Microbial Melanin: Recent Developments and Challenges in the Production, Extraction, Purification and Application of Microbial Melanin

ABSTRACT

A class of biopolymers known by the common name melanin has several potential uses in the medicinal sciences, bioelectronics, cosmeceutical and bioremediation. These pigments wide distribution indicates that they help a variety of life forms- fight biotic and abiotic stressors. Different types of melanin, such as eumelanin, pheomelanin, allomelanin, pyomelanin, and neuromelanin, are produced by the oxidative polymerization of phenolic compounds in fungi and bacteria, primarily via the 1,8-dihydroxynaphthalene [DHN] or 3,4-dihydroxyphenylalanine [DOPA] pathways. The tyrosinase, laccase, and polyketide synthase are the groups of enzymes that are primarily responsible for the production of melanin in many microorganisms. Research has indicated that utilising recombinant technologies in conjunction with culture parameter manipulation can enhance melanin yield for industrial production. Its low solubility has hindered the development of extraction techniques despite tremendous efforts, and its heterogeneous structural complexity has hindered structural elucidation, which limits the full potential of its biotechnological applications. The process of extraction may differ slightly depending on the kind of tissue and where the melanin is located.Melanin pigments from various taxa of life (like Sepia, bacteria, fungi) have been the subject of countless investigations to expand our understanding of them and enable their effective use in a wide range of applications. Due to these studies, there is an immediate need for a thorough review of melanin pigments that have been isolated from microorganisms. A review that covers biosynthesis, bioproduction, characterization, and possible applications will aid researchers from a variety of backgrounds in appreciating the significance of microbial melanins and in organizing their own melanin-related research projects. In order to achieve this, the current study contrasts traditional and cutting-edge concepts for environmentally sustainable melanin extraction processes.

Keywords: *Melanin, biopolymers, extraction, sustainable, bioremediation, etc.*

INTRODUCTION:

Compared to manufactured pigmentor synthetic pigments, natural pigments are thought to be safer and offer a multitude of advantages. Among them, "melanin" is a ubiquitous

heterogeneous polymer that has a wide variety of structural and functional diversity (Gosset, 2017). According to Greek history, the word "melanin" originated from the word "melanos," which means "dark." However, scientists Berzelius and others first used the term in 1840 to describe a pigment that was extracted from the membranes of the eyes that was dark in colour. The oxidation of phenolic or indolic substrates by enzymatic catalysis produces the polymeric colours. Melanin is a heterogeneous polymer that is produced when phenols are oxidized and then intermediate phenols and their quinones are polymerized (Solano, 2014). “Based on the structural monomers of melanin, there are three primary categories of melanin: eumelanin, pheomelanin, and allomelanin. While pheomelanin (found in the animals) gives cells their yellow/red pigmentation, eumelanin and allomelanin give cells their black/brown colour” (Pralea*et al.,* 2019). “In eumelanin, the indole-type units which results from the oxidation of L-tyrosine or L-DOPA (L-3,4-dihydroxyphenylalanine) are the main precursor units for the polymer(Fig 1). Comparably, oxidative polymerization of cysteinyl conjugates of DOPA via benzothiazine intermediates results in the formation of pheomelanins” (Ito *et al.*, 2020). “Conversely, nitrogen-free diphenols such catechol, 1,8-dihydroxynaphtalene, and γ-glutaminyl-3,4-dihydroxybenzene are converted to allomelanins through oxidation. Pyomelanin and neuromelanin are two more forms of melanin that are known to exist. While neuromelanin consists of both benzothiazine and indole units, pyomelanin is the result of the oxidation of homogentisic acid” (Pralea*et al.,* 2019). In addition to giving cells their colour, melanin also protects cells from UV radiation (Coelho *et al.,* 2005: Stepien *et al.,* 2013;) quenches free radicals (Meredith and Sarna, 2006); is involved in a variety of functions across multiple phyla (Singh S*. et al.,* 2021); aids in defense mechanisms in insects and mollusks (Vavricka *et al.,* 2014); increases virulence mechanisms in a variety of fungi and bacteria (Nosanchuk and Casadevall, 2003); and offers the benefit of antibacterial and antioxidant actions. Melanins can also be used in semiconductors (Bothma *et al.,* 2008), metal chelators (Abbas *et al.,* 2009), optical imagers, medicines and cosmeceuticals, MRI probes, soil bioremediations, and other fields (Martinez *et al.,* 2019). “Because of their physicochemical characteristics and heterogeneities, it is difficult to draw precise conclusions about their structures and properties despite their many and promising qualities. The absence of the genetic makeup necessary for sequential metabolic pathways and melanin manufacture is a key cause of variability. Moreover, microorganisms can synthesize melanin from a variety of precursors, which results in a more random polymerization process” (Cao *et al.,* 2021).

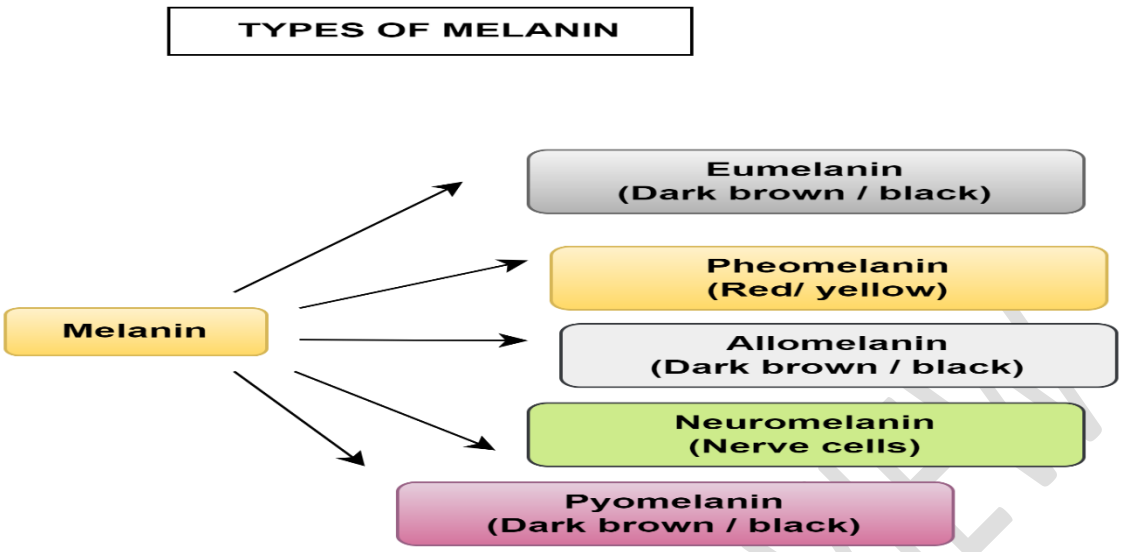

Fig 1: Types of melanin

Melanin's insolubility in most solvents and difficulties in understanding its structural properties could pose hurdles to its extraction and purification methods, hence impeding its cost-effective industrial production (Sun *et al.,* 2016). In conclusion, it is difficult to produce economical and environmentally acceptable methods of producing melanin from eukaryotic resources; under these circumstances, microbial melanin may be able to help (Pavan *et al.,* 2020). The efficiency of melanin production from microbial sources comes from simple fermentation processes, wherein melanin synthesis yield can be maximized by optimizing relevant parameters.

Despite its complex random polymeric structures, which give it its distinct features and utility, melanin has been used in a wide range of biological, physiological, and physical materials. As a result, a lot of work has gone into screening strains that produce melanin. For instance, there has been a lot of interest in the isolation of fungal strains that produce melanin and the large-scale synthesis of melanin. However, depending on the host strain and kind of melanin, different production titers and isolation techniques apply. Fungal strains are excellent hosts for the synthesis of melanin, but in order to reach the required production titer, a lengthy fermentation period is needed (Nosanchuk *et al*., 2015; Cordero and Casadevall, 2017). Furthermore, the methods of extraction and purification vary based on the physical characteristics, like solubility and intended application of the separated melanin (Pralea *et al.,* 2019; Singh *et al*., 2021). Fermentation and extraction are two common bioprocesses utilized in melanin biorefineries for biochemical manufacturing processes. The melanin biorefinery also includes the chemical

treatment procedure used in the extraction of melanin, such as organic solvent. As a result, knowledge of melanin formation in relation to the biorefinery process is essential.

The current review, which is based on a survey of the literature covering the last five years, focuses on the literature on melanin derived from microbiological sources, such as bacteria, recombinant bacterial strains and fungi. The biosynthesis, extraction, and purification methods, as well as the properties and practical uses of microbial melanin, are the main topics of this review. The paper was to address the ways in which various microbial melanin functional characteristics can be enhanced in industries.

SOURCES OF MELANIN

There are numerous common fruits and vegetables that are capable of producing melanin, including potatoes, apples, bananas, garlic, persimmons, and bananas (Qi *et al.,* 2020). Plants such as the callus of *Mucuna monosperma* (Wight) can also provide melanin (Inamdar *et al.,* 2014). Sepia extract or synthetic methods are used to prepare commercial melanin (Prados-Rosales *et al.,* 2015; Srisuk*et al.,* 2016).

However, the drawbacks of these approaches are their high manufacturing costs, limited flexibility, and potential for environmental damage. Consequently, there has been a lot of interest in the bioproduction of melanin by microorganisms like bacteria and fungus as a substitute source of melanin. Due to their rapid growth, they can be utilized in the process of scaling up for large-scale production. Additionally, a number of attempts have been undertaken to separate strains of melanin-producing bacteria from different settings in order to increase melanin production via reactions and host cell engineering.

MELANIN EXTRACTION FROM NATURAL SOURCES.

Traditionally, melanin is extracted from sepia ink or the dark fur or feathers of animals. The majority of melanins are produced inside melanosomes and are firmly attached to certain cellular components, such as proteins or minerals, which presents a difficulty for melanin formation and extraction from these sources (Prota, 1995). Consequently, in order to extract melanin, the complete protein fraction, cell debris, and unabsorbed nutrients, the separation process of melanin typically entails harsh chemical treatments. According to Pralea *et al*. (2019) and Liu and Simon (2005), these treatments often involve significant hydrolysis with boiling mineral acids or bases, followed by successive washing processes with organic solvents like acetone, chloroform, or pure ethanol. But in the latter step, there are molecular changes to the

melanin polymeric skeleton (Pralea *et al.,* 2019). Other approaches documented in the literature have explained the application of kinder isolation techniques like ultracentrifugation for mechanical separation; proteolytic digestion for the removal of the remaining protein matrix using enzymes; or a mix of the two approaches (Xiao *et al*., 2018). According to certain research, enzymatic extraction techniques outperform acid/base extraction strategies in preserving the melanin's appearance and structure as intact melanosomes (Liu *et al*., 2003).

Complete polymers, natural melanins have limited opportunities for alteration. Additionally, the way that melanin is ultimately dried can have a significant impact on its physical characteristics, including porosity, surface area-to-mass ratio, and aggregation (D'Ischia*et al*. 2013). Because of this source-dependency, natural melanin supply is costly and challenging to scale up, and depending on the source, it may cause contamination. For instance, melanin taken from bird feathers or the sepia ink sac may include higher concentrations of linked hazardous metals due to environmental exposure. Furthermore, since the animals from which melanin is taken may need to be killed, these sources of melanin raise ethical questions. The careful utilization of natural melanin for applied study is highlighted by all of these features.

CHEMICALLY SYNTHESIZED MELANIN

The synthesis of melanins with characteristics similar to those of natural melanins has been the subject of much research in the past 10 years (D'Ischia*et al*., 2014; Solano, 2017). In chemical synthesis, polydopamine is synthesized by oxidatively polymerizing dopamine. Because of their similar functional groups—such as catechol, amine, and imine groups—polydopamine and natural melanin share certain characteristics (Solano, 2017). Research on polydopamine has accelerated due of its excellent tunability (Liu *et al*., 2014). Interestingly, research on synthetic materials based on melanin is typically discussed in terms of polydopamine and its analogues.

Solution oxidation, enzymatic oxidation, and electropolymerization are the three common methods for synthesizing polydopamine (Liu *et al*., 2014). Solution oxidation under alkaline conditions is a commonly employed technique that entails the oxidation of dopamine monomers with oxygen and their subsequent self-polymerization. The second method frequently involves the use of the enzyme tyrosinase to oxidize L-tyrosine enzymatically. Another technique in this strategy is oxidizing dopamine's diphenolic groups and then employing the enzyme laccase to polymerize it into polydopamine. Finally, the production of polydopamine on an electrode has been the primary use of the electropolymerization technique. Applying the right electrical voltage in a deoxygenated solution can efficiently produce a high-thickness polymeric film. However,

this approach has a drawback in that polydopamine can only be placed on conductive materials because it requires the electrode's surface to be conductive.

Synthetic melanins frequently differ from natural melanins in terms of their structural and functional characteristics, even though there have been several attempts to replicate natural melanins (Ligonzo*et al.,* 2009; Bridelli and Crippa, 2010). According to several research, natural melanins work better than synthetic melanins in biotechnological applications. For example, in aqueous sodium-ion batteries, sepia melanin has a greater specific capacity (16.1 versus 7.9 mAhg−1) than polydopamine (Kim *et al*., 2013). The inherent characteristics of natural melanins, such as the carboxyl content of the starting precursor (tyrosine or L-DOPA vs. dopamine, which lacks a carboxylic group), the melanogenesis mechanism (Pezzella *et al.,* 1997), the distinct nanostructure of melanin granules attached to small amounts of proteins, and the higher efficiency of natural melanins, may be responsible for their superior performance and the distinct melanin granule nanostructure linked to trace levels of proteins, as well as the molecules' greater degree of hydration (Tran-Ly *et al.,* 2020).

MELANIN PRODUCTIONIN BACTERIA

There have also been reports of a number of microbial melanins (Perez-Cuesta *et al.,* 2020;Guo *et al.,* 2014 and Jalmi *et al*., 2012; Madhusudhan *et al*., 2014; Tarangini and Mishra, 2014; and Ganesh Kumar *et al*., 2013). Additionally, it has been reported that melanin can be produced in *E. coli* by expressing tyrosinase or by using wild-type strains of *Pseudomonas*, (Ahn *et al.,* 2021), *Bacillus, Amorphotheca*, and *Vibrio* (Tarangini and Mishra, 2014; Oh *et al.,* 2020; Ahn *et al*., 2021). When given 1 g/L of tyrosine, the *Klebsiella sp*. GSK46 strain, which was isolated from crop field soil, was able to produce roughly 0.13 g/L of eumelanin (Sajjan *et al.,* 2010). On the other hand, melanin might still be produced without tyrosine. For instance, it was shown that marine *Pseudomonas stutzeri*, which was isolated from seaweed, produced a notable amount of melanin—6.7 g/L—after 10 hours of incubation in sea water production medium without the need for tyrosine supplementation (Ganesh Kumar *et al.,* 2013). Fruit waste extract has been used to produce melanin because it offers good nutrients for biochemical development. *Bacillus safensis*, isolated from garden soil, was shown by Tarangini and Mishra to be able to produce 6.96 g/L of melanin after 10 hours of incubation (Tarangini and Mishra, 2014; Valdez-Calderón *et al.,* 2020). Along with the use of sugar-based fermentation, such as glucose, starch, and molasses, amino acids have also been used for whole cell biotransformation, which results in the generation of melanin (Ghadge *et al.,* 2020; Oh *et al.,* 2020; Eskandari and Etemadifar, 2021). For instance, *Pseudomonas koreensis* UIS19 in a molasses medium with tyrosine

supplementation was used by Eskandari and Etemadifar to produce melanin at a low cost (Mustafa *et al.,* 2020; Eskandari and Etemadifar, 2021) (Table 1). To obtain 5.4 g/L of dry cell mass, 32 g/L of sugar were supplied. Supplemented tyrosine was able to yield 0.44 g of dry melanin/g of weight. Furthermore, melanin was produced in several g/L using a variety of amino acid-based mediums, including peptone and yeast extract, using *Streptomyces kathirae, Streptomyces glaucescens, Streptomyces sp*. ZL-24, and *Amorphoteca resinae* (Guo J. *et al.,* 2014; El-Naggar and El-Ewasy, 2017; Wang *et al*., 2019; Eskandari and Etemadifar, 2021). Specifically, *S. kathirae* was capable to producing up to 13.7 g/L of melanin; however, the greatest titer needed 128 hours of incubation (Guo J. *et al.,* 2014).

It is important to remember that the synthesis of eumelanin depends on metal ions. For instance, it has been observed that ferrous and nickel ion supplementation increases tyrosinase activity or stimulates tyrosinase synthesis to propel melanin production (Wang *et al.,* 2019). The optimisation results showed that 4.24 g/L of soluble pure melanin and 189.9 mg/L of insoluble melanin could be produced with 1.33 g/L $FeSO_4$ and 3.05 mM $NiCl_2$. The activation of melanin production appeared to be positively impacted by the addition of metal ions; however, the melanin that was produced was also observed to have the ability to chelate or absorb metal ions, such as Cu(II) and Zn(II), which could lead to a metal-melanin complex and modify its properties (He *et al*., 2020 and Caldas *et al.,* 2020).

Table 1: Examples of Melanin producing bacteria

S.NO	Bacteria Name	References
1.	*Bacillus subtilis 4NP BL*	Ghadge *et al*., (2020)
2.	*Bacillus safensis*	Valdez- Calderón *et al.,* (2020)
3.	*Amorphothicaresinae*	Oh *et al*., (2020)
4.	*Pseudomonas korcensis UIS 19*	Eskandari and Etemadifar (2021)
5.	*Burkholderia cepacia*	Zughaier *et al*., (1999)
6.	*Bacillus thuringiensis*	Ruan *et al.,*(2004)
7.	*Bacillus cereus*	Zhang *et al.,* (2007)
8.	*Klebsiella sp. GSK*	Sajjan *et al*.,(2010)
9.	*Pseudomonas maltophilia*	Wan *et al.,* (2007)

10.	*Stenotrophomonas maltophilia*	Amoli *et al.,* (2017)
11.	*Pseudomonas stuzeri*	Ganesh Kumar *et al.,* (2013)
12.	*S. kathirae*	Guo *et al.,* (2014)

MELANIN PRODUCTION BY BACTERIAL RECOMBINANT STRAINS

Research has also been done extensively on the production of a recombinant strain that produces allomelanin. The co-expression of enoyl-CoA hydratase/aldolase (ECH) and feruloyl-CoA synthetase (FCS) in an*E. coli* strain that promotes the synthesis of allomelanin in the presence of caffeic acids was originally described by Jang *et al*.,(2018). These enzymes were previously employed in the ferulic acid-based vanillin production process (Gallage*et al.,* 2014). Contrary to ferulic acid, which has one hydroxyl group blocked by the methoxyl group, caffeic acid contains a catechol moiety in its core structure. This means that allomelanin could easily be formed through the enzymatic alteration of the other carboxylic moiety. According to Jang *et al.*, (2018), the FCS/ECH overexpressing recombinant strain could yield 0.2 g/L of allomelanin in a 12-hour reaction (about 40.9 mg/L/h). Ahn *et al.,* also produced caffeic acid-based allomelanin using the same strain and compared its chemical makeup to other natural and manufactured forms of melanin (Ahn *et al.,* 2019). The HEMA (hydroxyethyl methacrylate) polymer, which is often used for soft contact lenses, was interestingly dyed significantly by the caffeic acid-derived allomelanin, indicating the possibility of using melanin as a UV-blocking contact lens.

For melanin synthesis, recombinant strains offer a number of benefits in terms of extraction and purity as well as production rates and titers. Research on eumelanin and pyomelanin production (Jang *et al.,* 2018; Ahn *et al.,* 2019; Bolognese *et al.,* 2019; Park H. *et al.,* 2020; Seo and Choi, 2020) demonstrated this. The potential for a second source of melanin building blocks to regulate the chemical structure of melanin is another benefit; this enables the tailoring of functions based on application. For instance, the engineering of eumelanin through the co-expression of cytochrome P450 monooxygenase (CYP102G4) and bacterial tyrosinase (MelC), which may catalyse the hydroxylation of indole (Park H. *et al.*, 2020). But there are a few things to consider before using recombinant strains to produce melanin. For instance, there are concerns about the safety of genetic engineering used in medications and cosmetics. It's also

important to take into account how dependent the macroscopic structures and physical characteristics are on the generating host.

MELANIN SYNTHESIS IN MOLDS AND YEAST

According to reports, there are currently a number of fungal strains that produce melanin (Rosas *et al*., 2000; Gomez *et al.,* 2001; Nosanchuk *et al.*, 2002; Morris-Jones *et al.,* 2003). Although various forms of melanin are produced by fungal strains, nitrogen-deficient allomelanin is the most common variety. Tyrosinases, which are copper-dependent biocatalysts engaged in the ortho-specific hydroxylation and subsequent oxidation of monophenols like tyrosine, are the primary enzymes involved in the formation of melanin. Similar to tyrosinase, laccase is an additional enzyme that can catalyse the oxidation of a wide variety of substrates, such as quinones and dihydroxyphenols (Nagai *et al.,* 2003). Rather than in bacteria, these enzymes are typically found in large quantities in plants and fungi. As a result, fungus strains were a viable option for producing melanin. Furthermore, fungi's dynamic and complex membrane structure provides a more favourable environment for the synthesis and deposition of melanin. For instance, it has been revealed that the melanin of *Cryptococcus neoformans* is found in the cell walls of protein constructions and branching polysaccharides (Nosanchuk and Casadevall, 2003). Furthermore, it has been suggested that the existence of other cellular structures, including anchoring structures, melanosomes, and fungal vesicles, aids in the effective synthesis and distribution of fungal melanin (Camacho et al., 2019).

However, it should be noted that because of the fungus's slow rate of cell growth, the production of melanin-consuming fungi requires a relatively long incubation period; for instance, *Auricularia auricula* or *Gliocephalotrichum simplex* produced 2.97 g/L and 6.6 g/L of melanin in 8 and 6 days, respectively (Jalmi *et al.,* 2012; Sun *et al.,* 2016). Interestingly, in a 3% (w/v) tyrosine-supplemented medium, Ribera *et al.,*(2019)found that after 161 days, *Armillaria cepistipes* culture could produce 27.98 g/L of eumelanin, which was the greatest to our knowledge. Nevertheless, it required 161 days to reach this production titer(Table 2).

Using the polyketide route, eumelanin can be produced from L-tyrosine and allomelanin (Varga *et al.,* 2016). To achieve desired production titers, however, obstacles such low growth rate, sporulation, low extraction efficiency, and possible toxicity of fungal strains must be addressed. Genetic engineering has recently made it feasible to use recombinant fungus to enhance the production of different biochemicals. This is because to advancements in sequencing and

genetic manipulation technologies. Accordingly, it could be able to boost the production of fungal melanin by expressing an outside enzyme.

Table 2: Melanin production in Yeast and Molds

S.NO	Yeast and Molds	References
1.	*Cryptococcus neoformans*	Nosanchuk and Casadevall (2013)
2.	*Auricularia auricula*	Sun *et al.,* (2016)
3.	*Armillaria cepistipes*	Rubera *et al.,* (2019)
4.	*Penicillium marneffei*	Woo *et al.,* (2010)
5.	*Aspergillus femigates*	Heinekamp T *et al.,* (2012)
6.	*Amorphotheca resinae*	Oh *et al.*, (2021)
7.	*Rhizopus arrhizua*	White C. *et al.,* (2007)
8.	*Lasiodiplodia theobromae*	Lui Y. et al., (2020)

FERMENTATION, EXTRACTION AND PURIFICATION OF MELANIN

The fundamental steps in the production of melanin include host selection, liquid statefermentation or biotransformation, and the extraction and purification of crude melanin to produce pure melanin (Fig 2).

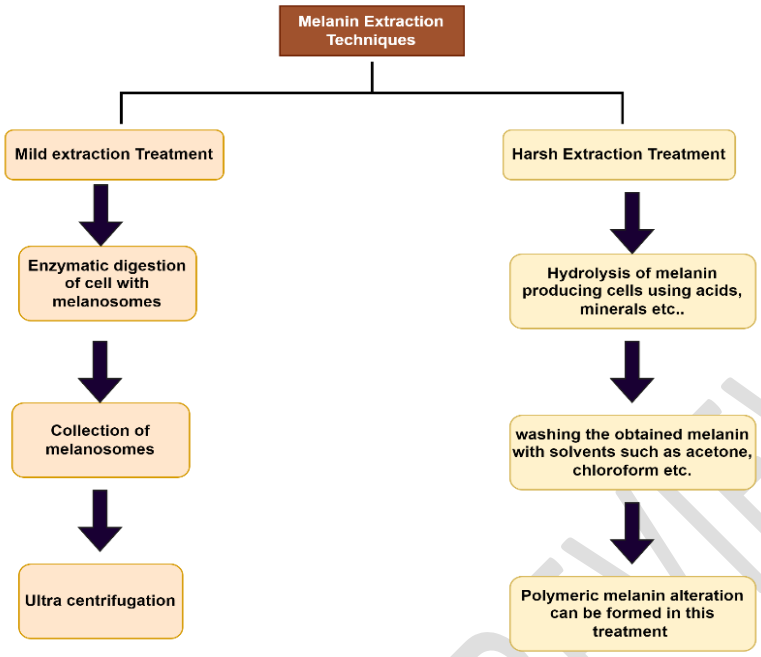

Fig 2: Extraction of melanin

The host cell that produces the melanin, the melanin's intracellular location, its structural characteristics, and its crystal structure all influence the extraction process. Early study on extracting melanin was carried out since melanin pigment is easily found in nature (Aneesh *et al.,* 2020). Particular techniques for removing melanin pigment from melanocytes and melanin organs; often, involves heating and dissolving the pigment in an alkali or strong acid solution. For instance, crude melanin was extractedfrom the liquid broth using a straightforward alkali process, although the yield was only 2.59% (Ma *et al*., 2018). The method used for extraction, the number of cycles that are repeated, and the kind of melanin—that is, pure or crude—all have an impact on the quality of the melanin that is extracted and purified.

Extracellular and intracellular melanin can be distinguished based on the source of the melanin. While extracellular melanin synthesis requires further alkali extraction, extracellular melanin extraction methods use acid precipitation. Ultrasonic or microwave aided techniques (450 W for 50 min, or 70 W for 3 min intervals with 30 cycles) were employed to help with alkali extraction (Sajjan *et al.,* 2010; Jalmi *et al.,* 2012;). Hou *et al*., reported that whereas 24.24% of the pure melanin was achieved without the use of ultrasonic assistance, 37.33% of the melanin was obtained using this method (Hou *et al.,* 2019).Similar to this, Lu *et al*.,2014showed that a microwave-assisted extraction method could provide a purification yield of 11.08%, which was

40.43% greater than that of alkali extraction and acid precipitation. To boost the extraction yields, a second phase of boiling at 80°C for two hours was also used (Oh *et al*., 2020).

Prior to acid precipitation, cell debris and byproducts were removed by filtering through different materials, including Millipore 0.2 µm polyether sulfone membrane, 0.45 µm nitrocellulose membrane, 0.22 µm membrane filter, (Jalmi *et al.,* 2012; Ribera *et al.,* 2019). A boiling or incubation stage for a few hours may be added to aid in precipitation (Liu *et al*., 2019). Following precipitation, deionized water was used for the washing stage. These methods of filtration, precipitation, centrifugation, and washing could be used to prepare crude melanin.

INCREASING THE PURITY OF EXTRACTED MELANIN

Several processes including redissolution, precipitation, boiling, and washing were used to improve the purity of the crude melanin that was separated. To put it briefly, centrifugation was used to collect the dissolved crude melanin in NaOH. After that, HCl was used to bring the obtained sample's pH down to about 2, and incubation ensued. Centrifugation was used to gather the resuspended melanin, which was then repeatedly cleaned with deionized water. The extracted melanin was then lyophilized after being cleaned with $CHCl_3$, DCM, EA, and pure EtOH. Additional boiling, acid-hydrolysis, and repeated washing procedures can be added to pure melanin, depending on the type and condition of melanin (Ribera *et al*., 2019; Oh *et al*., 2020).

Although a number of streamlined extraction techniques have been suggested, the acid precipitation-pH adjustment-washing-resuspension sequence is frequently employed (*Ghadge et al.,* 2020; Wang *et al*., 2020). Other helpful technologies have also been used in the extraction of melanin. For instance, rather than employing alkali extraction, protease or hydrolase enzymes have been used to enzymatically rupture the cell membrane. Furthermore, melanin extraction with good yields has been achieved using a range of organic solvents. Environmental issues pertaining to bioprocesses, such as the use of organic solvents and the disposal of effluent in the melanin isolation process, should be taken into consideration. It is possible to use many extraction techniques to distinct melanin kinds and sources, as previously said, indicating that there isn't a single best technique that can be used consistently. It seems reasonable to apply optimized procedures unique to each process, depending on the chemical structure, kind, solubility, and intended usage.

APPLICATIONSOF MICROBIAL MELANIN

Tyrosinase inhibitor-induced reduction of melanin synthesis has long been studied because of the skin's dark pigmentation. A lot of skin-whitening cosmetics contain these inhibitors as components. But because of its intriguing properties—such as its capacity to scavenge free radicals, defend against metal ions, and shield against UV rays—melanin is now produced in large quantities as a functional material with a wide range of potential uses in the pharmaceutical, cosmetic, and environmental industries (Fig 3). Furthermore, melanin's ability to store electrons has made it possible for it to be used as an electrode and supercapacitor (Park H*. et al.,* 2020).

Because of this, a pigment produced by bacteria is getting a lot of attention. Specifically, bio-pigments with biocompatibility can be applied in a number of industries, including the environment, pharmaceuticals, medicine, and cosmetics. Emphasizing the industrial use of melanin, a number of products have lately been introduced in the beauty sector of hair care that use water-soluble squid melanin for colouring and shampooing (Longo *et al.,* 2017).

The concurrent synthesis of melanin and biochemicals in a single cell could also be employed as a tactic to ensure melanin's high value-added application and productivity in biological processes. Ahn*et al.,* (2021), for instance, have documented co-producing melanin with important biochemicals, such cadaverine , a diamino pentane derived from the decarboxylation of lysine.

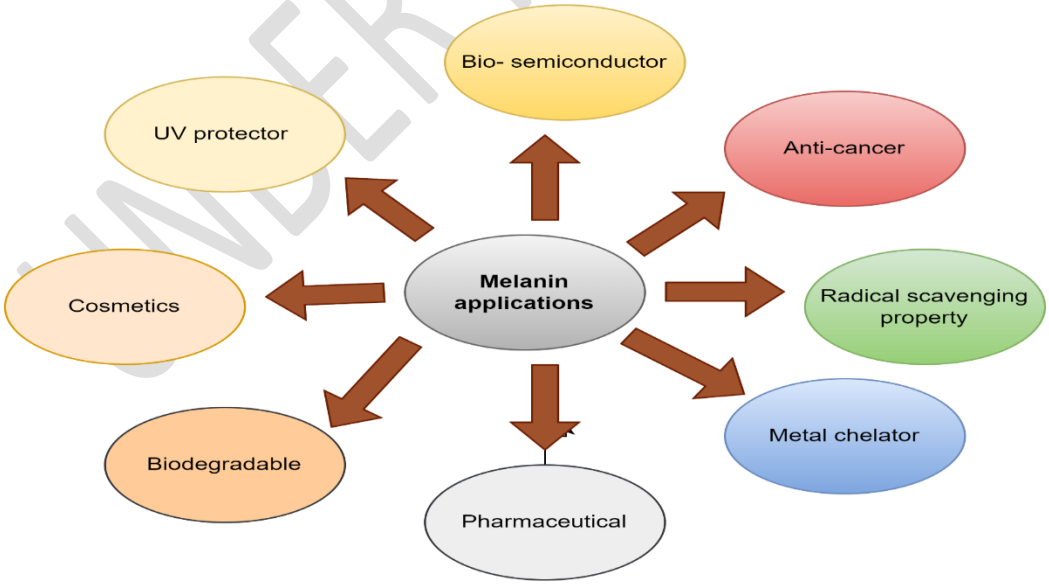

Fig 3: Applications of melanin

The cadaverine that was produced was directly integrated into the melanin polymerization process, as per the study. A competitive market pricing might be ensured by using this co-production method. It makes sense from the perspective of bioprocessing to synthesize biochemicals with this kind of functionality using only the expression of one enzyme. Future focus should also be on the study of how to get superior functionality in recombinant melanin-producing strains by making additional structural alterations based on building blocks.

CONCLUSION

The pigment that is most commonly found and a component of human skin tissue is called melanin. Numerous organisms are capable of melanogenesis, and the identification of bacteria that produce melanin and bioconversion processes have made it feasible to produce melanin in large quantities. Studies on the intricate chemical structure and physical characteristics of melanin have led to the optimisation of melanin extraction, separation, and purification processes. These investigations have led to the production of crude and pure melanin at concentrations of many g/L. Above all, maintaining price competitiveness in the tyrosine substrate bioconversion process and securing the substrate are two major obstacles that must be removed for the industrial application of melanin. Furthermore, to fully replicate the functional features of melanin and enable its development as a real biochemical product, a deeper understanding of the relationship between the structural complexity and biological function of melanin is required.

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