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*Review Article*

# Recent era of research: Microbial melanin in life sciences

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## Abstract

This review focuses on melanin pigment, its types, physical and chemical properties, melanogenesis, various microbial sources for melanin synthesis, current trends in melanin characterization, applications of melanin pigments in various fields, and their future approaches. It is also found that different bacterial and fungal melanins possess different properties and thus these sources are associated with different uses and applications. Oxidative polymerization of indolic and phenolic molecules leads to the biosynthesis of melanin. It occurs through two distinct pathways, principally through L-DOPA, which leads to the generation of various types of melanin such as eumelanin and pheomelanin. Various types of enzymes are also involved in microbial melanogenesis. Researchers are working to create melanin nanoparticles because melanin holds some unique properties and also has many possible medical or therapeutic uses. This review helps develop applications of melanin and its research aspects.

**Keywords**: melanin, melanogenesis, eumelanin, pheomelanin, oxidative polymerization

## 1. Introduction

Melanin, a naturally occurring pigment, is widely found in both microorganisms (bacteria and fungi) and higher species (plants, animals), where it serves a variety of essential and multipurpose functions. Melanin is produced when indolic or phenolic molecules undergo oxidative polymerization (Pavan, López, & Pettinari, 2020). Physically, melanin is amorphous, primarily appearing dark brown to black, while there have also occasionally been reports of reddish and yellowish hues. Human skin color is determined by melanin (Huang & Chang, 2012; Langfelder, Streibel, Jahn, Haase, & Brakhage, 2003). Chemically, melanin is highly thermostable; some melanin types can withstand thermolysis up to 600 °C. They also have a large molecular weight and a negatively charged hydrophobic character, which combine to produce complex polymers that withstand concentrated acids, light, and reducers (El-Naggar & El-Ewasy, 2017; Pavan *et al*., 2020). However, on the other hand, they are soluble in both phenols and alkalis, and vulnerable to oxidizing agents (bleaching processes) (Jacobson, 2000). Because of its combined ability to perform multiple functions, melanin has potential uses in a wide range of biological, environmental, and technological domains. These include liver protection, anti-inflammatory, antitumor, scavengers of free radicals, antimicrobial, neuroprotector, nanoparticle synthesizer, remediator of radioactive residuals, and digestive system protector (Apte, Girme, Bankar, RaviKumar, & Zinjarde, 2013; Sava, Hung, Blagodarsky, Hong, & Huang, 2003). Melanogenesis is hence an essential process for living things.

Although melanin shields pigmented cells and tissues from potentially harmful substances (drugs and chemicals), this process may have a downside because prolonged exposure to chemicals can result in high levels of accumulated chemicals, which may eventually cause degeneration in the cells that contain melanin and secondary lesions in surrounding tissues (Al Mofleh *et al*., 2008).

According to estimates, cancer is the most prevalent disease causing about one in six deaths worldwide (GBD 2016 Causes of Death Collaborators, 2017). There have been numerous attempts to enhance the prognosis for cancer, and the recent achievements of immune checkpoint inhibitors have undoubtedly raised expectations. These compounds, however, have only shown promise in a small number of

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patients. Although therapeutic vaccines are an appealing approach for cancer patients, they have not yet demonstrated a substantial level of clinical success. Provenge®, a vaccination recommended for patients with prostate cancer, is the only one that has received approval thus far (Cheever & Higano, 2011). Anti-tumor efficacy has been proven for a significant variety of vaccine formulations in different murine models as well as in early phase studies, but their efficacy has not yet been verified in phase III trials. The immunosuppressive tumor microenvironment and challenges in inducing a robust tumor-specific immune response are likely to be the main causes of this lack of effectiveness. Accordingly, adjuvant optimization in vaccine formulations is critical to enhance the immune response via multiple mechanisms, namely: (1) transporting the antigen into lymph nodes and extending its availability there; (2) stimulating innate immunity; and (3) counteracting tumor-associated immune suppression (Cuzzubbo *et al*., 2021).

The ability of nanoparticle systems to act as carriers of various antigens to the lymph nodes, thereby particularly targeting antigen-presenting cells, has garnered significant interest in the field of cancer vaccines. Furthermore, nanoparticles have certain immunostimulatory characteristics. In that field, bioinspired nanomaterials are particularly interesting due to their biocompatibility. In many cancer vaccine formulations, melanin-based nanoparticles demonstrate immunostimulatory properties and effectively localize into draining lymphoid tissues. Furthermore, melanin can be used as a photosensitizer in photothermal therapy due to its distinct photophysical and photochemical characteristics. But the word "melanin" refers to a broad range of compounds, both synthetic and natural, that have distinct biological structures and may not share the same immunological characteristics.

## 2. Types of Melanin

The quantity and bonding patterns of the smaller molecules that make up the melanin polymer have a significant impact on the kind of melanin. Based on color and structural classifications, the four primary types of melanin – eumelanin, pheomelanin, allomelanin, and occasionally formed others have been discovered.

### 2.1 Eumelanin

The majority of eumelanin pigments, which range from black to dark brown, are produced by some bacteria and fungi as well as by human skin and hair (Tarangini, & Mishra, 2014). Tyrosine and/or phenylalanine undergo oxidative polymerization to become L-3,4-dihydroxyphenylalanine (L-DOPA), which is then converted into dopachrome and ultimately melanin to produce eumelanin (Belozerskaya, Gessler, & Aver'yanov, 2017; Roy & Rhim, 2021).

### 2.2 Pheomelanin

Originally created similarly to eumelanin, pheomelanin is a red or yellow pigment found mostly in red human hair. However, due to the integration of cysteine into the polymer (cysteinylation) of L-DOPA, pheomelanin contains sulphur (Singh *et al*., 2021).

### 2.3 Allomelanin

Numerous fungi and plants contain allomelanin, a heterogeneous pigment that belongs to a category of heterogeneous polymers without nitrogen. They come from a variety of sources such as catechols, homogentisic acid, dihydrofolate, and others (El-Naggar, 2021; El-Naggar & El-Ewasy, 2017). This class contains water-soluble pyomelanin produced by the accumulation and polymerization of homogentisic acid, a byproduct of the tyrosine breakdown pathway, and melanin, which is derived from 1,8-dihydroxy naphthalene (DHN) molecules (Vasanthakumar, DeAraujo, Mazurek, Schilling, & Mitchell, 2015).

### 2.4 Other types

Occasionally, a divergence from the previous melanin types results in the creation of other, less common melanin. Because trichochrome pigments (formerly called trichosiderins) have a lower molecular weight than the initial melanin molecules, they are produced via the same metabolic pathway as eumelanin and pheomelanin (Jangir, Gadre, & Argade, 2015). Dark, insoluble pigments called neuromelanin are produced in a specific area of the brain by catecholaminergic neurons. Pheomelanin and eumelanin should be mixed together in the core of neutremelanin granules (Bush *et al*., 2006). Neuromelanin, which is found in smaller amounts in the brains of several other non-human primates but that is absent entirely from the brains of many lower animals, is most abundant in humans (Fedorow *et al.*, 2005). It has been shown that human neuromelanin can bind potentially dangerous substances like iron and other transition metals. It might therefore play an important role in Parkinson's disease, neurodegeneration, and apoptosis (Double, 2006). Comparing patients with Parkinson's disease to those of the same age without the illness, those with Parkinson's disease had half as much neuromelanin in the substantia nigra. Age-related increases in neuromelanin concentration suggest a role for the substance in neuroprotection (Double, 2006; Fedorow *et al*., 2005).

## 3. Microbial Sources of Melanin

From an environmental and quality perspective, synthesized melanin polymers cannot be compared to natural melanin. In particular, if the situations suggested in the current review are taken into account, the latter is more economical and safe than the artificial one. On the other hand, the use of microorganisms as a substitute for chemical melanin production has grown in favor recently. For a variety of reasons, numerous attempts have been made to develop microbial strains that are capable of synthesizing a large amount of melanin pigment. The melanin derived by bacteria is superior to synthetic, animal, and plant-based melanin in many ways.

After testing 102 fungal isolates in an intensive search for more affordable sources of melanin synthesis than the conventional sources, *Amorphotheca resinae* was identified as the most promising melanin producer on peptone yeast extract glucose broth. The melanin was produced by *Amorphotheca resinae* very quickly; in just 14 days, it reached 4.5 g/L. According to assays for free radical scavenging, the

structural characterization of the purified fungal melanin shows that it is similar to eumelanin and has a high antioxidant activity, indicating a promising fungal candidate for scalable production of industrially applicable melanin (Oh *et al*., 2020). Some groups of microorganisms which produce melanin are shown in Tables 1, 2, 3 and 4.

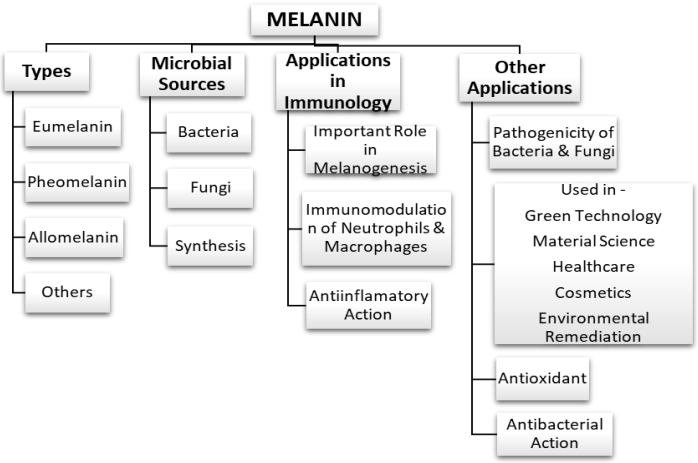


Figure 1. Flow chart of melanin types and resources

Table 1. Actinomycetes

| Sr. No. | Actinomycetes | Objective |
|---|---|---|
| 1. | *Sterptomyces spp.* | Separation, recognition, and evaluation of *Streptomyces* melanin production |
| 2. | *Nocardiopsis dassonvillei* | It is not common to extract bioactive melanin pigment from marine actinobacteria. |
| 3. | *Streptomyces cyaneus* | Using response surface methods, medium conditions are optimized for *Streptomyces cyaneus* to produce melanin, and gamma radiation is used to synthesise copper oxide nanoparticles. |

Table 2. Yeast

| Sr. No. | Yeast | Objective |
|---|---|---|
| 1. | *Cryptococcus neoformans* | The function of melanin in the virulence mechanism of *Cryptococcus neoformans* |
| 2. | *Yarrowia lipolytica* | *Yarrowia lipolytica* yeast produces a nontoxic pyomelanin pigment that is characterized. |
| 3. | *Hortaea werneckii* | For *Hortaea werneckii* to grow in a hypersaline environment, melanin is essential. |

Table 3. Bacteria

| Sr. No. | Bacteria | Objective |
|---|---|---|
| 1. | *Bacillus cereus* | Detection of melanin generated by a *Bacillus cereus* wild-type strain |
| 2. | *Bacillus thuringiensis* | The production of melanin pigment in high temperatures |
| 3. | *Burkholderia cepacia* | Diminished respiratory burst activity of monocytes |
| **4.** | *Klebsiella* SP. GSK | Melanin pigment purification and physicochemical characterisation |
| **5**. | *Pseudomonas stutzeri* | *Pseudomonas stutzeri*, isolated from the red seaweed *Hypnea musciformis*, produces melanin**.** |
| **6**. | *Pseudomonas maltophilia* *Aeromonas media* | Novel strain generating elevated DOPA-melanin levels and evaluation of the melanin's photoprotective function |
| **7**. | *Stenotrophomonas maltophilia* | Production of melanin pigment and isolation of *Stenotrophomonas maltophilia* from clinical samples |

Table 4. Fungi

| Sr. No. | Fungi | Objective |
|---|---|---|
| 1. | *Amorphotheca resinae* | Synthesis and evaluation of melanin pigments from *Amorphotheca resinae* |
| 2. | *Aspergillus bridgeri* | Melanin's physicochemical properties and antioxidant capacity |
| 3. | *Aspergillus fumigatus* | Pyomelanin synthesis through the tyrosine degradation route |

### 3.1 Bacterial melanin

Since it was discovered that bacteria can synthesize black pigments, a long time ago, a number of bacteria have been found to manufacture different types of melanin via specialized pathways or by exploiting enzymatic imbalances in altered metabolic channels (El-Naggar, 2021). While transcriptional and metabolic regulation play a role in the regulation of the melanin synthesis process in bacteria, numerous biosynthesis routes are still mostly unknown (Pavan *et al*., 2020). Nonetheless, both Gram-positive and Gram-negative bacteria, including *Ralstonia solanacearum* (Hernández-Romero, Solano, & Sanchez-Amat, 2005), *Bacillus licheniformis* (Ragab, Helal, & Esawy, 2019), and *Streptomyces griseus* (Funa, Ohnishi, Fujii, Shibuya, Ebizuka, & Horinouchi, 1999), are capable of synthesizing melanin. Melanin, in particular, is produced by a variety of *Streptomyces* species and is considered a helpful criterion for taxonomy research (Tarangini, & Mishra, 2014). Certain bacteria's physiological growth characteristics, including those of thermo-alkaliphilic Streptomyces sp., were well-described, calling for a pH of 9.0, an increase in temperature to 45 ◦C, and 3% sodium chloride (El-Naggar, 2021).

### 3.2 Fungal melanin

It has been claimed that fungi contain every known kind of melanin. Melanin is regarded as a secondary metabolite found in fungi, where it may occur naturally. While melanin is not necessary for the growth and development of a fungus, it can carry out a wide range of biological functions, including increasing the virulence of many fungi that are harmful to people and plants (Belozerskaya *et al*., 2017). The pigment is primarily found in the outermost layer of the majority of melanized fungus as granules, stacked in fibrils, embedded in the cell wall, attached to chitin in the cell wall, or produced extracellularly. *Aspergillus niger*, *Aspergillus nidulans*, *Alternaria alternata*, *Cladosporium carionii*, *Fonsecaea compacta*, *Exophiala jeanselmei*, *Hendersonula toruloidii*, and *Phaeoannellomyces wernickii* are among the fungi that have been documented to exude melanin (Belozerskaya *et al*., 2017).

## 4. Application of Melanin in Immunology

There are several reasons to believe that pigmentation and immunity may be related in some way:

a) By attaching to its receptor, the MC1R (melanocortin 1 receptor), alpha melanocyte-stimulating hormone (α-MSH), an endogenous peptide hormone of the melanocortin family plays a crucial role in melanogenesis. It seems that α-MSH is involved in a variety of processes, such as immunomodulation of neutrophils and macrophages and anti-inflammatory actions (Brzoska, Böhm, Lügering, Loser, & Luger, 2010). In numerous models of inflammatory or ischemia/reperfusion injury as well as bacterial endotoxin-induced inflammation, these anti-inflammatory properties have been thoroughly demonstrated (Lipton & Catania, 1998). Furthermore, α-MSH can stimulate regulatory T-lymphocytes (Treg), which control immunity by focusing on particular antigens (Taylor & Lee, 2011).

b) An important part of invertebrate innate immunity is melanogenesis. The process of melanization, which occurs when melanin is formed surrounding invasive microorganisms, is frequently triggered by insects (Viljakainen, 2015). Microbes are encapsulated in melanin within minutes of infection, and it is believed that the production of free radical byproducts during this capsule's creation helps to destroy the bacteria. Invertebrates' innate immunological defense mechanism against invasive infections includes this process, which does not exist in mammals. In mammals, the situation may even be the reverse, as *Cryptococcus neoformans* creation of melanin actually makes the fungus more virulent by shielding it from the host's phagocytic killing (Casadevall, Rosas, & Nosanchuk, 2000). Furthermore, there was no difference observed in the clinical course of malaria infection between mouse strains that differed just in the gene encoding tyrosinase, an essential enzyme in the manufacture of melanin (Waisberg, Vickers, Yager, Lin, & Pierce, 2012). Ultimately, when lipopolysaccharide stimulates macrophages, synthetic melanin inhibits the generation of cytokines (Mohagheghpour *et al*., 2000)

c) Because of their strategic placement in the epidermis and their dendritic character, melanocytes have been linked to adaptive protection against external infections (Plonka *et al*., 2009). It is true that melanocytes have phagocytic activities. Phagosomes are transferred from the cell surface to the melanosomes, which house a large number of lysosomal enzymes (Diment, Eidelman, Rodriguez, & Orlow, 1995; Le Poole *et al*., 1993). Additional research has demonstrated that melanocytes can function as antigen presenting cells (Gasque & Jaffar-Bandjee, 2015; Le Poole *et al*., 1993). Furthermore, Langerhans cells continuously collect and transfer melanin granules from the epidermis to local lymph nodes in mice with melanocytosis (Hemmi *et al*., 2006). There is a possibility that naturally existing melanin can stimulate an adaptive immune response *in vivo*, for example, by means of antibodies or cytotoxic T-lymphocytes in vivo.

d) In cancer therapy, photothermal therapy (PTT) is particularly interesting. The immune system's activation through immunogenic cell death (ICD) is linked to the direct lethal action of hyperthermia on cancer cells. PTT causes the production of immunostimulant molecules such heat shock proteins and damage-associated molecular patterns (DAMPs), as

well as tumor antigens. This draws and activates a variety of immune cells, including T cells, dendritic cells, and macrophages, into the tumor site. Through this immune activity, PTT helps to create an immune memory that prevents tumor recurrence and boosts the immediate killing effect on cancer cells (Chen *et al*., 2016).

## 5. Future Approaches

Traditional one-variable-at-a-time medium condition optimization techniques are tedious, time-consuming, and produce inconsistent yields because they ignore the interference between the production components under test. Melanin biosynthesis circumstances can now be optimized with the help of newly developed statistical experimental designs, which have overcome the limitations of previous approaches.

The first step in the statistical modelling approach is to simultaneously screen several tested factors for significant factors. The most popular technique for identifying significant factors is the use of Plackett-Burman designs (PBD). The significant factors are chosen for additional optimization tests, while the nonsignificant factors like melanin, for example, that have a very small effect on response values are left out of further experiments. In order to investigate the link between the tested variable and response (melanin production), the response surface methodology (RSM) technique is applied to the significant elements after the screening design and associated results have been considered. Several fixable designs are included in RSM to accommodate a range of experimental parameters, including the quantity and concentration of components examined, the structure of the design space, and the number of trials conducted. The Box-Behnken design and Central Composite Design (CCD) are the two primary popular methods for carrying out such optimization and maximization processes. It was evident that this modelling technique only needed two steps, after which the entire process needed to be validated (Ghoniem, El-Naggar, Saber, El-Hersh, & El-Khateeb, 2020; Singh *et al*., 2021).

Nonetheless, just a few studies mostly conducted in the past ten years have used PBD and RSM for melanin biosynthesis. For instance, PBD was carried out on 17 independent parameters, and three important factors influencing *Streptomyces glaucescens'* melanin synthesis were chosen in order to investigate and maximize their interaction using CCD, resulting in a maximum melanin production of 310.650 µg/1 mL (El-Naggar & El-Ewasy, 2017). Comparably, PBD and CCD techniques were used to investigate the enhancement of melanin biosynthesis by *Auricularia auricula*. In the optimized settings, the melanin yield was 1.08 g/L, compared to 306.52 mg/L in the inadequate conditions, signifying a 3.52-fold increase (Zhang *et al*., 2015)

The two aforementioned examples came to the conclusion that the statistical technique improves melanin biosynthesis by developing a quick and inexpensive fermentation process. This in turn motivates scientists to employ these methods more widely in the coming years.

## 6. Some Unique Applications of Melanin

1) Melanins play a significant role in the pathogenicity of fungi and bacteria (Cordero & Casadevall, 2017; Jacobson, 2000)
2) Melanin acts as a natural "sunscreen" by absorbing UV-visible light across its whole spectrum. This pigment not only blocks UV light but also acts as a potent antioxidant. Melanin also has hydration-dependent semiconductor properties. It is assessed as a component for organic electrical devices (Bothma, de Boor, Divakar, Schwenn, & Meredith, 2013)
3) Microbial melanin's bioavailability, biocompatibility, and biodegradability make it an attractive contender for biomedical applications, including implantable devices (Vahidzadeh, Kalra, & Shankar, 2018).
4) Melanin can also be used to synthesize silver nanostructures in an environmentally friendly way. Melanin-mediated silver nanostructures have broad-spectrum antibacterial activity against food pathogens, with potential applications in the food and healthcare industries (Kiran *et al*., 2018).
5) Most of the recent references have shown endophytic actinomycetal metabolites, where these metabolites were involved in production of microbial nanoparticles with their significances like as antagonistic activity (Chaugule, 2021b) and such type of nanoparticles is also involved in scavenging activity (Chaugule, 2021a) where melanin is one unique example of metabolites.

## 7. Conclusions

Melanin is a naturally occurring pigment that is responsible for pigmentation in organisms such as bacteria, fungi, and higher species. Many microorganisms, like bacteria, fungi, actinomycetes, yeasts, etc. are found to be essential sources of microbial melanin. It has a very crucial and multipurpose function. Due to its multipurpose ability, it is used in a wide range of biological, environmental, and technological domains. This review interpreted and focused on microbial resources for melanin. Many researchers are conducting a number of different experiments to create melanin nanoparticles because melanin pigment already possesses various medical uses and applications. As concerns the medical field, these melanin pigments also have various applications in immunology, such as immunomodulation of neutrophils and macrophages, anti-inflammatory actions, and the α-MSH (alpha melanocyte-stimulating hormone) can stimulate regulatory T-lymphocytes, which control immunity by focusing on particular antigens, etc. It also retains some other applications of melanin pigments which are likely to be used as important materials in many fields like material science, green technology, healthcare, etc. Very short research has been seen on melanin and melanin production. Researchers seek to establish development in its modifications

and production, so that it will reach up to the mark in the sense of multifunction use of melanin.

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