*, in which the production of eumelanin can also be used in taxonomic identification (Chen et al. 1992).

These macro-heterogeneity molecules can take two different forms during their synthesis and depend on the positions and couplings involved (Meredith and Sarna 2006). The proportion varies according to the type of eumelanin produced, which depends on where the melanin will be required, as shown in Fig. 1 (Chatterjee et al. 2012; Plonka and Grabacka 2006).

Pheomelanins

Pheomelanins are yellow-reddish biopolymers that are synthesized a priori as eumelanin. They are also derived from the precursor dopaquinone but differ in that during

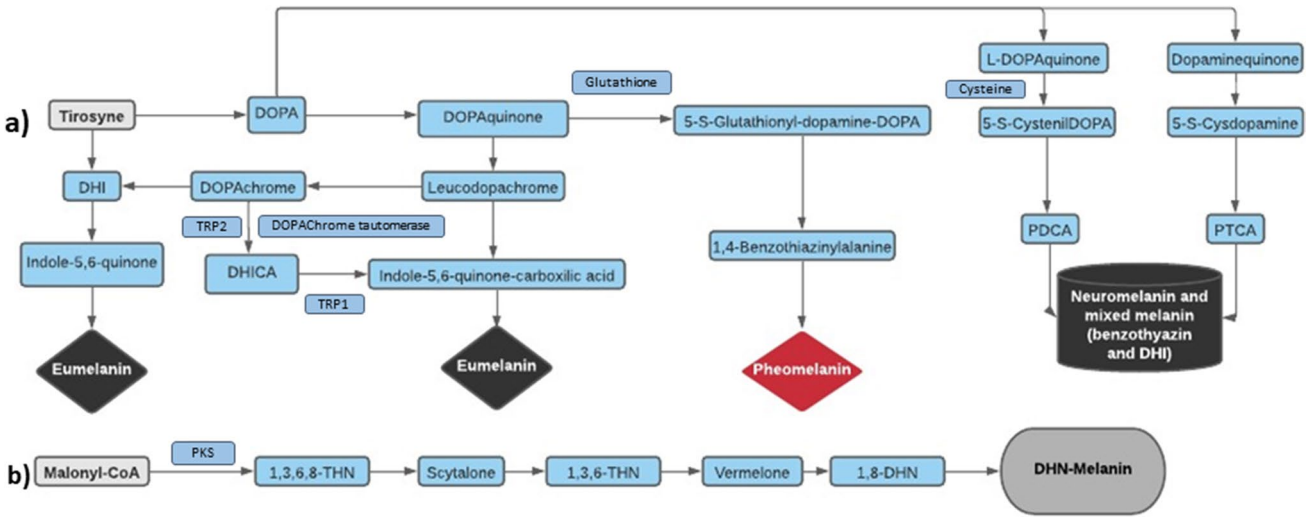

Fig. 1 Flowchart showing the synthesis of eumelanin, pheomelanin, neuromelanin (**a**) and allomelanin-DHN melanin (**b**). TRP1- tyrosinase-related protein 1; TRP2- tyrosinase-related protein 2; DOPA-3,4-dihydroxyphenylalanine; DHI-5,6-dihydroxyindole; DHICA-5,6-dihydroxyindole-2-carboxylic acid; PKS- polyketide synthase; 1,3,6,8-THN- 1,3,6,8-tetrahydroxynaphthalene; 1,3,6-THN-1,3,6-trihydroxynaphthalene; 1,8-DHN- 1,8-dihydroxynaphthalene; pyrrole-2,3-dicarboxylic acid (PDCA); and pyrrole-2,3,5-tricarboxylic acid (PTCA)

pheomelanogenesis, tyrosinase activity and expression are reduced (Barber et al. 1985). During pheomelanogenesis, thiols take on the role of tyrosinase for the cysteinylation of dopaquinone through cysteine in cysteinyldopa or by the intermediation of glutathione in glutathionedopa (Kobayashi et al. 1995). Subsequently, after a series of yet unknown reactions, they undergo polymerization to produce benzothiazine derivatives (5-hydroxy-1,4-benzothiazinylalanine) which result in the formation of high molecular weight macromolecules known as pheomelanins (Fig. 1) (Change 2009).

Many organisms have been described as pheomelanogenic including reptiles, mammals, and birds (Solano 2014). However, the occurrence of the pigment in fungi remains unclear and is only deduced due to the brownish-yellow color observed macroscopically in some species (Ye et al. 2011) and the presence of thiol groups associated with melanin oligomers (Ito and Wakamatsu 2003).

Allomelanins

Allomelanins are a type of non-animal pigment resulting from the oxidation of phenols that are devoid of nitrogen. They belong to a group of heterogeneous polymers that are present in plants, some Ascomycetes of the genus *Tuber* (De Angelis et al. 1996), *Aspergillus* (Wheeler 1983), and they also confer the staining seen in Deuteromycetes which range in color from dark brown to black (Change 2009; Solano 2014).

The synthesis of allomelanin begins with the entry of acetyl-CoA or malonyl-CoA (Adachi and Hamer 1998; Lee et al. 2019). Thus, the first step is the formation of 1,3,6,8-tetrahydroxinaphthalene (1,3,6,8-THN), which is catalyzed by polyketide synthases (PKS) in a series of reduction and dehydration reactions for the production of intermediates. Among these, scytalone stands out due to the action of the catalyst enzyme THN reductase (Eisenman and Casadevall 2012). The dehydration of scytalone produces 1,3,6-trihydroxinaphthalene and vermilion through other reductases leading to the polymerization of these compounds. This, in turn, leads to the formation of 1,8-dihydroxinaphthalene, DHN-melanin, as demonstrated in Fig. 1 (Langfelder et al. 2003).

Neuromelanins

Neuromelanin is a dark-colored, insoluble polymer that is present in different types of neurons in the central nervous system of animals. It is concentrated particularly in the dopaminergic neurons of the substantia nigra, the noradrenergic neurons of the locus coeruleus, and the dorsal motor nucleus of the vagus nerve (Halliday et al. 2014; Zecca et al. 2003; Nicolaus 2005; Zucca et al. 2014).

The process of synthesis of neuromelanin is complex and in many ways still unclear (Sulzer and Rayport 2000; Zecca et al. 2008). Currently, questions remain whether it is mediated enzymatically or by a process of auto-oxidation of dopamine derivatives (Zecca et al. 2001). Some authors suggest that tyrosinase is involved in the neuromelanin biosynthesis process since the tyrosinase enzyme and tyrosinase-related proteins (TRP-1 and TRP-2), which are usually expressed in melanosomes, have also been isolated in cells of the melanosome in the central nervous system and active during all stages of brain development (Tief et al. 1998).

The biosynthesis of neuromelanin occurs in the cytosol, through the accumulation of catecholamines, which are quickly oxidized to the quinones dopaquinone and DA-quinone. This likely occurs through enzymatic catalysis, iron, and reactive oxygen species (ROS) produced by oxidative stress (Ferrari et al. 2017). These quinones react with amino acids, i.e., cysteine (Cys) from peptides such as glutathione, to form 5-S-cysteinyldopa (5-S-Cys-DOPA) through the oxidation of L-DOPA. They are produced in the anterior region of the brain, including the motor cortex and the cerebellum, through the different types of neurons present in these regions due to the presence of diverse Cys-catechols. These sites are high in tyrosine hydroxylase expression (Zecca et al. 2008) and 5-S-cysteinyldopamine (5-S- Cys-DA) in the substantia nigra, due to the precursor dopamine-DA, with subsequent formation of residues such as pyrrole-2,3-dicarboxylic acid (PDCA) and pyrrole-2,3,5-tricarboxylic acid (PTCA) by alkaline oxidation of H_2O_2 (Wakamatsu et al. 2003; Zecca et al. 2001).

The melanic portion of neuromelanin contains polymerized catecholamine residues of Cys-DOPA, which correspond to eumelanin, and residues of Cys-DA, corresponding to pheomelanin. They are characterized by the dihydroxindol and benzothiazine unit, which suggest the presence of both types of melanin in these brain regions (Zecca et al. 2008), as shown in Fig. 1.

Enzymes Related to Melanin Synthesis in Microorganisms

Tyrosinase

Tyrosinase (Polyphenol-oxidase EC 1.14.18.1) appeared on Earth as a result of the chemical transformations occurring in the early atmosphere. They were likely formed during the transition from an exclusively reducing system to an oxidizing one because of the emergence of photosynthesis (Plonka and Grabacka 2006).

The first references to the possible role of tyrosinase in fungal melanogenesis were proposed in the nineteenth century. In 1895, Bourquelot and Bertrand observed the

presence of the enzyme in the fungus *Russula nigricans*. Since then, tyrosinase has been found widely, distributed across bacteria and mammals (Change 2009).

Tyrosinases belong to the family of hemocyanin that is present in mollusks and insects, as well as catechol oxidases in plants (Change 2009). Tyrosinases involved in the formation of melanin can come in three different forms: oxy, met, and deoxy-tyrosinase (Solano 2014). In these, two copper atoms (CuA and CuB) are linked to three histidine molecules and distributed over specific binding sites. These sites catalyze oxidation reactions in monophenols (creolase or monophenolase cycle) and diphenols (catecholase or diphenolase cycle). Both reactions use molecular oxygen, which directly participates in melanin synthesis to form quinones (Chang 2012; Langfelder et al. 2003).

In the monophenolase cycle, a monophenol reacts with the oxy form being catalyzed into an *o*-diphenol. This is oxidized to an *o*-quinone and results in a deoxy form ready for a subsequent dioxygen bond (Chang 2012). Oxy tyrosinase is then regenerated after binding molecular oxygen to deoxy tyrosinase. If only *o*-diphenol is present (diphenolase cycle), both oxy and met can react with *o*-diphenol, oxidizing it to form an *o*-quinone (Halaouli et al. 2006). Subsequently, *o*-diphenol binds to the oxy form and is oxidized to *o*-quinone, thus obtaining the met form of the enzyme. This in turn transforms another *o*-diphenol molecule into o-quinone which is finally reduced to the deoxy form (Change 2009; Chang 2012).

Tyrosinase acts on tyrosine to form a protein-cupric enzyme complex, whereby the amino acid is converted to L-DOPA (3,4-dihydroxyphenyl-L-alanine) by a series of reactions. Through this, L-DOPA is converted into dopachrome for later oxidation in dopaquinone and finally polymerized in DOPA-melanin (Kobayashi et al. 1995; Manivasagan et al. 2013).

In fungi, tyrosinases are cytosolic enzymes involved in melanogenesis with considerable heterogeneity from other enzymes that also have copper atoms attached to their molecules (Halaouli et al. 2006). They have a range of molecular weights and are generally associated with spore formation and stability, defense mechanisms, increased virulence, and tissue regeneration. In addition, they play a fundamental role in the darkening and pigmentation of some species of fungi (Halaouli et al. 2005; Selinheimo et al. 2007; Van Gelder et al. 1997).

Laccases

Together with ascorbate oxidase in plants, ceruloplasmin synthesized in the liver of mammals, and ferroxidases, laccases (benzenediol: oxygen-oxidoreductase EC 1.10.3.2) are metalloproteins that belong to a small group known as "blue proteins" (Thurston 1994). These proteins are characterized by having four copper atoms linked to histidine in their conformation. One of these is linked to a site called CuT1, which acts on the reduction of substrates. The other two tri-atoms under the CuT2/CuT3 domains, which are sites of oxygen reduction, are responsible for the blue-green color (Kunamneni et al. 2007).

Laccase is widely distributed among plants and fungi and is also found in insects and bacteria (Nagai et al. 2003). When mapping expression genes for polyphenol oxidases extracted from a microbial community in bovine rumen, Beloqui et al. (2006) found a gene that regulates an enzyme with characteristics similar to laccase, known as adenosine deaminase (RL5). This may suggest that this enzyme is also present in the melanin synthesis of these microorganisms, acting in some way on mammals.

Laccases show phenoloxidase activity. In addition, they can oxidize a wide range of aromatic constituents such as amines, N-heterocycles, phenothiazines, thiol groups, among others (Levasseur et al. 2010). In fungi, the main producers of laccase are Basidiomycetes, which play an important role in the degradation of organic matter. As such, laccases of white-rot or wood decay fungal species have been widely studied and characterized due to their fundamental role in lignin degradation (Copete et al. 2015).

Depending on the substrate, laccase melanogenesis can occur either by oxidation of *p*-diphenols or *o*-diphenols. Oxidation by *o*-diphenols results in the deposition of pigments on the cell wall, while oxidation by *p*-diphenols produces pigments that diffuse between cells (Williamson et al. 1998).

Melanin biosynthesis occurs through a series of oxy-reduction reactions where DOPA and dopaquinone are catalyzed by phenoloxidase. Subsequent melanogenic reactions occur via the classic Mason-Raper pathway (Mason 1955), by which the indole compounds dopachrome and 5,6-dihydroxyindole are rapidly formed and dependent on laccase (Polacheck and Kwon-Chung 1998).

Polyketide Synthase (PKS)

Polyketide synthases (PKS) constitute a large class of natural secondary metabolites found in bacteria, plants, and fungi (Pastre et al. 2007). PKS are synthesized by means of acetyl-CoA strikers and are structurally divided into three classes: type I are modular proteins; type II are often aromatic proteins; and type III are small aromatic molecules produced by different types of fungi. These enzymes are fully active in several biological systems (Plonka and Grabacka 2006).

Much of the interest in these synthases comes from the unique biological importance that these enzymes play in natural systems, which makes them potential candidates for the discovery of new drugs (Shen 2003). Therefore, PKS provide a range of research opportunities due to their powerful catalytic activity, their ability to transport ions between

biological membranes, and their remarkable versatility in the generation of new compounds by combining different mechanisms (Hill 2012).

In fungi, a limited number of PKS enzymes are involved in melanogenesis. In black-colored species, melanization occurs by means of a polythetic similar to 1,8-dihydroxinaphthalene (DHN-melanin). In dark brown fungi, melanin is synthesized through the polymerization of a polythetic originating from precursor 1,3, 6,8-tetrahydroxinphthalene (THN4) by successive dehydration reactions (Kroken et al. 2003). Therefore, the enzymes that belong to the PKS group responsible for melanogenic synthesis in fungi are type-I that produce unreduced aromatic polyketides (Fujii et al. 2004).

Synthesis of Melanin in Fungi

Melanin plays an important role in the protection, virulence, and pathogenesis of the organisms in which they occur due to its ability to modify many cytokine responses and decrease cell phagocytosis. It also reduces the toxicity of microbicidal peptides, limits the accumulation of ROS and free radicals in the cell, and alters responses to antifungals (Van de Sande et al. 2007; Lee et al. 2019). Thus, there is significant interest in the study of this pigment, which is reflected directly in the countless publications over the last few decades that analyze a range of topics related to the synthesis and function of this polymer in fungi (Belozerskaya et al. 2017; Eisenman and Casadevall 2012; Gessler et al. 2014; Henson et al. 1999; Lee et al. 2019; Plonka and Grabacka 2006; Pralea et al. 2019; Solano 2014; Tran-L et al. 2020).

The synthesis of melanin in free-living microorganisms may be related to the susceptibility of the organism to hostile environments with extreme weather conditions, which may offer certain advantages for survival (Steenbergen and Casadevall 2003).

The melanin pigment is very common in fungi, although melanogenesis is restricted to certain stages of development. Therefore, the biopolymer can be found in the mycelium, during sporulation, or as a defensive reaction to traumatic damage (Romero-Martinez et al. 2000; Treseder and Lennon 2015). Melanin is an abundant compound that can be found in the cell wall of fungi, indicating that it is produced inside the fungal cell and then transported to the cell wall (Solano 2014).

Fungal melanins are negatively charged and formed in various ways, including: oxidative polymerization of phenolic and indole compounds, such as glutaminyl-3,4-dihydroxybenzene (GDHB); through catecholamine; via the 1,8-dihydroxynaphthalene (DHN) pathway; or, in Basidiomycetes, via 3,4-dihydroxyphenylalanine (DOPA) (Nosanchuk and Casadevall 2006). However, most Ascomycota fungi synthesize DHN-melanin via the PKS pathway (Bell and Wheeler 1986). Some species of fungi may be able to synthesize the melanin polymer through the exogenous L-DOPA pathway, which is slightly different from the process of formation in mammalian cells (Eisenman and Casadevall 2012).

In mammals, melanin particles are produced inside organelles linked to the plasma membrane of cells, called melanosomes. Later, they are actively transported through a network of microtubules by melanocytes to keratinocytes by mechanisms of cell transfer that are not yet well understood. Finally, they are transferred to neighboring units, reaching the cell wall (Eisenman 2012; Hara et al. 2000).

Two main cell transfer models have received particular attention. In the shedding-phagocytosis model, the melanocyte discards packages rich in melanosome, enclosed in the plasma membrane, which are later internalized by the keratinocyte via phagocytosis. In the endocytosis model, the melanocyte releases the melanin nucleus from the melanosome in the extracellular space by exocytosis, and the keratinocyte internalizes this melanin from the melanosome by endocytosis (Wu and Hammer 2014). In addition, studies suggest that the transfer of melanin to keratinocytes is regulated by a protease-activated receptor type 2 (PAR-2), a keratinocyte receptor that controls melanin uptake (Seiberg 2001; Correia et al. 2018).

Melanosomes are intracellular organelles that have the exclusive function of synthesizing and storing melanin (Eisenman and Casadevall 2012; Freitas et al. 2019). These structures are derived from the initial endosomes of the endosomal membranes, compartments formed from endocytosis by the fusion of vesicles from the Golgi complex (Raposo and Marks 2007). In animal cells, these organelles mature within the melanocytes and pass through four morphologically distinct stages, as has been described in humans and other mammals (Slominski et al. 2004).

The first and second stages are called pre-melanosomes. They are characterized by the complete absence of melanin and the formation of interlamellar fibers that begin to form at stage I and are complete at the end of stage II. In this phase, there are high levels of tyrosinase activity coming from the ribosomes, which are sent via endoplasmic reticulum to the Golgi complex and stored in the melanosomes (Raposo and Marks 2007). As soon as the lamellar striations are formed, the synthesis of melanin begins with its deposition along the fibers. This results in its spacing and darkening, following maturation in stage III. In stage IV, the complete filling of its protein walls occurs (Fig. 2) with intense enzymatic activity in which the presence of Tyrosinase, TRP1, and dopachrome tautomerase, also known as DCT or TRP2 (Miot et al. 2009), is particularly notable. At stage IV, melanosomes are translocated along microtubules of actin dendritic cells and then

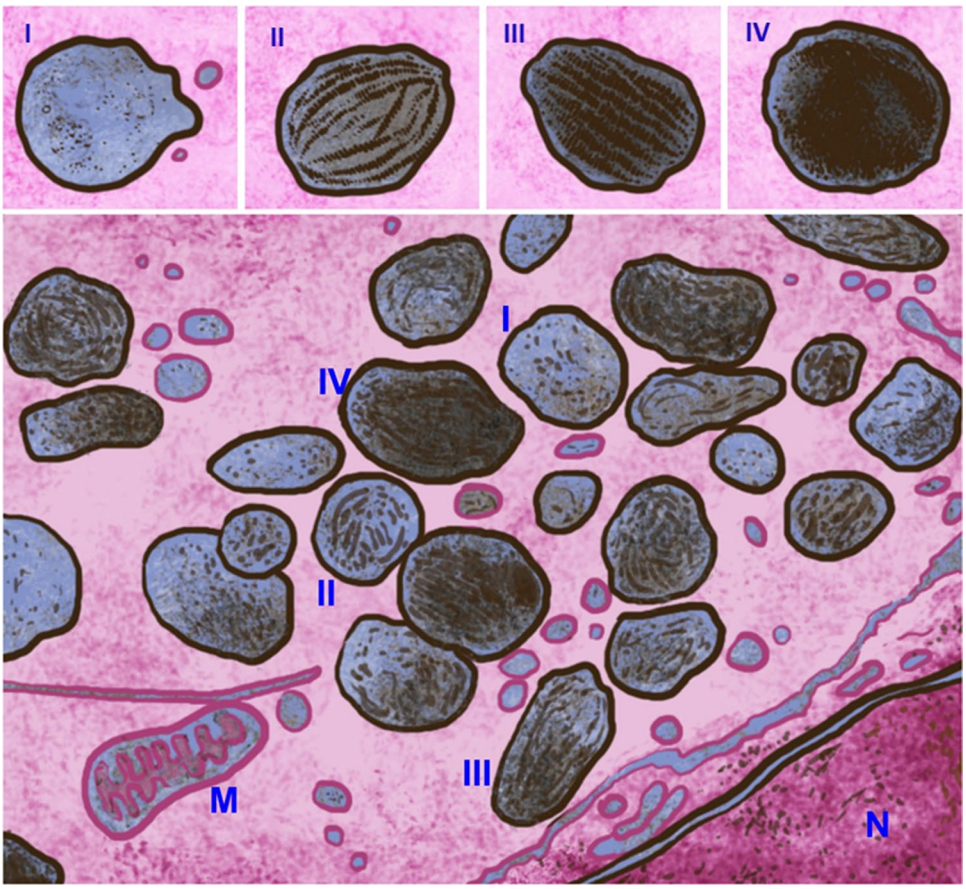

Fig. 2 Drawing adapted from Raposo and Marks (2007) showing the maturation stages of melanosomes. Designed by Jonathas Corrêa Botelho. Note the intraluminal vesicles of stage I melanosomes, the proteinaceous fibrils at stage II, and the melanin deposition in stages III and IV. Mitochondria (M), nucleus (N)

transferred to neighboring keratinocytes for later deposition in the cell wall (Van Den Bossche et al. 2006).

In fungi, the participation of melanosomes in the synthesis of melanin was suggested initially due to the discovery of transport vesicles (Rodrigues et al. 2007) in the cell wall complex. This indicates that fungal melanization may also occur in specialized organelles analogous to mammalian melanosomes (Eisenman et al. 2009; Frazen et al. 2008; Freitas et al. 2019; Walker et al. 2010).

Nematophagous Fungi

Nematophagous fungi are part of a diverse group of fungi that use refined traps to capture prey (predators), conidia, or zoospores (endoparasites) to infect nematodes and appressoria and penetrate nematode eggs (ovicides). Of these, more than 700 species (Li et al. 2015) have been described distributed across the phylum Ascomycota, Basidiomycota, Blastocladiomycota, Calcarisporiellomycota, Zoopagomycota, and Entomophthoromycota (Tedersoo et al. 2018). Species of *Oomycota* (Chromista kingdom), as well as some species of the genera *Myzocytium*, *Haptoglossa*, and *Gonimochaete* that are traditionally included in the fungi kingdom, can also kill nematodes. These fungi are cosmopolitan and inhabit soil organic matter, decomposing vegetation, and manure.

Predatory fungi produce a range of traps including: adhesive nodules that can be pedunculated or sessile; non-differentiated adhesive hyphae; hypha branches that undergo anastomosis, forming three-dimensional adhesive networks; adhesive ramifications that come together to form simple two-dimensional adhesive networks; and constrictive and non-constrictive rings (Barron 1977).

Phylogenetic studies demonstrate that the mechanisms involved in the construction of traps are linked to at least two distinct strains of fungi, one being efficient in the construction of rings, and another efficient in traps with adhesive buttons (Yang et al. 2008). These organisms may have evolved from cellulolytic and ligninolytic fungi as a possible response to nutrient deficiencies in habitats with significant nutritional scarcity (Barron 1992). In these locations, nematodes, which have a high carbon:nitrogen (C: N) ratio, could serve as a source of nitrogen while the fungi grow on substrates that contain large amounts of carbohydrates. As such, nematophagous fungi can use cellulose and other polysaccharides as a source carbon (Nordbring-Hertz et al. 2006).

The use of fungi for the biological control of gastrointestinal nematodes has been considered a viable alternative to control free-living parasites. The main objective of such

treatments is to decrease a given population of nematodes using a natural predator that is available in the environment whose activity focuses on attacking intermediate hosts, vectors, or larval stages of free life (Braga and Araújo 2014).

Nematophagous fungi have a unique ability to trap and infect nematodes through a process that may or may not include adhesion (adhesive traps and adhesive conidia vs. non-adhesive traps or ingestible conidia). Then, the cuticle or esophagus (ingestible conidia) is penetrated, and the nematode is immobilized. Finally, its entire contents are digested (Herrera-Estrella et al. 2016; Tunlid et al. 1992). In the case of adhesion, previous studies on the predatory fungus *Arthrobotrys oligospora* point out the participation of lectins in the traps joining with carbohydrates in the nematodes (Nordbringhertz and Mattiasson 1979; Tunlid et al. 1992). However, later studies did not find such a relationship in the adhesion process (Balogh et al. 2003; Yang et al. 2011).

This succession of events occurs through a combination of physical and enzymatic processes (Cruz et al. 2009). Several proteases are likely involved in the sequence of events that precede infection, including the release of nutrients for the growth of the fungal structures, thus facilitating penetration by solubilizing the cuticle of the nematode. This induces cytotoxic effects, which culminate in the total digestion of the host tissue and also inhibits the secondary invasion of microorganisms (Tunlid et al. 1994). Among proteases, serine proteases have been shown to participate in this process (Åhman et al. 2002; Cruz et al. 2015; Wang et al. 2006, 2009; Yang et al. 2005).

The nematophagous fungi that are classified as predators are well studied and more commonly assessed for use as a biological control of gastrointestinal nematodes in ruminants. Of these, the genus *Arthrobotrys* (Gomes et al. 2001; Grønvold et al. 1993; Zhang et al. 2013), including *A. oligospora* (Fresenius 1852) and *A. musiformis* (Dreschler 1937), is particularly well studied. The genus *Monacrosporium* also has also received attention and is represented mainly by *M. thaumasium* (Dreschler 1937), *M. sinense* (Liu and Zhang 1994), and *M. haptotylum* (Liu and Zhang 1994). Meanwhile, the genus *Duddingtonia* includes a single representative, *D. flagrans* (Braga et al. 2008; Duddington 1955), which has attracted the attention of researchers searching for alternative methods to control nematodes in farm animals (Jobim et al. 2008; Tavela et al. 2013). The species produces resistant structures known as chlamydospores and have a thick cell wall. As such, they can resist extreme stress conditions as they pass through the gastrointestinal tract of domestic animals and have a high environmental tolerance (Buzatti et al. 2015).

Knowledge about the interactions that occur between a fungus and its host can contribute to their application as a biological parasite control. Understanding the molecular mechanisms and the biochemistry involved in the interaction between fungi and their hosts is also key, as such fundamental information can contribute effectively to elucidating the details of the degree of activity in the relationships between these predators and their prey (Davies and Spiegel 2011).

Since the discovery of predatory activity in *A. oligospora* by Zopf (1888), nematode predatory fungi have attracted attention. Numerous species have been isolated and identified, and their ecological, nutritional, and physiological characteristics described. Charles Dreschler (1892–1986) contributed greatly to this field of study (Rubner 1996). However, gaps in the literature remain, especially regarding the presence and function of melanin in these microorganisms. Freitas et al. (2019) observed that *D. flagrans* produces melanin and noted that it is deposited on the cell wall of its hyphae and chlamydospores. Furthermore, the predatory activity of fungus treated with tricyclazole, an inhibitor of melanin biosynthesis, was drastically affected after 27 h of in vitro anaerobic stress with rumen inoculum. These results support the idea that melanin can effectively contribute to the protection of the fungus during fungus-nematode and environment interaction processes. In this same study, the authors found structures that may participate in the synthesis and deposition of melanin, known as melanosomes, and that the observed genesis suggests a possible analogy with the melanosomes of mammals. This is a pioneering find for nematophagous fungi.

Advances in Fungal Melanin Research with Proteomics

Proteomics is defined as a systematic reading of the proteome, which is a set of proteins and their variants, as expressed through a genome, cell, tissue, or organism (Bhadauria et al. 2007; Oliveira et al. 2009). Thus, proteomics offers the potential to advance scientific research since it is a fundamental tool that can be used to understand protein components, elucidating changes that occur in a given protein at the molecular level (Doyle 2011).

Proteomic analysis provides important information about cell signaling and is warranted considering the following: (i) gene function, which is translated into the information encoded in a gene, results in a gene product that is carried out by proteins; (ii) although most of the genes in a genome have a function, a large number of genes have no assigned function; (iii) the information that can be obtained using a transcriptomic approach is still incomplete, since the profile of the transcriptome varies according to time, physiological state, and physical, chemical, and biological stimuli. Therefore, proteomics is complementary to the transcriptome, as proteins are the final gene product and the direct executors of biological functions; and (iv) the correlation between mRNA and protein levels is remarkably low (Watson et al. 2003;

Jorrín et al. 2006; Koh et al. 2012). Furthermore, because post-transcriptional regulation and post-translational modification directly influence the proteome (Alam et al. 2010), there is no strict linear relationship between mRNAs and proteins (Gygi et al. 1999) thus making it difficult to predict the protein expression through transcriptional level analysis (Yan et al. 2017).

The intracellular proteome of a eukaryotic organism consists mainly of proteins that are present in the cytosol and within the organelles. Therefore, due to the heterogeneity of the Fungi kingdom, the procedures for extracting, separating, and identifying proteins require particular techniques (Doyle 2011). Currently, models based on mass spectrometry are generally considered the gold standard for proteomic analysis (although there are others, such as the microarray of proteins). These models can be classified as either "bottom-up", which consists of using liquid chromatography to separate proteins of the peptides obtained after tryptic digestion of complex protein solutions, followed by analysis with mass spectrometry, or "top-down", in which the intact proteins are subjected to mass spectrometry and then decoded by bioinformatics (Barbosa et al. 2012).

The development of "label-free" methodologies has also provided a new environment for proteomics research, where complex mixtures of proteins are digested by proteases then separated by multidimensional liquid chromatography. Subsequently, they are analyzed and identified through tandem mass spectrometry (Jorrín Novo et al. 2014).

Over the last few decades, proteomic studies on melanogenic fungi have started to emerge in the literature. Mainly, these studies aim to understand the key targets that are somehow involved in microorganism virulence and pathogenicity (Xu et al. 2017), although the proteomics associated with melanin synthesis remains unclear (He et al. 2021).

Proteomic approaches have been applied to some species of melanogenic fungi, such as *Aspergillus fumigatus* (Bruneau et al. 2001*), Saccharomyces cerevisiae* (Navarre et al. 2002), *Candida albicans* (Cabezón et al. 2009), and *Paracoccidioides brasiliensis* (Chaves et al. 2019). More recently, these methods have been used to assess the association between proteomics and the presence of melanin, for example in *P. brasiliensis*, *Paracoccidioides lutzii* (Almeida-Paes et al. 2020), and *Cryptococcus neoformans* (Camacho et al. 2019). This recent research aimed to determine aspects related to the adaptation and survival of the fungus in the host (i.e., fungus-macrophage interaction), which is understood as the success of the pathogen's intracellular mechanisms to evade macrophage activation (Almeida-Paes et al. 2020; Chaves et al. 2019). In such studies, several additional proteins linked to the virulence of these species have been found, such as phospholipases (PLC), proteases, superoxide dismutase (SOD), heat shock proteins, and adhesins and proteins related to vesicular transport in fungi treated with L-DOPA (Almeida-Paes et al. 2020). In *C. neoformans*, the synthesis of melanin granules was found to be closely associated with the expression of four proteins that may have some connection with the melanogenesis of this fungus, including the mannoprotein that plays a role in iron acquisition (Cig1), the hypothetical protein containing a Barwin-like domain (Blp1), the quorum-sensing peptide (Qsp1), and GPI-anchored protein (CNI3590).

For nematophagous fungi, there is still much to be understood in terms of proteomics. Yang et al. (2011) analyzed the proteome of *A. oligospora* by linking the identified proteins to molecular mechanisms involved in the production of nematode capture and predation structures. They found that some of these proteins (i.e., PLC, MAPK, PP2A, CACYBP, CaMK) were involved in translation, post-translation modification, amino acid and carbohydrate metabolism, energy conversion, cell wall synthesis, and membrane biogenesis, which suggests an intense level of metabolic activity during the formation of traps for the species. In another study on nematophagous fungi, Andersson et al. (2013) identified and quantified proteins related to the mechanisms involved in the formation of predation structures in *M. haptotylum*. They observed that 16.07% of these proteins are expressed in greater quantity during the formation of nematode predation traps and involve peptidases, adhesin surfaces with carbohydrate-binding domains (including the domain of stress-responsive cell wall proteins - CWPs), tyrosinase, and proteins with functions that until then were unknown.

There is a wide range of studies relating to genomics and pathogenicity in nematophagous fungi. Although *A. oligospora* has been particularly well studied (Meerupati et al. 2013; Yang et al. 2011; Yang et al. 2020; Zhang et al. 2020), analyses of other species include: *M. haptotylum* (Meerupati et al. 2013); *Dactylellina stenobrocha* (Liu et al. 2014); *Hirsutella minnesotensis* (Lai et al. 2014); *D. flagrans* (Youssar et al. 2019); *A. conoides, D. appendiculata, D. drechsleri, D. haptotyla, D. stenobrocha* (Zhang et al. 2020) and *D. cionopaga* (Deng and Yu 2019).

Recent advances in fungal proteomics research are mainly due to the availability of powerful techniques based on proteomics and the advent of second-generation processing for high performance DNA sequencing (Muggia et al. 2020). Furthermore, the molecular singularity of the species has stimulated considerable interest in the search for proteins that play important roles in survival and stress responses (Kroll et al. 2014). The use of these new technologies can accelerate our understanding of the biology of melanogenic fungi, especially those with nematophagous activity.

Another point that must be considered is genetic editing, i.e., the procedure in which specific stretches of DNA are manipulated, allowing their replacement by new gene sequences, including insertion, deletion, or alteration of certain regions of the genome. With the development of

projected nucleases, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and more recently clustered regularly interspaced short palindromic repeats (CRISPR), a revolution has begun in several fields of biotechnology, including agriculture and biopharmaceutical production, research on the structure, regulation, and function of the genome, and the creation of transgenic organisms and cell lines (Saha et al. 2019).

The CRISPR technique is based on the adaptive immune system of bacteria. When bacteria undergo an invasion by viruses or plasmids, they capture a fragment of approximately 20 base pairs from the invader to form the CRISPR sequence. Once inserted into the bacterial genome, the CRISPR sequence can be transcribed into a type of RNA that will bind to CRISPR RNAs. The protein complex formed by RNAs and the associated Cas9 protein creates an active endonuclease that will degrade a 23 base pair target DNA using a Protospacer-Adjacent Motif (PAM) sequence. Therefore, it is possible to build guide RNAs associated with the Cas9 protein that are complementary to the sequences to be cut (Saha et al. 2019).

The discovery of the CRISPR/Cas9 immune system in bacteria and archaea and its reuse for genome editing offers new prospects for the genetic engineering of filamentous fungi. Several successful applications have been reported for fungal cell factories, including *A. niger*, *Penicillium chrysogenum*, *Trichoderma reesei*, and *Termothelomyces thermophilus* (Kwon et al. 2019). Similarly, this methodology has been applied to studies related to fungal melanin, for example to create mutant strains deficient in the production of melanin to study its effects on conidia resistance to food, heat, and UV-C radiation stressors (Seekles et al. 2021), or studies of melanic biosynthesis for a better understanding of the regulators involved (Zhang et al. 2019). To date, no studies have used CRISPR to assess the relationship between melanin and nematophagous fungi.

Conclusions

Melanin is a compound that is not only associated with numerous defense mechanisms, but also offers a series of advantages for the carrier organisms. Due to its unique characteristics, the melanin polymer has attracted significant interest worldwide, and further study on its synthesis, structure, location within cells, and role in the biology and survival of nematophagous fungi are needed. Notable advances in molecular biology, including the application of proteomics and gene editing, can contribute to such analyses and provide a better understanding of how this ancient macromolecule can act so effectively in biological processes and in fungi-nematode and environment interactions. Furthermore, in-depth analyses on the molecular basis associated with melanin can contribute to the development of more effective biocontrol agents.

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Declarations

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Consent to Participate Not applicable.

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References

Abad A, Fernández-Molina JV, Bikandi J, Ramírez A, Margareto J, Sendino J, Hernando FL, Pontón J, Garaizar J, Rementeria A (2010) What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. Rev Iberoam Micol 27:155–182. https://doi.org/10.1016/j.riam.2010.10.003

Adachi K, Hamer J (1998) Divergent cAMP signaling pathways regulate growth and pathogenesis in the rice blast fungus Magnaporthe grisea. Plant Cell 10:361–1373. https://doi.org/10.1105/tpc.10.8.1361

Åhman J, Johansson T, Punt PJ OM, Van Den Hondel CAMJJ, Tunlid A (2002) Improving the pathogenicity of a nematode-trapping fungus by genetic engineering of a subtilisin with nematotoxic activity. Appl Environ Microbiol 68:3408–3415. https://doi.org/10.1128/AEM.68.7.3408-3415.2002

Alam I, Sharmin SA, Kim KH, Yang JK, Choi MS, Lee BH (2010) Proteome analysis of soybean roots subjected to short-term drought stress. Plant Soil 333:491–505. https://doi.org/10.1007/s11104-010-0365-7

Almeida-Paes R, Almeida MA, Baeza LC, Marmello LAM, Trugilho MRO, Nosanchuk JD, Soares CMA, Valente RH, Zancopé-Oliveira RM (2020) Beyond melanin: proteomics reveals virulence-related proteins in *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii* yeast cells grown in the presence of L-dihydroxyphenylalanine. J Fungi 4:1–18. https://doi.org/10.3390/jof6040328

Andersson KM, Meerupati T, Levander F, Friman E, Ahrén D, Tunlid A (2013) Proteome of the nematode-trapping cells of the fungus *Monacrosporium haptotylum*. Appl Environ Microbiol 16:4993–5004. https://doi.org/10.1128/AEM.01390-13

Balogh J, Tunlide A, Rosén S (2003) Deletion of a lectin gene does not affect the phenotype of the nematode-trapping fungus *Arthrobotrys oligospora*. Fungal Genet Biol 39:128–135. https://doi.org/10.1016/s1087-1845(03)00023-9

Barber JI, Townsed D, Olds DP, King RA (1985) Decreased DOPAchrome oxidoreductase activity in yellow mice. J Hered 76:59–60. https://doi.org/10.1093/oxfordjournals.jhered.a110019

Barbosa EB, Vidotto A, Polachini GM, Henrique T, Marqui ABT, Tajara EH (2012) Proteômica: metodologias e aplicações no estudo de doenças humanas. Rev Assoc Med Bras 3:366–375. https://doi.org/10.1590/S0104-42302012000300019

Barron GL (1977) The nematode-destroying fungi. Canadian Biological Publications Ltd., Guelph

Barron GL (1992) Lignolytic and cellulolytic fungi as predators and parasites. In: Carroll GC, Wicklow DT (eds) The fungal community: its organization and role in ecosystem. Marcel Dekker, New York, pp 311–326

Bashkatov AN, Genina EA, Kochubei VI, Tuchin VV (2006) Estimate of the melanin content in human hairs by the inverse Monte-Carlo method using a system for digital image analysis. Quantum Electronics 12:1111–1118. https://doi.org/10.1070/QE2006v036n12ABEH013336

Bell AA, Wheeler MH (1986) Biosynthesis and functions of fungal melanins. Annu Rev Phytopathol 24:411–451. https://doi.org/10.1146/annurev.py.24.090186.002211

Beloqui A, Pita M, Polaina J, Martínez-Arias A, Golyshina OV, Zmárraga M, Yakimov MM, García-Arellano H, Alcalde M, Fernández VM, Elborough K, Andreu JM, Ballesteros A, Plou FJ, Timmis KN, Ferrer M, Golyshi PN, Elborough K (2006) Novel polyphenol oxidase mined from a metagenome expression library of bovine rumen biochemical properties, structural analysis, and phylogenetic relationships. J Biol Chem 281:22933–22942. https://doi.org/10.1074/jbc.M600577200

Belozerskaya TA, Gessler NN, Aver'yanov AA (2017) Melanin Pigments of Fungi. In: Mérillon JM, Ramawat K (eds) Fungal Metabolites. Reference Series in Phytochemistry. Springer, Cham, pp. 263–291

Berzelius JJ (1840) Lehrbuch der Chemie, 9, 522 (Aus der Schwedischen Handschriff des Verfassers Übersetzt von F. Wöhler), Dritte Umgearbeitete und Vermehrte Original- Auflage. Arnoldischen Buchhandlung, Dresden u Leipzig

Bhadauria V, Zhao WS, Wang LX, Zhang Y, Liu JH, Yang J, Kong LA, Peng YL (2007) Advances in fungal proteomics. Microbiol Res 162:193–200

Bonser RHC (1995) Melanin and the abrasion resistance of feathers. The Condor 2:590–591. https://doi.org/10.2307/1369048

Braga FR, Araújo JV (2014) Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals. Appl Microbiol Biotechnol 98:71–82. https://doi.org/10.1007/s00253-013-5366-z

Braga FR, Araújo JV, Campos AK, Araújo JM, Carvalho RO, Silva AR, Tavela AO (2008) In vitro evaluation of the action of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on Fasciola hepatica eggs. World J Microbiol Biotechnol 24:1559–1564. https://doi.org/10.1007/s11274-007-9643-9

Bruneau JM, Magnin T, Tagat E, Legrand R, Bernard M, Diaquin M, Fudali C, Latgé JP (2001) Proteome analysis of *Aspergillus fumigatus* identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis. Electrophoresis 22: 2812–2823. https://doi.org/10.1002/1522-2683(200108)22:13<2812::AID-ELPS2812>3.0.CO;2-Q

Buzatti A, Santos CP, Fernandes MAM, Yoshitani UY, Sprenger LK, Dos Santos CD, Molento MB (2015) *Duddingtonia flagrans* in the control of gastrointestinal nematodes of horses. Exp Parasitol 159:1–4. https://doi.org/10.1016/j.exppara.2015.07.006

Cabezón V, Llama-Palacios A, Nombela C, Monteoliva L, Gil C (2009) Analysis of *Candida albicans* plasma membrane proteome. Proteomics 20:4770–4786. https://doi.org/10.1002/pmic.200800988

Camacho E, Vij R, Chrissian C, Prados-Rosales R, Gil D, O'Meally RN, Cordero RJB, Cole RN, McCaffery JM, Stark RE, Casadevall A (2019) The structural unit of melanin in the cell wall of the fungal pathogen *Cryptococcus neoformans*. J Biol Chem 27:10471–10489. https://doi.org/10.1074/jbc.RA119.008684

Casadevall A, Nakouzi A, Crippa PR, Eisner M (2012) Fungal melanins differ in planar stacking distances. PLos One. 2:1–6. https://doi.org/10.1371/journal.pone.0030299

Chang TS (2012) Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity. Materials 5:1661–1685. https://doi.org/10.3390/ma5091661

Change TS (2009) An updated review of tyrosinase inhibitors. Int J Mol Sci 10:2440–2475. https://doi.org/10.3390/ijms10062440

Chatterjee S, Prados-Rosales R, Frases S, Itin B, Casadevall A, Stark RE (2012) Using solid-state NMR to monitor the molecular consequences of *Cryptococcus neoformans* melanization with different catecholamine precursors. Biochemistry 5:6080–6088. https://doi.org/10.1021/bi300325m

Chaves EGA, Parente-Rocha JA, Baeza LC, Araújo DS, Borges CL, Oliveira MAP, Soares CMA (2019) Proteomic analysis of *Paracoccidioides brasiliensis* during infection of alveolar macrophages primed or not by interferon-gamma. Front Microbiol 10:1–14. https://doi.org/10.3389/fmicb.2019.00096

Chen LY, Leu WM, Wangg KT, Lee YH (1992) Copper transfer and activation of the Streptomyces apotyrosinase are mediated through a complex formation between apotyrosinase and its trans-activator MelC1. J Biol Chem 267:20100–20107. https://doi.org/10.1016/S0021-9258(19)88671-4

Clusella Trullas S, Wyk JHV, Spotila JR (2007) Thermal melanism in ectotherms. J Therm Biol 32:235–245. https://doi.org/10.1016/j.jtherbio.2007.01.013

Copete LS, Chanagá X, Barriuso J, López-lucendo MF, Martínez MJ, Camarero S (2015) Identification and characterization of laccase-type multicopper oxidases involved in dye-decolorization by the fungus *Leptosphaerulina* sp. BMC Biotechnol 15:74. https://doi.org/10.1186/s12896-015-0192-2

Correia MS, Moreiras H, Pereira FJC, Neto MV, Festas TC, Tarafder AK, Ramalho JS, Seabra MC, Barral DC (2018) Melanin transferred to keratinocytes resides in nondegradative endocytic compartments. J Investig Dermatol 138:637–646. https://doi.org/10.1016/j.jid.2017.09.042

Cruz DG, Silva CP, Carneiro CNB, Retamal CA, Thiébaut JTL, Damatta RA, Santos CP (2009) Acid phosphatase activity during the interaction of the nematophagous fungus *Duddingtonia flagrans* with the nematode *Panagrellus* sp. J Invertebr Pathol 102:238–244. https://doi.org/10.1016/j.jip.2009.08.003

Cruz DG, Costa LM, Rocha LO, Retamal CA, Vieira RA, Seabra SH, Silva CP, Damatta RA, Santos CP (2015) Serine proteases activity is important for the interaction of nematophagous fungus *Duddingtonia flagrans* with infective larvae of trichostrongylides and free-living nematodes *Panagrellus* spp. Fungal Biol 119:672–678. https://doi.org/10.1016/j.funbio.2015.03.005

Dadachova E, Bryan RA, Howell RC, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A (2008) The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. Pigment Cell Melanoma Res 21:192–199. https://doi.org/10.1111/j.1755-148X.2007.00430.x

Davies KG, Spiegel Y (2011) Biological control of plant-parasitic nematodes: towards understanding field variation through molecular mechanisms. In: Jones J, Gheysen G, Fenollg C (eds) Genomics and molecular genetics of plant-nematode interactions. Springer, Dordrecht, pp 493–516

De Angelis F, Arcadi A, Marinelli F, Paci M, Boti D, Pacioni G, Miranda M (1996) Partial structures of truffle melanins. Phytochemistry 43:1103–1106. https://doi.org/10.1016/S0031-9422(96)00451-7

Deng C, Yu ZF (2019) The complete mitochondrial genomes of the nematode-trapping fungus *Dactylellina cionopaga*.

Mitochondrial DNA B Resour 1:866–867. https://doi.org/10.1080/23802359.2019.1573115
D'ischia M, Wakamatsu K, Napolitano A, Briganti S, Garcia-Borron JC, Kovacs D, Meredith P, Pezzella A, Picardo M, Sarna T, Simon JD, Ito S (2013) Melanins and melanogenesis: methods, standards, protocols. Pigment Cell Melanoma Res 26:616–633. https://doi.org/10.1111/pcmr.12121
Double KL, Maruyama W, Naoi M, Gerlach M, Riederer P (2011) Biological role of neuromelanin in the human brain and its importance in Parkinson's disease. Melanins and melanosomes: biosynthesis, biogenesis, physiological, and pathological functions 8:225–246. https://doi.org/10.1002/9783527636150.ch8
Doyle S (2011) Fungal proteomics: from identification to function. FEMS Microbiol Lett 1:1–9. https://doi.org/10.1111/j.1574-6968.2011.02292.x
Dreschler C (1937) Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447–552. https://doi.org/10.1080/00275514.1937.12017222
Duddington CL (1955) Fungi that attack microscopic animals. Bot Rev 21:377–439
Duff GA, Roberts JE, Foster N (1988) Analysis of the structure of synthetic and natural melanins by solid-phase NMR. Biochemistry 27:7112–7116. https://doi.org/10.1021/bi00418a067
Eisenman HC, Casadevall A (2012) Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 93:931–940. https://doi.org/10.1007/s00253-011-3777-2
Eisenman HC, Frases S, Nicola AM, Rodrigues ML, Casadevall A (2009) Vesicle-associated melanization in Cryptococcus neoformans. Microbiology 155:3860. https://doi.org/10.1099/mic.0.032854-0
Ferrari E, Capucciati A, Prada I, Zucca FA, D'arrigo G, Pontiroli D, Bridelli MG, Sturini M, Bubacco L, Monzani E, Verderio C, Zecca L, Casella L (2017) Synthesis, structure characterization, and evaluation in microglia cultures of neuromelanin analogues suitable for modeling Parkinson's disease. ACS Chem Neurosci 8:501–512. https://doi.org/10.1021/acschemneuro.6b00231
Figueiredo-Carvalho MHG, Dos Santos FB, Nosanchuk JD, Zancope-oliveira RM, Almeida-Paes R (2014) L-Dihydroxyphenylalanine induces melanin production by members of the genus *Trichosporon*. FEMS Yeast Res 14:988–991. https://doi.org/10.1111/1567-1364.12174
Frazen AJ, Cunha MML, Mirand K, Hentschel J, Plattner H, Silva MB, Salgado CG, Souza W, Rozental S (2008) Ultrastructural characterization of melanosomes of the human pathogenic fungus *Fonsecaea pedrosoi*. J Struct Biol 162:75–84. https://doi.org/10.1016/j.jsb.2007.11.004
Freitas DF, Vieira-da-Motta O, Mathias LDS, Franco RWDA, Gomes RDS, Vieira RAM, Santos CP (2019) Synthesis and role of melanin for tolerating in vitro rumen digestion in *Duddingtonia flagrans*, a nematode-trapping fungus. Mycology 10:229–242. https://doi.org/10.1080/21501203.2019.1631896
Fresenius G (1852) Beiträge zur Mykologie. Heft 1-2:1–80
Fujii I, Yasuoka Y, Tsai HF, Chang YC, Kwon-Chung KJ, Ebizuka Y (2004) Hydrolytic polyketide shortening by ayg1p, a novel enzyme involved in fungal melanin biosynthesis. J Biol Chem 279:44613–44620. https://doi.org/10.1074/jbc.M406758200
Gessler NN, Egorova AS, Belozerskaya TA (2014) Melanin pigments of fungi under extreme environmental conditions (review). Appl Biochem Microbiol 50:105–113. https://doi.org/10.1134/S0003683814020094
Glass K, Ito S, Wilby PR, Sota T, Nakamura A, Bowers CR, Vintherf J, Dutta S, Summons R, Briggs DEG, Wakamatsub K, Simon JD (2012) Direct chemical evidence for eumelanin pigment from the Jurassic period. PNAS 109:10218–10223. https://doi.org/10.1073/pnas.1118448109
Gomes APS, Vasconcellos RS, Ramos ML, Guimarães MP, Yatsuda AP, Vieira-Bressan MCR (2001) In vitro interaction of Brazilian strains of the nematode-trapping fungi *Arthrobotrys* spp. on *Panagrellus* sp. and *Cooperia punctata*. Mem Inst Oswaldo Cruz 96:861–864. https://doi.org/10.1590/S0074-02762001000600021
Grønvold J, Wolstrup J, Larsen M, Henriksen SA, Nansen P (1993) Biological control of *Ostertagia ostertagi* by feeding selected nematode-trapping fungi to calves. J Helminthol 67:31–36. https://doi.org/10.1017/S0022149X00012827
Gygi S, Rochon Y, Franza RB, Aebersold R (1999) Correlation between protein and mrna abundance in yeast. Mol Cell Biol 3:1720–1730. https://doi.org/10.1128/MCB.19.3.1720
Halaouli S, Asther M, Sigoillot JC, Hamdi M, Lomascolo A (2006) Fungal tyrosinases: new prospects in molecular characteristics, bioengineering and biotechnological applications. J Appl Microbiol 100:219–232. https://doi.org/10.1111/j.1365-2672.2006.02866.x
Halaouli S, Asther MI, Kruus K, Guo L, Hamdi M, Sigoillot JC, Asther M, Lomascolo A (2005) Characterization of a new tyrosinase from Pycnoporus species with high potential for food technological applications. J Appl Microbiol 98:332–343. https://doi.org/10.1111/j.1365-2672.2004.02481.x
Halliday GM, Leverenz JB, Schneider JS (2014) Adler CH (2014) the neurobiological basis of cognitive impairment in Parkinson's disease. Mov Disord 29(5):634–650. https://doi.org/10.1002/mds.25857
Hara M, Yaar M, Byers HR, Goukassian D, Fine RE, Gonçalves J (2000) Gilchrest BA (2000) Kinesin participates in melanosomal movement along melanocyte dendrites. J Investigat dermatol 114:438–443. https://doi.org/10.1046/j.1523-1747.2000.00894.x
He X, Liu D, Chen Q (2021) Proteomic analysis on the regulation of DOPA-melanin synthesis in Talaromyces marneffei. Microb Pathog 150:1–10. https://doi.org/10.1016/j.micpath.2020.104701
Henson JM, Butler MJ, Day AW (1999) The dark side of the mycelium: melanins of phytopathogenic fungi. Annu Rev Phytopathol 37:447–471. https://doi.org/10.1146/annurev.phyto.37.1.447
Herrera-Estrella A, Casas-Flores S, Kubicek CP (2016) Nematophagous Fungi. In: Druzhinina IS, Kubicek CP (eds) Environmental and microbial relationships. Springer, Cham, pp 247–267. https://doi.org/10.1007/978-3-319-29532-9
Hill AM (2012) Polyketide Polyethers. In: Civja N (Ed) Natural Products in Chemical Biology Wiley, New York. pp.189–206. https://doi.org/10.1002/9781118391815
Hoekstra HE (2006) Genetics, development and evolution of adaptive pigmentation in vertebrates. Heredity 3:222–234. https://doi.org/10.1038/sj.hdy.6800861
Ito S, Wakamatsu K (2003) Quantitative analysis of Eumelanin and Pheomelanin in humans, mice, and other animals: a comparative review. Pigment Cell Res 16:523–531. https://doi.org/10.1034/j.1600-0749.2003.00072.x
Jobim MB, Santurio JM, La Rue D, Luiz M (2008) Duddingtonia flagrans: controle biológico de nematódeos de bovinos a campo. Cienc 8:2256–2263. https://doi.org/10.1590/S0103-84782008000800026
Jorrín Novo JV, Pascual J, Lucas RS, Romero-Rodriguez C, Ortega MR, Lenz C, Valledor L (2014) Fourteen years of plant proteomics reflected in proteomics: moving from model species and 2DE-based approaches to orphan species and gel-free platforms. Proteomics 15:1089–1112. https://doi.org/10.1002/pmic.201400349
Jorrín JV, Rubiales D, Dumas-Gaudot E, Recobert G, Maldonado A, Castillejo MA, Curto M (2006) Proteomics: a promising approach to study biotic interaction in legumes. A review. Euphytica 147:37–47. https://doi.org/10.1007/s10681-006-3061-1

Kejzar A, Gobec S, Plemenitas A, Lenassi M (2013) Melanin is crucial for growth of the black yeast Hortaea werneckii in its natural hypersaline environment. Fungal Biol 117:368–379. https://doi.org/10.1016/j.funbio.2013.03.006

Kobayashi T, Vieira WD, Potterf B, Sakai C, Imokawa G, Hearing VJ (1995) Modulation of melanogenic protein expression during the switch from eu-to pheomelanogenesis. J Cell Sci 6:2301–2309

Kollias N, Baqer AH (1988) The role of human melanin in providing photoprotection from solar mid-ultraviolet radiation (280–320 nm). J Soc Cosmet Chem 39:347–354

Koh J, Chen S, Zhu N, Yu F, Soltis PS, Soltis DE (2012) Comparative proteomics of the recently and recurrently formed natural allopolyploid *Tragopogon mirus* (Asteraceae) and its parents. New Phytol 196:292–305. https://doi.org/10.1111/j.1469-8137.2012.04251.x

Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG (2003) Phylogenomic analysis of type Ipolyketide synthase genes in pathogenic and saprobic ascomycetes. PNAS 26:15670–15675. https://doi.org/10.1073/pnas.2532165100

Kroll K, Pähtz V, Kniemeyer O (2014) Elucidating the fungal stress response by proteomics. J Proteome 97:151–163. https://doi.org/10.1016/j.jprot.2013.06.001

Kunamneni A, Ballesteros A, Plou FJ, Alcalde M (2007) Fungal laccase-a versatile enzyme for biotechnological applications. CRMICR 1:233–245. https://doi.org/10.1007/978-3-030-10480-1_13

Kwon MJ, Schütze T, Spohner S, Haefner S, Meyer V (2019) Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi. Fungal Biol Biotechnol 6:15. https://doi.org/10.1186/s40694-019-0079-4

Lai Y, Liu K, Zhang X, Zhang X, Li K, Wang N, Shu C, Wu Y, Wang C, Bushley KE, Xiang M, Liu X (2014) Comparative genomics and transcriptomics analyses reveal divergent lifestyle features of nematode endoparasitic fungus *Hirsutella minnesotensis*. Genome Biol Evol 11:3077–3093. https://doi.org/10.1093/gbe/evu241

Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 2:143–158. https://doi.org/10.1016/S1087-1845(02)00526-1

Larsen M (2006) Biological control of nematode parasites in sheep. J Anim Sci 13:133–139. https://doi.org/10.2527/2006.8413_supplE133x

Lee D, Jang E, Lee M, Kim S, Lee Y, Lee K, Bahn Y (2019) Unraveling melanin biosynthesis and signaling networks in Cryptococcus neoformans. M Bio 5:2267–2219. https://doi.org/10.1128/mBio.02267-19

Levasseur A, Saloheimo M, Navarro D, Andberg M, Pontarotti P, Kruus K, Record E (2010) Exploring laccase-like multicopper oxidase genes from the ascomycete Trichoderma reesei: a functional, phylogenetic and evolutionary study. BMC Biochem 11:1–10. https://doi.org/10.1186/1471-2091-11-32

Li J, Zou C, Xu J, Ji X, Niu X, Yang J, Huang X, Zhang KQ (2015) Molecular mechanisms of nematode- nematophagous microbe interactions, basis for biological control of plant-parasitic nematodes. Annu Rev Phytopathol 53:67–95. https://doi.org/10.1146/annurev-phyto-080614-120336

Liu XZ, Zhang KQ (1994) Nematode-trapping species of Monacrosporium with special reference to two new species. Mycol Res 8:862–868. https://doi.org/10.1016/S0953-7562(09)80255-4

Liu K, Zhang W, Lai Y, Xiang M, Wang X, Zhang X, Liu X (2014) *Drechslerella stenobrocha* genome illustrates the mechanism of constricting rings and the origin of nematode predation in fungi. BMC Genomics 15:2–14. https://doi.org/10.1186/1471-2164-15-114

Lopes JR (2013) Avaliação in vitro da melatonina como agente terapêutico em tumores mamários estrógeno-dependentes ou não. Dissertation, Universidade Estadual Paulista Julio de Mesquita Filho

Magarelli M, Passamonti P, Renieri C (2010) Purification, characterization and analysis of sepia melanina from commercial sepia ink (sepia Officinalis). Ces Med Vet Zootec 2:18–29

Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim SK (2013) Isolation and characterization of biologically active melanin from Actinoalloteichus sp. MA-32. Int J Biol Macromol 58:263–274. https://doi.org/10.1016/j.ijbiomac.2013.04.041

Mason HS (1955) Comparative biochemistry of the phenolase complex. Adv Enzymol Relat Areas Mol Biol 16:105–184. https://doi.org/10.1002/9780470122617.ch3

Meunier J, Figueiredo Pinto S, Burri R, Roulin A (2011) Eumelanin-based coloration and fitness parameters in birds: a meta-analysis. Behav Ecol Sociobiol 65:559–567. https://doi.org/10.1007/s00265-010-1092-z

Meerupati T, Andersson KM, Friman E, Kumar D, Tunlid A, Ahrén D (2013) Genomic mechanisms accounting for the adaptation to parasitism in nematode-trapping fungi. PLoS Genet 11:1–20. https://doi.org/10.1371/journal.pgen.1003909

Meredith P, Sarna T (2006) The physical and chemical properties of eumelanin. Pigment Cell Res 6:572–594. https://doi.org/10.1111/j.1600-0749.2006.00345.x

Miot LDB, Miot HA, Silva MG, Marques MEA (2009) Physiopathology of melasma. An Bras Dermatol 6:623–635. https://doi.org/10.1590/S0365-05962009000600008

Moses DN, Mattoni MA, Slack NL, Waite JH, Zok FW (2006) Role of melanin in mechanical properties of *Glycera jaws*. Acta Biomater 5:521–530. https://doi.org/10.1016/j.actbio.2006.05.002

Muggia L, Ametrano CG, Sterflinger K, Tesei D (2020) An overview of genomics, Phylogenomics and proteomics approaches in Ascomycota. Life 10:1–77. https://doi.org/10.3390/life10120356

Nagai M, Kawata M, Watanabe H, Ogawa M, Saito K, Takesawa T, Katsuhiro K, Sato T (2003) Important role of fungal intracellular laccase for melanin synthesis: purification and characterization of an intracellular laccase from Lentinula edodes fruit bodies. Microbiology 9:2455–2462. https://doi.org/10.1099/mic.0.26414-0

Nappi AJ, Sugumaran M (1993) Some biochemical aspects of Eumelanin formation in insect immunity. Insect Immunity 48:131–148. https://doi.org/10.1007/978-94-011-1618-3_10

Navarre C, Degand H, Bennett KL, Crawford JS, Mørtz E, Boutry M (2002) Subproteomics: identification of plasma membrane proteins from the yeast *Saccharomyces cerevisiae*. Proteomics 2:1706–1714. https://doi.org/10.1002/1615-9861(200212)2:12<1706::AID-PROT1706>3.0.CO;2-K

Nicolaus RA (1968) Melanins. Herman, Paris, p 311

Nicolaus BJR (2005) A critical review of the function of neuromelanin and an attempt to provide a unified theory. Med Hypotheses 4:791–796. https://doi.org/10.1016/j.mehy.2005.04.011

Nordbringhertz B, Mattiasson B (1979) Action of a nematode-trapping fungus shows lectin-mediated host-microorganism interaction. Nature 281:477–479. https://doi.org/10.1038/281477a0

Nordbring-Hertz B, Jansson HB, Tunlid (2006) Nematophagous fungi. eLS:1–11. https://doi.org/10.1038/npg.els.0004293

Nordlund JJ, Abdel-Malek ZA, Boissy ER, Rheins LA (1989) Pigment cell biology: An historical review. J Invest Dermatol 4:53S–60S. https://doi.org/10.1111/1523-1747.ep13074988

Nosanchuk JD, Casadevall A (2006) Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Antimicrob Agents Chemother 11:3519–3528. https://doi.org/10.1128/AAC.00545-06

Oliveira DM, Ferreira Júnior DA, Prado EG, Soares MV, Faria MLST (2009) Proteomas. Praxis 2:55–57

Palumbo A, Yeh J (1994) In situ localization of apoptosis in the rat ovary during follicular atresia. Biol Reprod 5:888–895. https://doi.org/10.1095/biolreprod51.5.888

Pastre R, Marinho AM, Rodrigues-Filho E, Souza AQ, Pereira JO (2007) Diversidade de policetídeos produzidos por espécies de Penicillium isoladas de Melia azedarach e Murraya paniculata. Quim Nova 8:1867–1871. https://doi.org/10.1590/S0100-40422007000800013

Pavan ME, López NI, Pettinari MJ (2020) Melanin biosynthesis in bacteria, regulation and production perspectives. Appl Microbiol Biotechnol 104:1357–1370. https://doi.org/10.1007/s00253-019-10245-y

Plonka PM, Grabacka M (2006) Melanin synthesis in microorganisms-biotechnological and medical aspects. Acta Biochim Pol 3:429–443

Polacheck I, Kwon-Chung KJ (1998) Melanogenesis in Cryptococcus neoformans. Microbiology 4:1037–1041. https://doi.org/10.1099/00221287-134-4-1037

Pralea IE, Moldovan RC, Petrache AM, Ilieș M, Hegheș SC, Ielciu I, Nicoară R, Moldovan M, Ene M, Radu M, Uifălean A, Iuga CA (2019) From extraction to advanced analytical methods: the challenges of melanin analysis. Int J Mol Sci 20:31–37. https://doi.org/10.3390/ijms20163943

Raposo G, Marks MS (2007) Melanosomes-dark organelles enlighten endosomal membrane transport. Nat. Rev Mol Cell Biol 10:786–797. https://doi.org/10.1038/nrm2258

Robbins CR (2012) Chemical and physical behavior of human hair. Springer, Heidelberg, pp 105–176

Rodrigues ML, Nimrichter L, Oliveira DL, Frases S, Miranda K, Zaragoza O, Alvarez M, Nakouzi A, Feldmesser M, Casadevall A (2007) Vesicular polysaccharide export in Cryptococcus neoformans is a eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell 6:48–59. https://doi.org/10.1128/EC.00318-06

Romero-Martinez R, Wheeler M, Guerrero-Plata A, Rico G, Torres-Guerrero H (2000) Biosynthesis and functions of melanin in *Sporothrix schenckii*. Infect Immun 6:3696–3703. https://doi.org/10.1128/iai.68.6.3696-3703.2000

Rosa LH, Vieira MDLA, Santiago IF, Rosa CA (2010) Endophytic fungi community associated with the dicotyledonous plant *Colobanthus quitensis* (Kunth) Bartl (Caryophyllaceae) in Antarctica. FEMS Microbiol Ecol 1:178–189. https://doi.org/10.1111/j.1574-6941.2010.00872.x

Roulin A, Mafli A, Wakamatsu K (2013) Reptiles Produce Pheomelanin: Evidence in the Eastern Hermann's Tortoise (*Eurotestudo boettgeri*). J Herpetol 2:258–261. https://doi.org/10.1670/12-028

Rubner A (1996) Revision of predacious hyphomycetes in the *Dactylella-Monacrosporium* complex. Stud Mycol 39:1–134

Saha SK, Saikot FK, Rahman S, Jamal MAHM, Rahman SMK, Islam R, Kim KH (2019) Programmable molecular scissors: applications of a new tool for genome editing in biotech. Mol Ther Nucleic Acids 14:212–238. https://doi.org/10.1016/j.omtn.2018.11.016

Sarna T, Plonka PM (2005) Biophysical studies of melanin. In: Eaton SR, Eaton GR, Berliner LJ (eds) Biomedical EPR, Part A: free radicals, metals, medicine, and physiology. Biological Magnetic Resonance, Springer, Boston, pp. 125–146

Seekles SJ, Teunisse PPP, Punt M, van den Brule T, Dijksterhuis J, Houbraken J, Wösten HAB, Ram AFJ (2021) Preservation stress resistance of melanin deficient conidia from Paecilomyces variotii and Penicillium roqueforti mutants generated via CRISPR/Cas9 genome editing. Fungal Biol Biotechnol 8:4. https://doi.org/10.1186/s40694-021-00111-w

Seiberg M (2001) Keratinocyte–melanocyte interactions during melanosome transfer. Pigment Cell Res 4:236–242. https://doi.org/10.1034/j.1600-0749.2001.140402.x

Selinheimo E, Nieidhin D, Steffensen C, Nielsen J, Lomascolo A, Halaouli S, Record E, O'beirne D, Buchert J, Kruus K (2007) Comparison of the characteristics of fungal and plant tyrosinases. J Biotechnol 4:471–480. https://doi.org/10.1016/j.jbiotec.2007.05.018

Shalaby ASG, Ragab TI, Helal MM, Esawy MA (2019) Optimization of Bacillus licheniformis MAL tyrosinase: in vitro anticancer activity for brown and black eumelanin. Heliyon 5:1–8. https://doi.org/10.1016/j.heliyon.2019.e01657

Shen B (2003) Polyketide biosynthesis beyond the type I, II and III polyketide synthase paradigms. Chem Biol Current Opinion 2:285–295. https://doi.org/10.1016/S1367-5931(03)00020-6

Shindler M, Sawada H, Tietjen K, Hamada T, Hagiwara H, Banaba S (2019) Melanin synthesis in the cell wall. In: Jeschke P, Witschel M, Krämer W, Schirmer U (eds) Modern Crop Protection Compounds. 9 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim pp. 879–909. https://doi.org/10.1002/9783527699261.ch22

Simon JD, Peles D, Wakamatsu K, Ito S (2009) Current challenges in understanding melanogenesis: bridging chemistry, biological control, morphology, and function. Pigment Cell Melanoma Res 5:563–579. https://doi.org/10.1111/j.1755-148X.2009.00610.x

Slominski A, Tobin DJ, Shibahara S, Wortsman J (2004) Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev 4:1155–1228. https://doi.org/10.1152/physrev.00044.2003

Smythies J (1996) On the function of neuromelanin. Proc R Soc Lond 1369:487–489

Solano F (2014) Melanins: skin pigments and much more types, structural models, biological functions, and formation routes. New J Sci 2014:1–28. https://doi.org/10.1155/2014/498276

Steenbergen JN, Casadevall A (2003) The origin and maintenance of virulence for the human pathogenic fungus *Cryptococcus neoformans*. Microbes Infect 7:667–675. https://doi.org/10.1016/S1286-4579(03)00092-3

Sulzer D, Rayport S (2000) Dale's principle and glutamate corelease from ventral midbrain dopamine neurons. J Amino Acids 1:45–52. https://doi.org/10.1007/s007260070032

Suwannarach N, Kumla J, Watanabe B, Matsui K, Lumyong S (2019) Characterization of melanin and optimal conditions for pigment production by an endophytic fungus, *Spissiomyces endophytica* SDBR-CMU319. PLoS One 9:e0222187. https://doi.org/10.1371/journal.pone.0222187

Takano Y, Kubo Y, Kawamura C, Tsuge T, Furusawa I (1997) The Alternaria alternata melanin biosynthesis gene restores Appressorial Melanization and penetration of cellulose membranes in the melanin-deficient albino mutant of Colletotrichum lagenarium. Fungal Genet Biol 1:131–140

Tavela AO, Araújo JV, Braga FR, Silveira WF, Silva VHD, Júnior MC, Borges LA, Araujo JM, Benjamin LA, Carvalho GR, Paula AT (2013) Coadministration of sodium alginate pellets containing the fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* on cyathostomin infective larvae after passing through the gastrointestinal tract of horses. Res Vet Sci 94:568–572. https://doi.org/10.1016/j.rvsc.2012.11.011

Tedersoo L, Sánchez-Ramírez S, Koljalg U, Bahram M, Döring M, Schigel D, May T, Ryberg M, Abarenkov K (2018) High-level classification of the fungi and a tool for evolutionary ecological analyses. Fungal Divers 1:135–159. https://doi.org/10.1007/s13225-018-0401-0

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 1:19–26. https://doi.org/10.1099/13500872-140-1-19

Tian S, Garcia-Rivera J, Yan B, Casadevall A, Stark RE (2003) Unlocking the molecular structure of fungal melanin using 13C biosynthetic labeling and solid-state NMR. Biochemistry 27:8105–8109. https://doi.org/10.1021/bi0341859

Tief K, Schmidt A, Beermann F (1998) New evidence for presence of tyrosinase in substantia nigra, forebrain and midbrain. Mol Brain Res 1-2:307–310. https://doi.org/10.1016/S0169-328X(97)00301-X

Tran-L AN, Ribera J, Francis WMRS, Brunelli M, Fortunato G (2020) Fungal melanin-based electrospun membranes for heavy metal detoxification of water. SM&T 23:e00146. https://doi.org/10.1016/j.susmat.2019.e00146

Treseder KK, Lennon JT (2015) Fungal traits that drive ecosystem dynamics on land. Microbiol Mol Biol Rev 2:243–262. https://doi.org/10.1128/MMBR.00001-15

Tunlid A, Jansson H, Nordbring-Hertz B (1992) Fungal attachment to nematodes. Mycol Res 6:401–412. https://doi.org/10.1016/S0953-7562(09)81082-4

Tunlid A, Rosen S, Ek BO, Rask L (1994) Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora*. Microbiology 7:1687–1695. https://doi.org/10.1099/13500872-140-7-1687

Turick CE, Tisa LS, Caccavo F (2002) Melanin production and use as a soluble electron shuttle for Fe(III) oxide reduction and as a terminal electron acceptor by *Shewanella algae* BrY. Appl Environ Microbiol 5:2436–2444. https://doi.org/10.1128/AEM.68.5.2436-2444.2002

Van De Sande WW, De Kat J, Coppens J, Ahmed AO, Fahal A, Verbrugh H, Van Belkum A (2007) Melanin biosynthesis in *Madurella mycetomatis* and its effect on susceptibility to itraconazole and ketoconazole. Microbes Infect 9:1114–1123. https://doi.org/10.1016/j.micinf.2007.05.015

Van den Bossche P, Gijselaers WH, Segers M, Kirschner PA (2006) Social and cognitive factors driving teamwork in collaborative learning environments: team learning beliefs and behaviors. Small Group Res 5:490–521. https://doi.org/10.1177/1046496406292938

Van Gelder CW, Flurkey WH, Wichers HJ (1997) Sequence and structural features of plant and fungal tyrosinases. Phytochemistry 7:1309–1323. https://doi.org/10.1016/S0031-9422(97)00186-6

Wakamatsu K, Fujikawa K, Zucca FA, Zecca L, Ito S (2003) The structure of neuromelanin as studied by chemical degradative methods. J Neurochem 4:1015–1023. https://doi.org/10.1046/j.1471-4159.2003.01917.x

Walker CA, Gómez BL, Mora-Montes H.M, Mackenzie KS, Munro CA, Brown AJ, Gow NAR, Christopher C.C, Odds FC (2010) Melanin externalization in Candida albicans depends on cell wall chitin structures. Eukaryot Cell 9:1329–1342. https://doi.org/10.1128/EC.00051-10

Wang RB, Yang JK, Lin C, Zhang Y, Zhang KQ (2006) Purification and characterization of an extracellular serine peptidases from the nematode-trapping fungus Dactylella shizishanna. Lett Appl Microbiol 6:589–594. https://doi.org/10.1111/j.1472-765X.2006.01908.x

Wang B, Xiao YL, Wenping W, Xingzhong L, Shindong L (2009) Purification, characterization, and gene cloning of an alkaline serine protease from a highly virulent strain of the nematode-endoparasitic fungus *Hirsutella rhossiliensis*. Microbiol Res 6:665–673. https://doi.org/10.1016/j.micres.2009.01.003

Watson BS, Asirvatham VS, Wang L, Summer LW (2003) Mapping the proteome of barrel medic (*Medicago truncatula*). Plant Physiol 131:1104–1123. https://doi.org/10.1104/pp.102.019034

Wheeler MH (1983) Comparisons of fungal melanin biosynthesis in ascomycetous, imperfect and basidiomycetous fungi. Trans Brit Mycol Soc 1:29–36. https://doi.org/10.1016/S0007-1536(83)802009

Williamson PR, Wakamatsu K, Ito S (1998) Melanin biosynthesis in Cryptococcus neoformans. J Bacteriol 6:1570–1572. https://doi.org/10.1128/JB.180.6.1570-1572.1998

Wogelius RA, Manning PL, Barden HE, Edwards NP, Webb SM, Sellers WI, Taylor KG, Larson PL, Dodson P, You H, Da-Qing L, Bergmann U (2011) Trace metals as biomarkers for eumelanin-pigment in the fossil record. Science 333:1622–1626. https://doi.org/10.1126/science.1205748

Wu X, Hammer JA (2014) Melanosome transfer: it is best to give and receive. Curr Opin Cell Biol 29:1–7. https://doi.org/10.1016/j.ceb.2014.02.003

Xu CG, Yang YB, Zhou YH, Hao MQ, Ren YZ, Wang XT, Chen JQ, Muhammad I, Wang S, Liu D, Li XB, Li YH (2017) Comparative proteomic analysis provides insight into the key proteins as possible targets involved in aspirin inhibiting biofilm formation of *Staphylococcus xylosus*. Front Pharmacol 8:1–12. https://doi.org/10.3389/fphar.2017.00543

Yan L, Fan G, Deng M, Zhao Z, Dong Y, Li Y (2017) Comparative proteomic analysis of autotetraploid and diploid *Paulownia tomentosa* reveals proteins associated with superior photosynthetic characteristics and stress adaptability in autotetraploid *Paulownia*. Physiol Mol Biol Plants 23:605–617. https://doi.org/10.1007/s12298-017-0447-6

Yang J, Huang X, Tian B, Wang M, Niu Q, Zhang K (2005) Isolation and characterization of a serine protease from the nematophagous fungus, Lecanicillium psalliotae, displaying nematicidal activity. Biotechnol Lett 27:1123–1128. https://doi.org/10.1007/s10529-005-8461-0

Yang SF, Li XY, Yu HQ (2008) Formation and characterisation of fungal and bacterial granules under different feeding alkalinity and pH conditions. Process Biochem 43:8–14. https://doi.org/10.1016/j.procbio.2007.10.008

Yang J, Wang L, Ji X, Feng Y, Li X, Zou C, Xu J, Ren Y, Mi Q, Wu J, Liu S, Liu Y, Huang X, Wang H, Niu X, Li J, Liang L, Luo Y, Ji K, Zhou W, Yu Z, Li G, Liu Y, Li L, Qiao M, Feng L, Zhang KQ (2011) Genomic and proteomic analyses of the fungus *Arthrobotrys oligospora* provide insights into nematode-trap formation. PLoS Pathog 7:e1002179. https://doi.org/10.1371/journal.ppat.1002179

Yang CT, Ulzurrun GVD, Gonçalves AP, Lin HC, Chang CW, Huang TY, Chen SA, Lai CK, Tsai PJ, Schroeder FC, Stajich JE, Hsueh YP (2020) Natural diversity in the predatory behavior facilitates the establishment of a robust model strain for nematode-trapping fungi. PNAS 12:6762–6770. https://doi.org/10.1073/pnas.1919726117

Ye Y, Wang H, Chu JH, Chou GX, Chen SB, Mo H, Fong WF, Yu ZL (2011) Atractylenolide II induces G1 cell-cycle arrest and apoptosis in B16 melanoma cells. J Ethnopharmacol 136:279–282. https://doi.org/10.1016/j.jep.2011.04.020

Youssar L, Wernet V, Hensel N, Yu X, Hildebrand HG, Schreckenberger B, Kriegler M, Hetzer B, Frankino P, Dillin A, Fischer R (2019) Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. PLoS Genet 3:1–31. https://doi.org/10.1371/journal.pgen.1008029

Zalar P, Novak M, De Hoog GS, Gunde-Cimerman N (2011) Dishwashers-a man-made ecological niche accommodating human opportunistic fungal pathogens. Fungal Biol 115(10):997–1007. https://doi.org/10.1016/j.funbio.2011.04.007

Zecca L, Tampellini D, Gerlach M, Riederer P, Fariello RG, Sulzer D (2001) Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour. Mol Pathol 54:414

Zecca L, Zucca FA, Wilms H, Sulzer D (2003) Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. Trends Neurosci 26:578–580. https://doi.org/10.1016/j.tins.2003.08.009

Zecca L, Casella L, Albertini A, Bellei C, Zucca FA, Engelen M, Sarna T (2008) Neuromelanin can protect against iron-mediated oxidative damage in system modeling iron overload of brain aging and Parkinson's disease. J Neurochem 106:1866–1875. https://doi.org/10.1111/j.14714159.2008.05541.x

Zhang Y, Qiao M, Xu J, Cao Y, Zhang KQ, Yu ZF (2013) Genetic diversity and recombination in natural populations of the nematode-trapping fungus *Arthrobotrys oligospora* from China. Ecol Evol 3:312–325. https://doi.org/10.1002/ece3.450

Zhang P, Zhou S, Wang G, An Z, Liu X, Li K, Yin WB (2019) Two transcription factors cooperatively regulate DHN melanin biosynthesis and development in *Pestalotiopsis fici*. Mol Microbiol 112:649–666. https://doi.org/10.1111/mmi.14281

Zhang Y, Yang G, Fang M, Deng C, Zhang KQ, Yu Z, Xu J (2020) Comparative analyses of mitochondrial genomes provide evolutionary insights into nematode-trapping fungi. Front Microbiol 11:1–13. https://doi.org/10.3389/fmicb.2020.00617

Zhdanova NN, Zakharchenko VA, Vember VV, Nakonechnaya LT (2000) Fungi from Chernobyl mycobiota of the inner regions of the containment structures of the damaged nuclear reactor. Mycol Res 104:1421–1426. https://doi.org/10.1017/S0953756200002756

Zopf WF (1888) Zur Kenntniss der Infections-Krankheiten niederer Thiere und Pflanzen. Nova Acta Lep Carol 52:314–376

Zucca FA, Basso E, Cupaioli FA, Ferrari E, Sulzer D, Casella L, Zecca L (2014) Neuromelanin of the human substantia nigra: an update. Neurotox Res 25:13–23. https://doi.org/10.1007/s12640-013-9435-y