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REVIEW

Actinobacterial melanins: current status and perspective for the future

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Abstract Melanins are enigmatic pigments that are produced by a wide variety of microorganisms including several species of bacteria and fungi. Melanins are biological macromolecules with multiple important functions, yet their structures are not well understood. Melanins are frequently used in medicine, pharmacology, and cosmetics preparations. Melanins also have great application potential in agriculture industry. They have several biological functions including photoprotection, thermoregulation, action as free radical sinks, cation chelators, and antibiotics. Plants and insects incorporate melanins as cell wall and cuticle strengtheners, respectively. Actinobacteria are the most economically as well as biotechnologically valuable prokaryotes. However, the melanin properties are, in general, poorly understood. In this review an evaluation is made on the present state of research on actinobacterial melanins and its perspectives. The highlights include the production and biotechnological applications of melanins in agriculture, food, cosmetic and medicinal fields. With increasing advancement in science and technology, there would be greater demands in the future for melanins produced by actinobacteria from various sources.

Keywords Actinobacteria · Melanins · Pigments · L-Tyrosine · Cosmetic · *Streptomyces*

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Introduction

Actinobacteria represents one of the largest taxonomic units among the 18 major lineages currently recognized within the bacteria domain, including five subclasses and 14 suborders (Stackebrandt 2000). Among the five subclasses, actinobacteria-bacteria belonging to the Order Actinomycetales (commonly called actinomycetes) account for approximately 7,000 of the metabolites reported in the *Dictionary of Natural Products*. Actinomycetes have a high GC content in their deoxyribonucleic acid (DNA) and grow as aerial mycelia (Yoshida et al. 2008). They are responsible for the production of about half of the discovered secondary metabolites (Bull 2004; Berdy 2005), notably antibiotics (Strohl 2004), pigments (Dastager et al. 2006), antitumor agents (Olano et al. 2009), immunosuppressive agents (Mann 2001) and enzymes (Oldfield et al. 1998).

Actinomycetes are a prolific source of secondary metabolites and the vast majority of these compounds are derived from the single genus *Streptomyces*. *Streptomyces* species are distributed widely in marine and terrestrial habitats (Pathom-Aree et al. 2006) and are of commercial interest due to their unique capacity to produce novel metabolites. It was also expected that *Streptomyces* species will have a cosmopolitan distribution, as they produce

abundant spores that are readily dispersed (Antony-Babu et al. 2008). These filamentous bacteria are well adapted to the marine environment and are able to break down complex biological polymers. The genus *Streptomyces* was classified under the family *Streptomycetaceae*, which includes Gram-positive aerobic members of the order Actinomycetales and suborder *Streptomycineae* within the new class Actinobacteria (Stackebrandt et al. 1997; Anderson and Wellington 2001), with a DNA G + C content of 69–78 mol %. In fact, the genus *Streptomyces* alone accounts for a remarkable 80 % of the actinomycete natural products reported to date, a biosynthetic capacity that remains unrivaled in the microbial world (Watve et al. 2001). *Streptomyces* include some of the most common soil, freshwater and marine life, playing an important role in decomposition of organic materials, such as cellulose and chitin.

Melanins are dark-brown to black pigments of macromolecules formed by oxidative polymerization of phenol and/or indolic compounds, which widely exist in animals, plants and microorganisms. They showed a broad spectrum of biological roles, including antioxidant (Goncalves and Pombeiro-Sponchiado 2005), antimicrobial activity (Casadevall et al. 2000), antitumor activity (El-Obeid et al. 2006), antivenin activity (Hung et al. 2004), anti-virus (Montefiori and Zhou 1991), liver protecting activity (Sava et al. 2003) and radio protective (Dadachova et al. 2007) etc. They are widely used in medicine, pharmacology, cosmetics and other fields.

Actinobacteria have long been described as capable of producing dark-brown coloured substances in culture media. Generally refered to as melanins, or melanoid pigments, these brown-black metabolic polymers are important not only as a useful criterion in taxonomic studies but also because of their similarity to soil humic substances. Melanins are not essential for the growth and development of the organisms but play an important role in improving their survival and competitiveness. Melanins have been reported to act as "armour" and function in the protection of actinobacteria against environmental stress such as UV radiation, temperature extremes (Butler and Day 1998). Some melanized actinobacteria inhabit remarkably extreme environments including high altitude, Arctic and Antarctic regions.

There is worldwide interest in the development of processes for the production of pigments from natural sources due to the serious safety problem with many artificial synthetic colourants, which have been widely used in foodstuff, cosmetic and pharmaceutical manufacturing processes (Kim et al. 1995). In actinobacteria, synthesis and excretion of dark pigments, melanin or melanoid, are considered to be a useful criterion for taxonomical studies (Zenova 1965; Arai and Mikami 1972; Dastager et al. 2006). In this review, we summarize recent studies on the production, application and importance of melanins from actinobacteria.

Melanins

Melanin compounds are irregular, dark brown polymers that have the radioprotective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation (Vinarov et al. 2002). Melanins are frequently used in medicine, pharmacology, and cosmetics preparations (Dastager et al. 2006). Melanins also have great application potential in agriculture industry. Melanins are complex natural pigments, widely dispersed in animals, plants, and microorganisms. They have several biological functions including photoprotection, thermoregulation, action as free radical sinks, cation chelators, and antibiotics. Plants and insects incorporate melanins as cell wall and cuticle strengtheners, respectively (Riley 1997). The function of melanin in microbes is believed to be associated with protection against environmental stress. For example, bacteria producing melanins are more resistant to antibiotics (Lin et al. 2005), and melanins in fungi are involved in fungal pathogenesis of plants (Butler and Day 1998).

Types of melanins

In mammals, two types of melanin can be distinguished: a dark eumelanin and a yellow to red pheomelanin (Riley 1997).

Eumelanin

Eumelanin polymers have long been thought to comprise numerous cross-linked 5,6-dihydroxindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) polymers (Fig. 1); recent research into the electrical properties of eumelanin, however, has indicated that it may consist of more basic oligomers adhering to one another by some other mechanism (Nicolaus 1968). Eumelanin is found in hair and skin, and colors hair grey, black, yellow, and brown (Prota 1992; Ito 1993). In humans, it is more abundant in peoples with dark skin. There are two different types of eumelanin, which are distinguished from each other by their pattern of polymer bonds. The two types are black eumelanin and brown eumelanin, with black melanin being darker than brown. Black eumelanin is in mostly non-Europeans and aged Europeans, while brown eumelanins is in mostly young Europeans. A small amount of black eumelanin in the absence of other pigments causes grey hair. A small amount of brown eumelanin in the absence of other pigments causes yellow (blond) color hair (Prota 1992; Ito 1993).

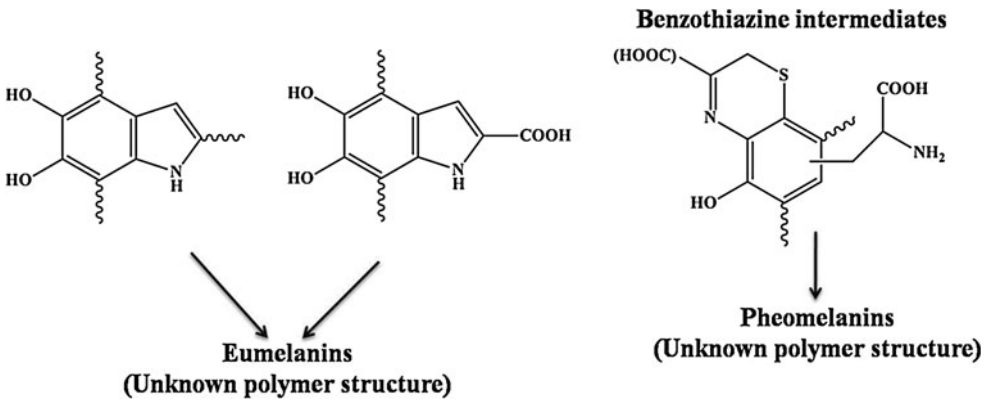

Fig. 1 Eumelanin is a polymer of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while pheomelanin is a polymer of benzothiazine intermediates (*wavy lines* indicate bonding sites for polymerization). For both melanin types, the detailed polymer structure is undetermined

Pheomelanin

Pheomelanin is also found in hair and skin and is both in lighter-skinned and darker-skinned humans respectively. In general women have more pheomelanin than men, and thus women's skin is generally redder than men's. Pheomelanin imparts a pink to red hue and, thus, is found in particularly large quantities in red hair. Pheomelanin is particularly concentrated in the lips, nipples, glans of the penis, and vagina. Pheomelanin also may become carcinogenic when exposed to the ultraviolet rays of the sun. Chemically, pheomelanin differs from eumelanin in that its oligomer structure incorporates benzothiazine units (Fig. 1) which are produced instead of DHI and DHICA when the amino acid L-cysteine is present (Prota 1992; Ito 1993).

L-Tyrosine

The aromatic amino acid L-Tyrosine is a precursor for valuable compounds in the food, pharmaceutical, chemical and cosmetic industries (Para et al. 1985; Riley 1997; Bourke and Kohn 2003; Lütke-Eversloh et al. 2007; Sariaslani 2007 Nagatsu and Sawada 2009). Besides chemical synthesis (Breuer et al. 2004; Leuchtenberger et al. 2005), aromatic compounds can be produced by microbes via the aromatic amino acid biosynthesis or shikimate pathway. Starting with the condensation of the central metabolites phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) to 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP), shikimate is formed via 3-dehydroquinate (DHQ) and 3-dehydroshikimate. Shikimate is then phosphorylated and converted to chorismate after the addition of another PEP molecule (Fig. 2). Chorismate is the biosynthetic branch point for aromatic amino acids, as well as for folate, ubiquinone, menaquinone, and siderophores synthesis (Frost and Draths 1995; Pittard 1996; Dosselaere and Vanderleyden 2001).

L-Tyrosine also serves as an important starting material for a variety of high-value compounds. For example, it is an important precursor for 3,4-dihydroxy-L-phenylalanine (L-DOPA or levodopa), which is currently the most powerful symptomatic drug for the treatment of Parkinson's disease, a condition caused by a deficiency in the action and formation of dopamine by the dopaminergic neurons of the brain. In newer drug formulations, levodopa is combined with the structurally similar chemical carbidopa, which helps prevent the metabolism of levodopa in the bloodstream before it is able to reach the brain (Bonuccelli and Del Dotto 2006; Rajput and Rajput 2006). L-Tyrosine is also the precursor for a variety of industrially relevant compounds. Melanins, which can be synthesized from L-Tyrosine by a single enzyme, possess physiochemical properties that make them particularly suitable for use as UV absorbers, cation exchangers, drug carriers, and amorphous semiconductors (Bell and Wheeler 1986; della-Cioppa et al. 1990; Cabrera-Valladares et al. 2006). Additionally, L-Tyrosine can be easily converted to *p*-hydroxycinnamic acid and *p*-hydroxystyrene, both of which are important components for a variety of novel polymers, adhesives and coatings, pharmaceuticals, biocosmetics, and health and nutrition products (Qi et al. 2007; Sariaslani 2007; Vannelli et al. 2007).

L-3,4-dihydroxyphenylalanine (L-DOPA)

L-DOPA is the precursor of the neurotransmitter dopamine; it is the preferred drug for treating Parkinson's disease (Nagatsu and Sawada 2009). Approximately 250 metric tons of this compound are produced every year by chemical and enzymatic methods (Koyanagi et al. 2005). This drug can be synthesized by following two different enzymatic methods, which do not produce significant amounts of side products and do not require optical resolution of the product. One of these methods utilizes tyrosine phenol-lyase (Tpl) as a catalyst. Tpl generally enables the use of L-Tyrosine as a carbon and nitrogen source in bacteria during the reversible catalysis of L-Tyrosine to pyruvate, ammonia and phenol. L-DOPA (Fig. 3a) can be synthesized if pyruvate, ammonia and catechol are used as the starting materials (Koyanagi et al. 2005).

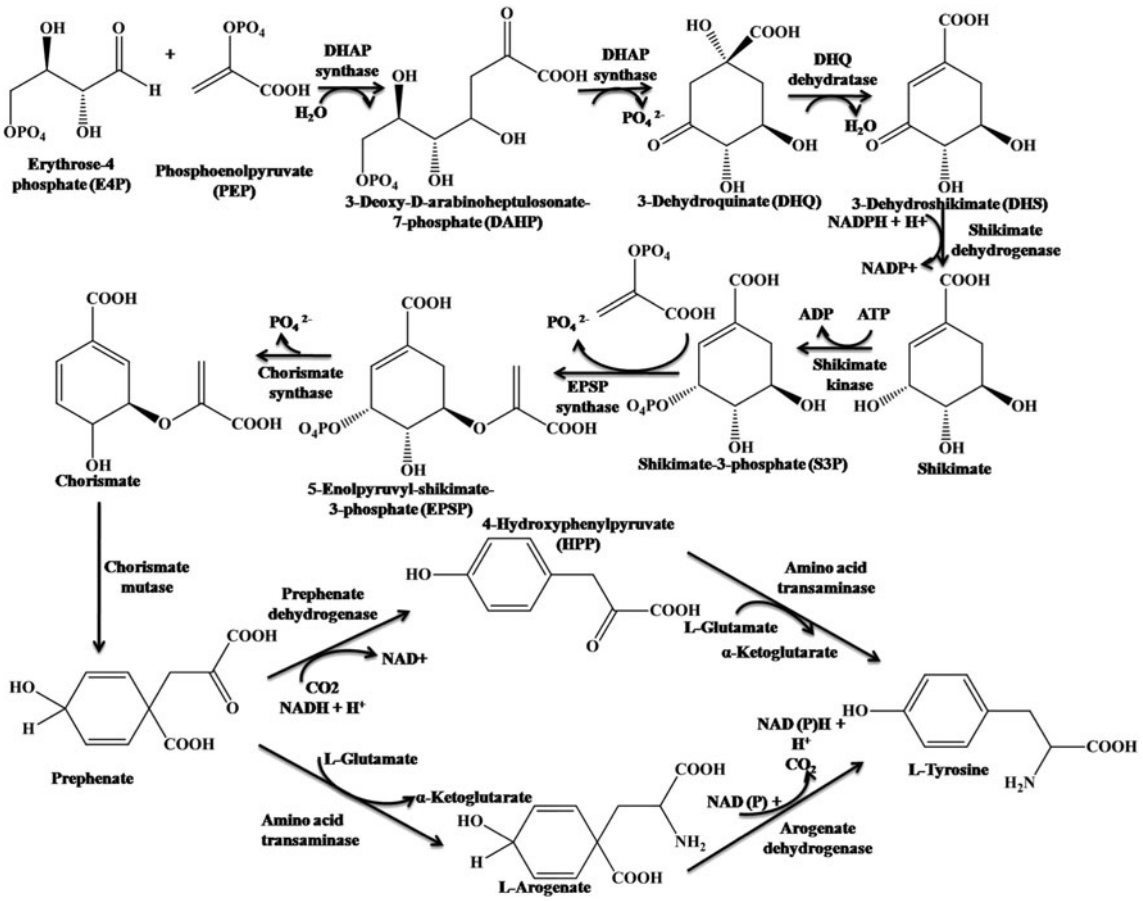

Fig. 2 Biosynthetic pathway for L-Tyrosine in bacteria

L-DOPA can also be synthesized by the enzyme tyrosinase, which participates in generating melanin in a large number of organisms, including bacteria and humans. Tyrosinase is a copper enzyme containing two cupric ions in the active site in regions of the proteins named CuA and CuB. This enzyme catalyzes two reactions: the hydroxylation of L-Tyrosine to L-DOPA (cresolase activity) and the oxidation of L-DOPA to dopaquinone (catecholase activity) (García-Borrón and Solano 2002). Dopaquinone is a very reactive molecule that spontaneously polymerizes to form melanin. During L-DOPA production, the oxidation of tyrosinase to dopaquinone must be avoided by using a reductant, usually ascorbic acid (Ros et al. 1993). Due to the high cost of purified tyrosinase, this enzyme is frequently immobilized so that it can be reused. Different immobilization methods and supports have been studied, each with particular advantages (Ho et al. 2003). Additionally, whole bacterial cells have been used as a source of tyrosinase (Krishnaveni et al. 2009; Ali and Haq 2010; Surwase and Jadhav 2011).

The processes described above require expensive raw materials. A novel approach that could reduce the L-DOPA production costs involves applying metabolic engineering to generate *E. coli* strains that produce L-DOPA from simple carbon sources. Several genetic modifications have been carried out to direct carbon flow from glucose to L-Tyrosine biosynthetic pathway because L-Tyrosine is the direct precursor of L-DOPA. The L-Tyrosine was converted to L-DOPA by the enzyme *p*-hydroxyphenylacetate 3-hydroxylase. L-DOPA producer strains have been modified by overexpressing genes encoding DAHPsyn^{fbr}, TyrC and the Cm domain from PheA. These and other genetic modifications have made it possible to produce L-DOPA from inexpensive carbon sources like glucose (Muñoz et al. 2011).

Tyrosinases

Tyrosinases (E.C. 1.14.18.1) are copper-containing enzymes which are ubiquitously distributed in nature (Mayer and Harel 1979; Van Gelder et al. 1997). They are essential for the formation of melanin (Butler and Day 1998; Nosanchuk and Casadevall 2003; Riley 2003) and various other functions. Tyrosinase and laccase catalyze oxidation of substrate using molecular oxygen as a terminal electron acceptor with concomitant reduction of oxygen to

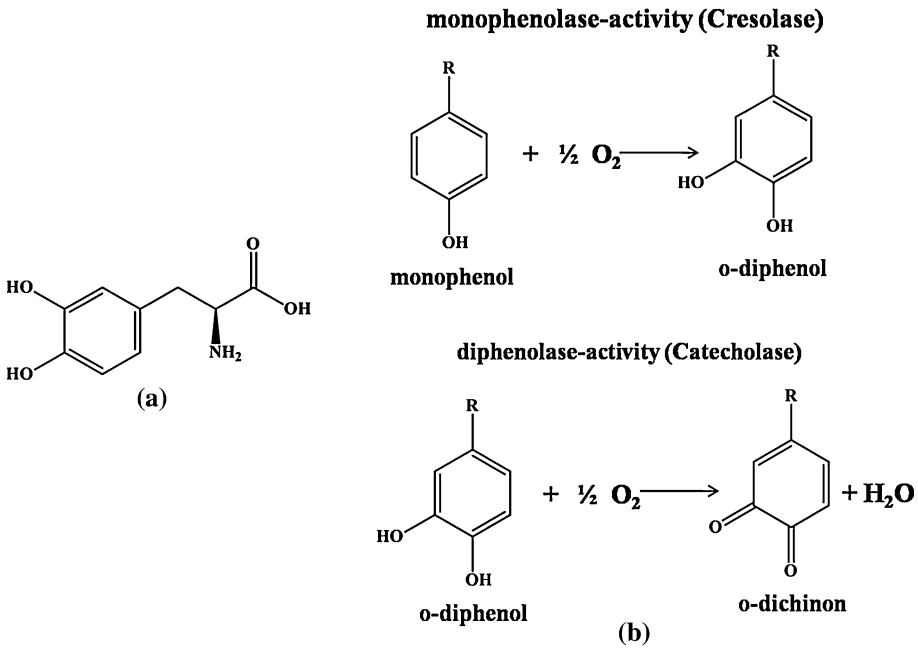

Fig. 3 **a** Chemical structure of L-3,4-dihydroxyphenylalanine (L-DOPA); **b** enzymatic activities of tyrosinases

water. Tyrosinases are found in prokaryotic and eukaryotic microbes, in mammals, invertebrates and plants. The most extensively investigated tyrosinases are, however, from mammals (Kwon et al. 1987; Kwon et al. 1988; Spritz et al. 1997; Kong et al. 2000b).

Tyrosinases are bifunctional enzymes that catalyzes two types of reactions in the presence of molecular oxygen: the *ortho*-hydroxylation of monophenols to its corresponding o-diphenol (monophenolase, cresolase activity) and the oxidation of diphenols to its correspondent *ortho*-quinones (diphenolase, catecholase activity). Quinones are highly susceptible to non-enzymatic reactions, which may lead to formation of mixed melanins and heterogeneous polymers (Solomon et al. 1996; Van Gelder et al. 1997; Edward et al. 2003) (Fig. 3b).

Streptomyces tyrosinases are the most thoroughly characterized enzymes of bacterial origin (della-Cioppa et al. 1994, 1998; Matoba et al. 2006). The first bacterial tyrosinases have been purified from cell extracts of *Streptomyces nigrifaciens* (Nambudiri et al. 1972) and *Streptomyces glaucescens* (Lerch and Ettlinger 2005). Members of the genus *Streptomyces* are involved in the formation and/or degradation of complex biopolymers like lignin, melanins, and humic substances. In addition, they are important industrial sources of antibiotics and other secondary metabolites.

About 40 % of *Streptomyces* species produce melanin as exopigments on tyrosine-containing agar media, which mostly but not always correlate with the appearance of an extracellular tyrosinase activity (Claus and Decker 2006). The typical double enzymatic activity of tyrosinases could be demonstrated in melanin-positive species, whereas melanin-negative mutants lost the cresolase activity, but sometimes retained some catecholase activity (Claus and Decker 2006). Tyrosine methylester and caffeic acid proved to be the best substrates for measuring both enzymatic activities of *Streptomyces* tyrosinase. Electrophoretic characterizations revealed that the intra- and extracellular tyrosinase of one species were identical, but that the enzymes of different species were not. After isoelectric focusing, several tyrosinase bands appeared in some species, indicating the presence of isoenzymes. The heterogeneity of *Streptomyces* tyrosinases was also reflected by different K_m values and temperature stabilities (Claus and Decker 2006).

mel genes

The frequent occurrence of a melanin-negative phenotype in *S. glaucescens* and *Streptomyces reticuli* has been attributed to insertion elements (Hintermann et al. 1985; Schrempf 1983). In the potato pest *Streptomyces scabies* and in *Rhizobium meliloti*, the tyrosinase genes are plasmid located (Gregory and Huang 1964). Genetic studies with melanin-negative *Streptomyces* mutants revealed a polycistronic organization of the chromosomal *melC* operon (Fig. 4). Besides the structure gene for the tyrosinase *(melC2)*, at least one more upstream located gene (*melC1*) is needed for the expression of the melanin phenotype in different *Streptomyces* species (Crameri et al. 1984; Hintermann et al. 1985; Held and Kutzner 1991; Ikeda et al. 1996; Kawamoto et al. 1993).

Melanin biosynthesis pathway

The pathway of eumelanogenesis may be divided into two phases, one proximal and the other distal (Fig. 5) (Kobayashi et al. 1995; Prota 2006). The proximal phase consists of the enzymatic oxidation of tyrosine or L-DOPA to its corresponding dopaquinone catalyzed by tyrosinase. This nascent dopaquinone can undergo two different types of reactions: intramolecular 1,4-addition to the benzene ring or a water addition reaction. The amino group of an *o*-dopaquinone side chain first undergoes an intramolecular 1,4-addition to the benzene ring, which causes its cyclization into leucodopachrome, as shown in Fig. 5. This intermediate is quickly oxidized to dopachrome by another *o*-dopaquinone, which is reduced back to L-DOPA (Cánovas et al. 1982; Neptuno Rodríguez-López et al. 1991; Cooksey et al. 1997).

The second reaction occurs with cyclizable and non-cyclizable quinones and consists of a water addition to the benzene ring, which leads to the formation of a three-hydroxylated phenol, 2,4,5-drihydroxyphenylalanine, which is chemically oxidized to *p*-dopaquinone by another *o*-dopaquinone (Cánovas et al. 1982). This *p*-topaquinone evolves through a series of slow reactions to dopachrome, which is the final product of the proximal phase.

The distal phase is represented by chemical and enzymatic reactions which occur after dopachrome formation and lead to the synthesis of eumelanins (Fig. 5). This phase starts with the slow chemical decarboxylation of dopachrome to 5,6-dihydroxyindole (DHI) and its subsequent oxidation to indole-5,6-quinone. As an alternative to this chemical evolution in the distal phase, dopachrome may be enzymatically transformed into 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by dopachrome tautomerase (Aroca et al. 1990; Pawelek 2006).

DHICA is further oxidized by a redox reaction with *o*-dopaquinone to form indole-5,6- quinone carboxylic acid, which can exist in three tautomeric forms, including the quinone-imine and the corresponding highly reactive quinone-methide (Lambert et al. 1989; Sugumaran and Semensi 1991). Properties of DHI-derived and DHICA-derived melanins differ in each other; the former are black and flocculent, while the latter are yellowish-brown and finely dispersed. This thiol group can be added to different ring positions, although the 5-position is the favored position. Subsequent cyclization and polymerization of cysteinyldopa or glutathionyldopa in an uncharacterized series of reactions result in the production of pheomelanins and trichochromes (Ito 1993). The interaction between the eumelanin and pheomelanin compounds gives rise to a heterogeneous pool of mixed-type melanins.

Melanin production

Melanins are a group of pigments formed by the polymerization of phenolic compounds. These polymers have been found in bacteria, plants and animals, where they help protect the organism from environmental stresses. The most common type of melanin is called eumelanin, which results from the tyrosinase-catalyzed oxidation of L-Tyrosine. As a result of its chemical composition, eumelanin has physicochemical properties that allow it to function as an UV absorber, cation exchanger, amorphous semiconductor and X-ray and X-ray absorber (Bell and Wheeler 1986). These properties impart eumelanin with many potential applications. For this reason, there has recently been considerable interest in finding and developing sources of eumelanin and other types of melanins. Melanins can be extracted and purified from plant and animal tissues (Sava et al. 2001). Although this is a relatively inexpensive method, maintaining reproducibility in the chemical composition of the product is difficult. Another source of melanins is microbes that have the natural capacity to synthesize these pigments (Aurstad and Dahle 1972; Hoti and Balaraman 1993; Mercado-Blanco et al. 1993; Kong et al. 2000a; Aghajanyan et al. 2005; Wang et al. 2006). The microbial production of melanins in liquid cultures under controlled conditions allows better control of the product's composition and purity. However, the capacity of microbial wild-type strains to synthesize melanin is usually very limited for commercial applications. For this reason, mutagenesis and recombinant DNA technology have been applied to generate microbial melanogenic production strains. *E. coli* lacks tyrosinase activity; therefore, to generate melanin-producing strains, a gene encoding this enzyme must be isolated from another organism and expressed in this host. In one case, the genes encoding a tyrosinase and a tyrosinase activator sequence from *Streptomyces antibioticus* were expressed in *E. coli*. The resulting *E. coli* recombinant strain produced eumelanin when L-Tyrosine was fed in the culture medium (Della-Cioppa et al. 1990). In a similar approach, the *melA* gene encoding a tyrosinase from the soil bacterium *Rhizobium etli* was isolated and expressed in *E. coli* (Cabrera-Valladares et al. 2006). A fermentation process using this recombinant strain produced 6 grams per liter of eumelanin in 80 h (Lagunas-Muñoz et al. 2006).

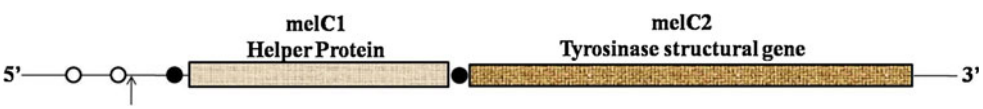

Fig. 4 Schematic drawing of the *mel* operon from *Streptomyces* sp

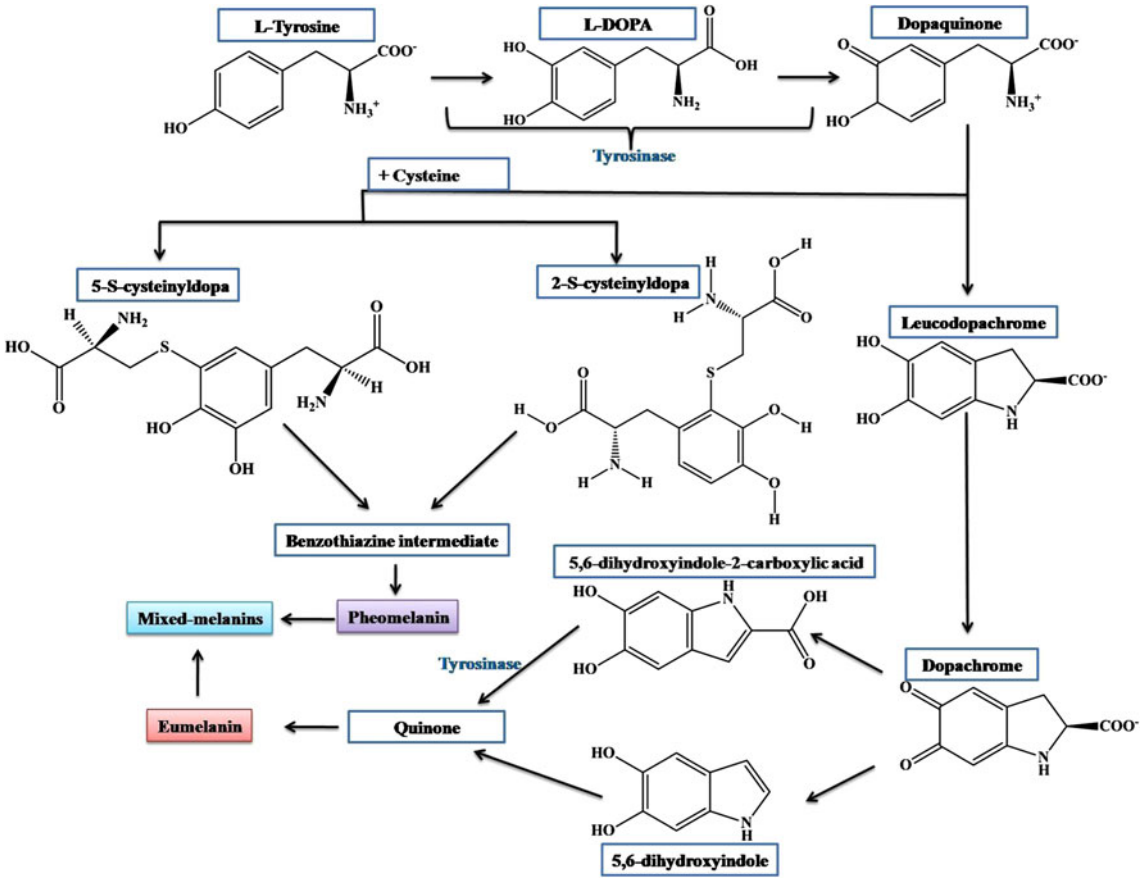

Fig. 5 Biosynthetic pathway of melanin

Purification of melanin

Procedures used for isolation and purification of melanin produced by the actinobacterial strains are shown in Fig. 6. The culture broth was harvested by centrifugation at 12,000 rpm for 10 min at 4 °C. The culture medium was centrifuged to remove the cells, and melanin was precipitated from the supernatant by adjusting the pH to 3.0 with 5 N HCl. The precipitated melanin was re-dissolved in distilled water at pH 8.0, centrifuged again and dialyzed against distilled water. The dialysis was conducted for at least 24 h and stopped when the dialyzed solution reached the pH 4.5. The dialyzed preparation of melanin was lyophilized (Sava et al. 2001). Further purification was carried out using ion exchange chromatography (Sephadex LH-20). The melanin powder was applied to a Sephadex LH-20 column, pre-equilibrated with 20 mM potassium phosphate buffer (pH 7.0). After washing the column with 3 vol. of equilibration buffer, bound melanins were eluted stepwise using phosphate buffers of increasing molarity and decreasing pH values at room temperature (approx. 25 °C). The flow rate was adjusted to 24 ml h^{-1} and fractions (1 ml each) were collected. The fractions showing high melanin content were pooled (Gadd 1982; David et al. 1996). To determine the UV–visible light absorption spectrum of purified melanin, 0.1 mg of sample was dissolved in alkaline distilled water at pH 8.0, and the resulting solution was scanned with a spectrophotometer (Shimadzu, UV-1800) to determine its absorption spectrum in the UV–visible light at wavelengths ranging from 200 to 800 nm. The purified melanin showed the absorption peak at 297 nm.

Tyrosinase inhibitors

Melanin plays a crucial protective role against skin photocarcinogenesis; however, the production of abnormal melanin pigmentation is a serious esthetic problem in human beings (Priestley 1993). The cytotoxicity of L-DOPA has been attributed to its selective uptake by melanocytic cells and to the formation of reactive quinones and semiquinones formed in situ during metabolic activation of L-DOPA by tyrosinase (Wick et al. 1977; Graham et al. 1978; Korytowski et al. 1987). The triphenolic amino acid TOPA also shows cytotoxic properties which seem to

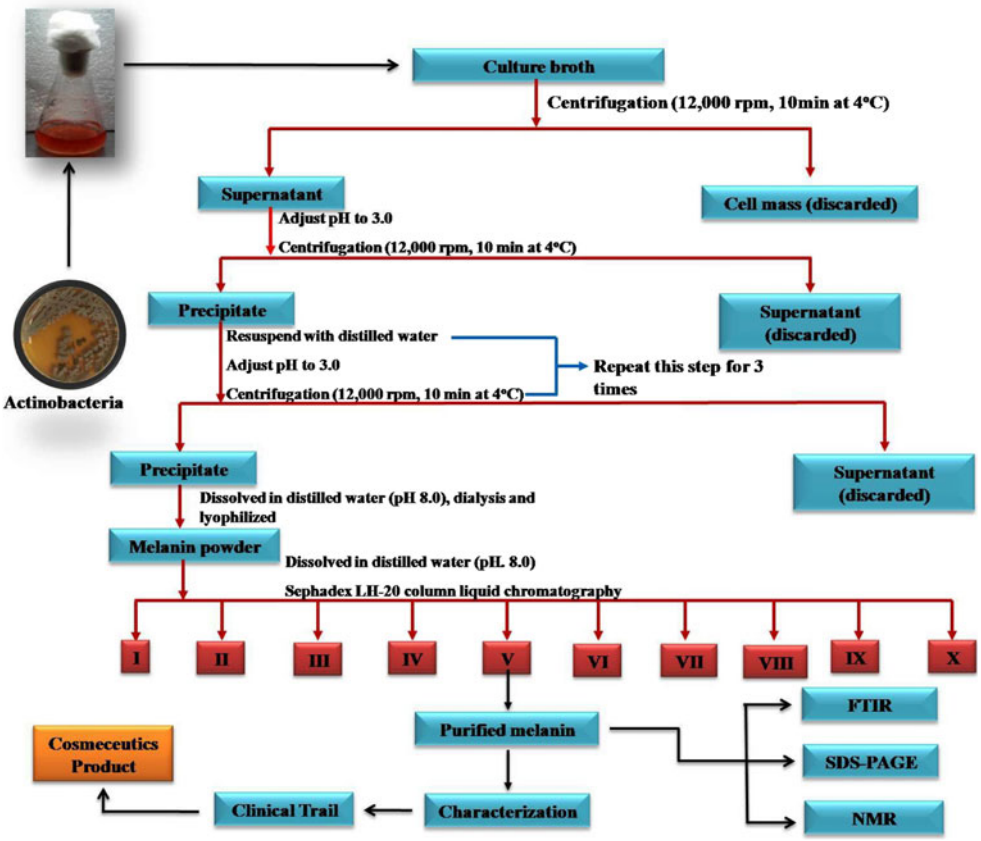

Fig. 6 Procedures for isolation, purification and applications of melanins

be due to the susceptibility of this substance to oxidation (Sachs and Jonsson 1975; Wick et al. 1979). It is possible that the cytotoxicity is mediated by oxygen radical or H_2O_2 formed on oxidation of TOPA (Heikkila and Cohen 1973). Another explanation of the cytotoxicity of TOPA could be the reaction of the quinone formed by oxidation of TOPA with nucleophilic groups of cellular macromolecules (Saner and Thoenen 1971; Rotman et al. 1976).

The oxidation products of L-DOPA and TOPA, quinones, are chemically reactive compounds that are potentially harmful, but in melanocytes the normal process of melanogenesis is not usually associated with significant toxicity due to the compartmentation of the reaction within membrane-limited organelles (melanosomes) and because of the rapid cyclization of the quinone intermediate. The discovery that certain substituted phenols have a depigmenting action due to their ability to act as substrates for tyrosinase, resulting in the generation of quinones, has led to the examination of this system as a possible targeted antimelanoma therapeutic strategy in the case of disseminated melanoma (Morgan et al. 1981; Riley 1991; Riley 2005; Naish et al. 2006). For such a chemotherapeutic agent to be useful, the prodrug must evade hepatic metabolism and other potentially toxic reactions (Schiller et al. 1991; Stolze and Nohl 1991), reach the tumor tissue and enter the malignant melanocytes. Moreover, this prodrug has to avoid alternative cellular metabolism and enter melanosomes, leading to oxidation by tyrosinase to generate significant amounts of the quinone product, which requires the ability to initiate reactions damaging to the melanoma cell. Melanin biosynthesis can be inhibited by avoiding UV exposure, the inhibition of tyrosinase, the inhibition of melanocyte metabolism and proliferation, or the removal of melanin with corneal ablation (Seiberg et al. 2000a; Seiberg et al. 2000b). Standard topical treatments for hyperpigmentation disorders such as melasma and postinflammatory hyperpigmentation include bleaching with hydroquinones, anti-inflammatory therapy by retinoids and use of tyrosinase inhibitors.

Biotechnological applications and importance of melanins

Agricultural and food fields

Amino-carbonyl and related interactions of food constituents encompass those changes commonly termed browning reactions. Specifically, reactions of amines, amino acids, peptides and proteins with quinones (enzymatic browning) cause deterioration of food during storage and commercial or domestic processing. The loss of nutritional quality is

attributed to the destruction of essential amino acids, decrease in digestibility, and inhibition of proteolytic and glycolytic enzymes. The production of antinutritional and toxic compounds may further reduce the nutritional value and possibly the safety of foods (Carpenter 1981). Thus, it is necessary to identify various methods to stop enzymatic browning caused by tyrosinase. Current conventional techniques to avoid browning include autoclave and blanching methods, whereby the food products were immersed in a liquid at 80–90 °C for 10–12 min or passed through a forced steam flow. These conventional processes are inherently linked to important weight and nutritional quality losses in the product (Konanayakam and Sastry 1988). One of the alternatives that have been proposed is microwave energy. The most restrictive factor for the application of microwave heating techniques to industrial blanching processes is the temperature gradient generated within the samples during microwave heating (Decareau and Mudgett 1985). Hence, the enzyme inactivation effect was exhibited in overheated areas, while in colder areas the enzyme might not completely be inactivated. To overcome this restriction, combined conventional microwave heating techniques are envisaged, since surface enzymes may not be completely inactivated by microwave treatment alone. The application of combined microwave hot-water treatment has shown some improvement on final quality, weight loss and processing times (Devece et al. 1999). Nevertheless, the rigorous control of microwave heating effects on enzymes requires homogenous heating of the sample and strict temperature control.

Compounds capable of inhibiting enzymatic browning in food products through the interference of tyrosinase-mediated reactions or through the reduction of *o*-quinones to *o*-diphenols have been identified (Langdon 1987; Santerre et al. 2006; Dudley and Hotchkiss, 2007). However, the number of chemicals that can actually be used in food systems to inhibit enzymatic browning is limited due to off-flavors, off-odors, toxicity and economic feasibility. Sulfiting agents have widely been used to prevent enzymatic browning in agricultural and seafood products. Due to health concerns, the use of sulfiting agents as food additives is being re-evaluated by the Food and Drug Administration (FDA) and, in some products, banned for use (FDA 1990). Most of the alternatives in the food industry are formulations of ascorbic and citric acids (Hsu et al. 2006). But they are less effective than sulfiting agents, since ascorbic acid is quickly consumed in the process of reducing quinones formed by tyrosinase. Recently the use of 4-hexylresorcinol is considered to be safe in the food industry and is quite effective in the prevention of shrimp melanosis and for browning control in fresh and dried fruit slices (Frankos et al. 1991; Iyengar et al. 1991; McEvily et al. 1992). However, as safety is of prime concern for an inhibitor to be used in the food industry, there is a constant search for better inhibitors from natural sources as they are largely free of any harmful side effects (Fig. 7).

Cosmetic and medicinal fields

Use of tyrosinase inhibitors is becoming increasingly important in the cosmetic and medicinal industries due to their preventive effect on pigmentation disorders. Tyrosinase inhibitors may result in a reduction in melanin biosynthesis and are used in cosmetic products for hyperpigmentation-related concerns, including the formation of freckles (Claus and Decker 2006). Tyrosinase and its inhibitors may also be targets for developing medicines to treat hypopigmentation-related problems, such as albinism and piebaldism. A number of tyrosinase inhibitors have been reported from both natural and synthetic sources, but only a few of them are used as skin-whitening agents, primarily due to various safety concerns (Kim and Uyama 2005; Kamkaen et al., 2007; Likhitwitayawuid 2008).

Among the skin-whitening agents, 1,4-dihydroquinone is one of the most widely prescribed (Dooley 1997; Hermanns et al. 2000). It causes reversible inhibition of cellular metabolism by affecting both DNA and RNA synthesis. It is also a poor substrate for tyrosinase, thereby competing for tyrosinase oxidation in active melanocytes. Hence, 1,4-dihydroquinone can be considered to be a potent melanocyte cytotoxic agent and has also been reported to induce mutations. As a result of these and other side effects, such as chronosis in African nations, the use of 1,4-dihydroquinone has been forbidden in cosmetics by most countries, and there has been increasing pressure to find alternative herbal and pharmaceutical depigmentation agents.

Currently arbutin and aloesin are used in the cosmetic industry as skin-whitening agents because they show strong inhibition toward tyrosinase, which is responsible for pigmentation in human beings. *b*-Arbutin inhibited both tyrosinase activities from mushroom and mouse melanoma noncompetitively, while *a*-arbutin only inhibited tyrosinase from mouse melanoma by mixed-type inhibition (Funayama et al. 1995). On the other hand, the co-treatment of arbutin and aloesin exhibited the inhibitory effect on tyrosinase in a synergistic manner by acting through different mechanisms: arbutin inhibited competitively, whereas aloesin inhibited noncompetitively (Jin et al. 1999). Taken together they inhibit melanin production synergistically by a combined mechanism of competitive and noncompetitive inhibition. This result indicates that it is beneficial to use arbutin and aloesin as a mixture in depigmentation because the co-treatment reduces the doses of these agents for the same inhibitory effect on tyrosinase activity and can diminish

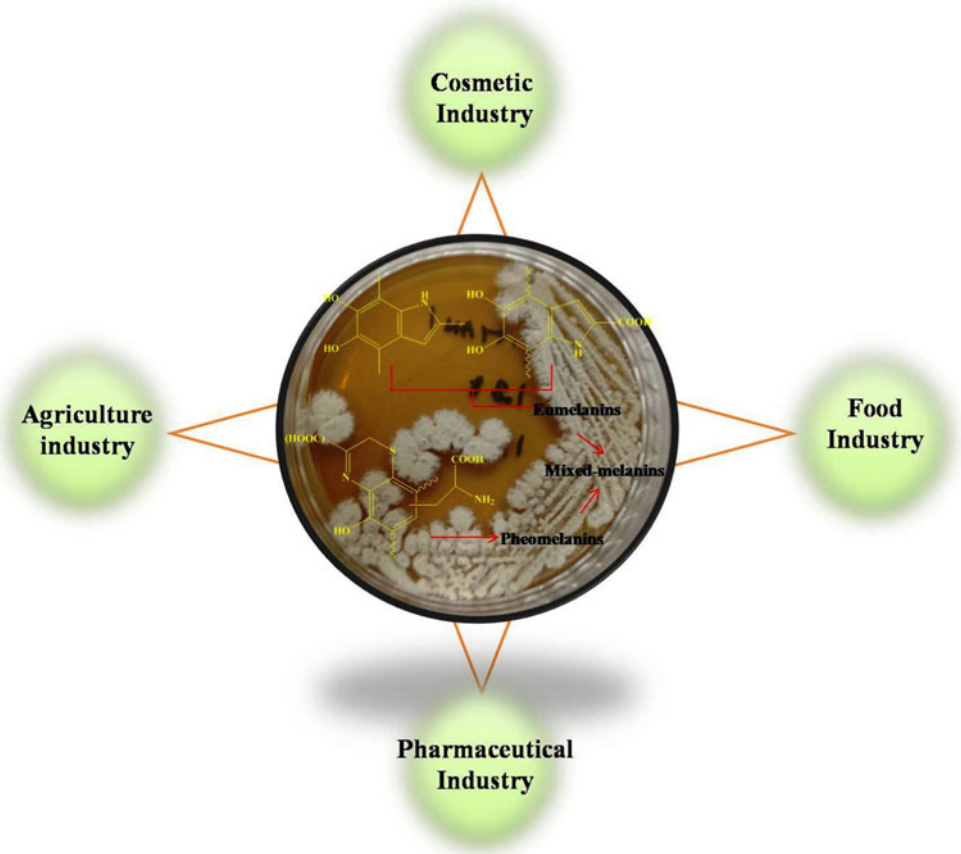

Fig. 7 Biotechnological application of melanins

adverse side effects. In addition, methimazole has proven effective as a depigmenting agent both in vitro and in vivo, and is noncytotoxic and nonmutagenic. Therefore, methimazole could serve as a lead compound for the discovery of safe and efficient skin depigmenting agents in the future (Fig. 7) (Kasraee et al. 2004).

Concluding remarks

In recent years, the harms of synthetic pigment to human body were found continuously; therefore, more and more natural pigments were used in cosmetic industry and food industry. In the international market the production of pigment is increasing at a rate of 10 % every year. In this review, we recovered the production, application and importance of melanins from actinobacteria.

Although the main objective of metabolic engineering is the rational design of producer strains, the current knowledge of cellular metabolism is still incomplete, which prevents precise predictions of the effects of specific genetic modifications. Thus, general random mutagenesis strategies are still valuable for generating melanins overproducing strains. The genomic and biochemical characterization of such mutants will help identify the modifications responsible for the increased melanins production phenotype, thus enabling the rational production of new strains by reverse engineering.

Strain generation is the first step in developing a melanins industrial production process. To obtain the best performance, the culture conditions must be designed for each particular strain phenotype. However, these types of optimization studies are scarce. Further research on this topic is necessary to increase our understanding of the behavior of the engineered strains in an industrial production context.

Tyrosinases are exceptionally versatile enzymes and more investigations are needed for a better understanding of their physiological importance and to further define their great biotechnological potential.

The discovery and characterization of new tyrosinase inhibitors are useful for their potential applications in improving food quality and nutritional value, controlling insect pests, and preventing pigmentation disorders and other melanin-related health problems in human beings. Additionally, understanding tyrosinase activators and inhibitors is of crucial importance in finding novel and more consumer-compatible approaches toward regulation of the discoloration process. To obtain better inhibitors, different types of compounds from both natural and synthetic sources have been investigated. However, on

balance, much more research on tyrosinase inhibitors is needed to confirm their structure and activity, and to improve their safety and effectiveness in various applications. On the whole, the melanin has shown the enormous development potentiality and it is hopeful to develop better methods to obtain melanin from natural sources. This review suggested that the melanin could potentially be used as a natural antioxidant in the food, cosmetic and pharmaceutical industries.

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