"An expert is a man who has made all the mistakes which can be made in a very narrow field"

- Edward Teller
2. Literature review

2.1. Melanin structure

Melanins are the polymers of phenolic compounds. In the structure of melanin indolic domains may be stacked by van der Waal’s interactions giving approximately 3.4 Å interlayer spacing in X-ray diffraction (Thathachari, 1976), but the irregular interposition of other residues makes many regions of the polymer essentially amorphous. The indolic melanin shows 4'–7 linked substituted indoles indicating the high degree of conjugation of such domains. Carboxylic acid groups are attached to the C2 and the S6 functionalities are shown as carbonyls in the orthoquinone form, deprotonated hydroxyls in the catecholic form, and an equilibrium form of linked semiquinones (Figure 1). The three major functions are indicated as electronic reactions, which render the polymer a potential free radical generator or scavenger, photon absorption and cation binding through the carboxyl groups. Some cationic bindings may involve deprotonated hydroxyl groups or semiquinones (Riley, 1997).

![Figure 1. Structure of melanin (Riley, 1997)](image-url)
2.2 Melanin synthesis pathways in microorganisms

There are three main types of melanin: Eumelanins (black or brown) which are produced in the course of oxidation of tyrosine to o-dihydroxyphenylalanine (DOPA) and dopaquionone (Figure 2), which further undergoes cyclization to 5,6-dihydroxyindole (DHI) or 5,6-dihydroxyindole-2-carboxylic acid (DHICA) (del Marmol and Beermann, 1996; Langfelder et al., 2003).

Allomelanins are the least studied and the most heterogeneous group of polymers, which emerge through oxidation or polymerization of di-(DHN) or tetrahydroxynaphthalene, via the pentaketide pathway leading through flavioline to various colored polymers of DHN-melanins (Figure 3A), and homogentisic acid (pyomelanins) (Figure 3B) (Gibello et al., 1995; Kotob et al., 1995; Espin et al., 1999; Funa et al., 1999; Jacobson, 2000).

Pheomelanins (yellow-red) which are initially synthesized just like eumelanins (Figure 4), but DOPA undergoes cysteinylation, directly or by the mediation of glutathione. The end product of this reaction, cysteinylDOPA, further polymerizes into various derivatives of benzothiazines (Kobayashi et al., 1995; Nappi and Ottaviani, 2000).

In general, pheomelanins contain sulphur, whereas most types of allomelanins do not contain nitrogen. The second important difference concerns configuration of quinone or quinonimine residues in melanins, ortho configuration is present in eumelanins and pheomelanins, and para (polymers of \(\gamma\)-glutaminyl-4-hydroxybenzene) or meta (polymers of DHN) present in allomelanins (Plonka and Grabacka, 2006).
A novel approach for biotransformation of L-tyrosine to melanin by microbial system

Figure 2. Eumelanin synthesis; T, Tyrosinase; TRP1, Tyrosinase related protein 1; TRP2, Tyrosinase related protein 2; DOPA, 3, 4-dihydroxyphenylalanine; DHI, 5, 6-dihydroxy indole; DHICA, 5, 6-dihydroxyindole-2-carboxylic acid (Plonka and Grabacka, 2006)
Figure 3. Allomelanins synthesis (A) synthesis of DHN melanin. PKS, polyketide synthase; 1,3,6,8-THN, 1,3,6,8-tetrahydroxynaphthalene; 1,3,6-THN, 1,3,6-trihydroxy naphthalene; 1,8-DHN, 1,8-dihydroxynaphthalene; (B) synthesis of pyomelanin from tyrosine, TyrAT, tyrosyl aminotransferase; HPPD, hydroxyl phenylpyruvate dehydrogenase (Plonka and Grabacka, 2006)
2.3 Microbial enzymes of melanogenesis

2.3.1 Tyrosinase

Tyrosinases (E.C. 1.14.18.1) are copper-containing enzymes which are ubiquitously distributed in nature (Mayer and Harel, 1978; Lerch, 1995). They are essential for the formation of melanin and various other functions (Butler and Day, 1998; Nosanchuk and Casadevall, 2003). In plants, sponges and many invertebrates, they are important components of wound healing and the primary immune response (Gelder et al., 1997; Muller et al., 2004; Cerenius and Soderhall, 2004). In arthropods they are also involved in sclerotization of the cuticle after molting or injury. In mammals tyrosinases are found in melanocytes of the retina and skin (Garcia-Borro and Solano, 2002). Tyrosinases use molecular oxygen to catalyze two different enzymatic reactions (Figure 5) (i) the ortho-hydroxylation of monophenols to o-diphenols (monophenolase, cresolase activity) and (ii) the oxidation of o-diphenols to o-quinones (diphenolase, catecholase activity). The reactive quinones polymerize nonenzymatically to the macromolecular melanins (Lerch, 1995; Solomon et al., 1996; Land et al., 2003). It should be noted that often the name phenoloxidase is used.
Literature review

A novel approach for biotransformation of L-tyrosine to melanin by microbial system

in literature, which summarizes tyrosinases, catecholoxidases and laccases as well. Because of their overlapping substrate specificities, a positive test for catecholase activity does not necessarily mean that the enzymes exhibit cresolase activity. In spite of intensive biochemical investigations since decades, there exist only limited informations about the protein structure and the exact reaction mechanisms.

**monophenolase activity (Cresolase)**

\[
\begin{align*}
\text{monophenol} & \quad + \quad \frac{1}{2} \text{O}_2 \\
\rightarrow & \\
\text{o-diphenol}
\end{align*}
\]

**diphenolase activity (Catecholase)**

\[
\begin{align*}
\text{o-diphenol} & \quad + \quad \frac{1}{2} \text{O}_2 \\
\rightarrow & \\
\text{o-diquinone} + \text{H}_2\text{O}
\end{align*}
\]

**Figure 5.** Monophenolase and diphenolase activity of tyrosinase (Claus and Decker, 2006)

Some reasons for these deficits are difficulties in purification of sufficiently high amounts of tyrosinases from eukaryotic sources due to low enzyme concentrations, contamination with pigments and occurrence of isoenzymes or post-translational modifications. The copper binding sites of tyrosinases share a high sequence homology with the haemocyanins, the oxygen carrier proteins of the mollusks and arthropods (Gelder et al., 1997; Decker and Tuczek, 2000; Holde et al., 2001). During evolution, a functional change of this protein family has been proposed from enzymatic oxygen detoxification towards oxygen transport (Jaenicke and Decker, 2004). The common feature is a ‘type 3 copper centres’, a diamagnetic spin-coupled copper pair (Lerch, 1995; Solomon et al., 1996; Land et al., 2003). Each of the two metal atoms, CuA and CuB, of the active site are coordinated by three conserved histidines which are located in a ‘four a-helix bundle’. During the catalytic
cycle the ‘type 3 copper centre’ can adopt different functional forms: the oxy-state \([\text{Cu (II)} - \text{O}_2 - 2\text{Cu (II)}]\), deoxy-state \([\text{Cu (I)} \text{Cu (I)}]\), half-met state \([\text{Cu(I)}\text{Cu(II)}]\) and the met state \([\text{Cu(II)} - \text{OH-Cu(II)}]\). In the latter case the two copper atoms are bridged by hydroxy ions. The valences of the two copper atoms change from Cu (I) to Cu (II), which can be followed spectroscopically. In the oxy-state the molecular oxygen is reversibly bound as peroxide between the two copper atoms in a ‘side-on’ conformation. In the absence of any substrate more than 85% of the enzyme is in the met state, which can be regarded as the resting form of tyrosinase. According to current conceptions, both, the met and the oxy state of tyrosinases enable the diphenoloxidase activity, whereas the monohydroxylase reaction requires the oxy state (Claus and Decker, 2006).

2.3.2 Laccase

Melanization in *Streptomyces* species and other microorganisms not only catalyzed by tyrosinases but also via different pathways and enzymes which include laccase (Butler and Day, 1998; Castro-Sowinski et al., 2002). Laccases (EC1.10.3.2, p-diphenol: dioxygen oxidoreductases) are multi-copper proteins that use molecular oxygen to oxidize various aromatic and non-aromatic compounds. (Solomon et al., 1996; Claus, 2003). Until recently, laccases have been only found in eukaryotes (fungi, higher plants and insects) but now there is strong evidence for their wide distribution in prokaryotes (Claus, 2003). For the catalytic activity a minimum of four copper atoms (type 1, type 2 and type 3) per active protein unit is needed (Solomon et al., 1996; Claus, 2003). The occurrence of a spin coupled copper pair (type 3 copper) is the common feature of the multi-copper oxidases and the protein super family of tyrosinases and haemocyanins (Lerch, 1995; Decker and Tuczek, 2000). In contrast to tyrosinases, laccases exhibit no monophenol hydroxylase activity, but oxidize phenols by a radical-generating reaction mechanism (Claus, 2003). Some microorganisms show both tyrosinase and laccase activities, and care must be taken to avoid confusion because of overlapping substrate specificities. In *Sinorhizobium meliloti*, a plasmid-encoded tyrosinase and a laccase have been demonstrated (Mercado-Blanco et al., 1993; Castro-Sowinski et al., 2002). In addition to a typical tyrosinase, a ‘multipotent’ phenoloxidase with both tyrosinase and laccase activities has been purified from the marine bacterium *Marinomanas mediterranea*. The protein
shows some extra histidine-rich copper-binding domains that are very likely related to its unique enzymatic properties (Sanchez-Amat et al., 2001). The phenoxazinone synthase of *Streptomyces antibioticus* which is involved in the biosynthesis of actinomycin is a multi-copper enzyme with laccase activity (Freeman et al., 1993). In the meanwhile, more laccases have been isolated and characterized from *Streptomyces cyaneus* (Arias et al., 2003), *Streptomyces griseus* (Endo et al., 2003) and *Streptomyces lavendulae* (Suzuki et al., 2003).

### 2.3.3 Polyketide synthases (PKS)

It produces DHN melanins, and belongs to an old family of multidomain proteins related to the animal fatty acid synthases (Kroken et al., 2003). The PKS family is diversified and plentiful. Numerous microorganisms employ these enzymes to produce pigments, antibiotics, toxins and other products of intermediate metabolism (Hutchinson, 2003; Snyder et al., 2003). There are only few DHN-melanin-producing PKS enzymes, which belong to the PKS-type-I group producing aromatic, not reduced polyketides (Kroken et al., 2003).

### 2.4 Properties of Melanin

#### 2.4.1 Light absorbance

The melanin polymer has many interesting properties, among which the most conspicuous is the wide spectral absorbance due to high degree of conjugation in the molecule. The darkness of the pigment is a result of the fact that much of the visible spectrum is absorbed, including radiation with low quantal energy. The lowest energy transitions are from nonbonding to anti-bonding pi-orbitals that occur predominantly in carbonyl bonds, which are abundant in most melanins. Melanins also absorb in the ultra-violet (UV) region of the spectrum, involving transitions from bonding to anti-bonding pi-orbitals, which occur in unsaturated carbon bonds. Transitions from bonding to anti-bonding orbitals are facilitated by conjugation permitting electronic delocalization. As the degree of conjugation increases, lower quanta energies are required for absorption; an effect termed bathochromicity. The majority of the visible spectral energy absorbed by melanin is converted into heat through photon-phonon coupling. Melanins with high levels of indole quinones (eumelanins) appear darker
because of the strong absorbance in the red part of the spectrum. This low frequency light absorption is largely through the carbonyls (Riley 1997).

2.4.2 Redox properties

Melanins, especially eumelanins, exhibit marked redox properties, and electron delocalization between orthoquinone and catecholic moieties of the polymer give rise to semiquinone free radicals, which can be detected by electron spin resonance spectroscopy (Sealy et al., 1980). Melanins can take part in one-electron and two-electron redox reactions and one of the effects of light absorption is photo-oxidation of the pigment, which, by increasing the carbonyl content, changes the absorbance properties of melanin, the so called immediate pigment darkening (IPD) reaction. This photo-oxidation process generates super oxide radicals (Sarna and Sealy, 1984). Melanins also have powerful cation chelating properties (Sarna et al., 1976) through the anionic functions such as the carboxyl and the deprotonated hydroxyl groups.

2.5 Roles of melanin synthesis in microorganisms

2.5.1 Electron acceptor

In the process of anaerobic respiration, reducing, dissimilating bacteria use a wide spectrum of electron acceptors replacing dioxygen in the last step of the respiratory chain. Among the substances utilized as alternative electron acceptors, there are mainly hydrated ferrous oxide, nitrates, sulphates, as well as organic compounds, in particular the ones containing quinone groups, e.g. related to melanin humic substances (Coates et al., 2002). Melanin, similarly to humic substances, is a polymer of various groups able to donate or to accept an electron. Therefore it can act as a final acceptor or a shuttle in the electron exchange with insoluble compounds of iron (Menter and Willis, 1997).

The facultatively anaerobic bacterium *Shewanella algae* produce pyomelanin and reduce it simultaneously with the oxidation of gaseous hydrogen. As *Shewanella algae* is unable to carry out fermentation, its survival in the conditions of variable oxygen concentration strongly depends on the presence of appropriate electron acceptors. In the mineralized marine deposits the availability of such soluble
compounds is limited; therefore production of melanin is an important evolutionary adaptation. Moreover, like other organisms, *Shewanella algae* produces melanin also for the protection from ultraviolet irradiation (Turick et al., 2002).

2.5.2 Virulence factors

2.5.2.1 Escaping respiratory burst

The pathogenic bacterium *Burkholderia cepacia* serves as an example of how melanin production increases virulence (Zughaier et al., 1999). This microorganism causes serious lung infections, which often develop to sepsis, mainly due to the presence of lipo-polysaccharide (LPS) which strongly enhances production and release of pro inflammatory cytokines. Although LPS does not trigger an oxygen burst directly, it stimulates the immunological system to an accelerated oxidative response to other stimuli. Melanin isolated from *Burkholderia cepacia* reveals a dose-dependent ability to sweep oxygen radical $\text{O}_2^-$ produced by leukocytes during oxygen burst. However, melanin does not influence the release or kinetics of the production of reactive oxygen species (ROS). The ability to remove superoxide anion allows *Burkholderia cepacia* to survive phagocytosis, so the host phagocytes are not able to eliminate the pathogen. They remain in the state of a permanent stimulation by the bacterial LPS, which causes a chronic inflammation. Similarly to *Legionella pneumonias*, *Burkholderia cepacia* is able not only to survive in the phagocytes (alveolar macrophages), but also to proliferate intracellularly, which leads to cell destruction and the infection of secondary macrophages (Saini et al., 1999; Abu-Zant et al., 2005).

2.5.2.2 Escaping phagocytosis

The ability to produce melanin as an important factor of virulence is well documented and confirmed *in vivo* for *Cryptococcus neoformans*. The presence of melanin in the cell wall of *C. neoformans* is correlated with less efficient phagocytosis, both in the case of *C. neoformans*, and *S. schenckii* (Nosanchuk and Casadevall, 1997; Romero-Martinez et al., 2000). A likely explanation is that phagocytosis is impaired by the decrease of the negative electric charge of the cell
A novel approach for biotransformation of L-tyrosine to melanin by microbial system

wall, which is caused by melanin deposition (Wang et al., 1995; Nosanchuk and Casadevall, 1997).

2.5.2.3 Antibiotic resistance

The melanin can increase the resistance of pathogenic microbes though binding with potential antibiotics such as tetracycline and vancomycin (Ikeda et al., 2003; Lin et al., 2005; Nosanchuk and Casadevall, 2006).

2.5.2.4 Resistance against UV and gamma radiations

Melanins confer resistance to UV light by absorbing a broad range of the electromagnetic spectrum and preventing photo induced damage (Hill, 1992). Melanin protects several fungal and bacterial species from UV, solar or gamma radiation. Increased melanin production is associated with the greater resistance of pigmented fungi to radiation (Vasilevskaya, 1970; Zhdanova et al., 1973). The protective properties of melanin against radiation injury could account for the growth of black fungi in the highly contaminated atomic reactors (Nosanchuk and Casadevall, 2006).

2.5.2.5 Heavy metal resistance

Melanins are able to bind with the heavy metals that are routinely found in the environment (Zunino and Martin, 1977; Rizzo et al., 1992; Fogarty and Tobin, 1996). The carboxyl, phenolic, hydroxyl, and amine groups on melanin provide numerous potential binding sites for metal ions (Fogarty and Tobin, 1996). Melanized Cryptococcus neoformans cells are more resistant to killing by silver nitrate, a compound highly toxic to bacteria and fungi, than nonmelanized cells (Garcia-Rivera and Casadevall, 2001).

2.6 Applications of melanin

2.6.1 Cosmetics

The melanin is used in Sun screen creams and other skin whitening creams basically for its UV radiations protecting and free radical scavenging properties. It is
also used in photoprotective eye glasses (Riley 1997; Nosanchuk and Casadevall, 2006). The inhibitory effect of skin whitening creams was studied earlier on L-DOPA oxidation and tyrosinase activity. The melanin producing microorganisms can be used as test organism to find out the skin whitening agents (Jeon et al., 2005).

### 2.6.2 Bioinsecticide protection from UV radiations

The bioisecticidal endotoxin produced from *Bacillus thuringiensis* is easily gets inactivated by solar radiation in nature. Natural sunlight, especially the UV-A (400 nm to 320 nm) and UV-B (320 nm to 290 nm) portions of the spectrum, is responsible for the inactivation of microbial insecticides (Pusztai et al., 1991). The melanin produced from *Aeromonas media* and *Bacillus cereus* 58 when added with bioinsecticidal preparations the result indicates that the melanin can protect the insecticidal crystal proteins from degradation caused by UV radiation (Wan et al., 2007; Zhang et al., 2007)

### 2.6.3 Vaccine against human melanocyte cancer (Melanoma)

The melanin can be used as vaccine against melanoma (Human melanocyte cancer). Melanoma is a malignant tumor of melanocytes which are found predominantly in skin but also in the bowel and the eye. It is one of the less common types of skin cancer but causes the majority (75%) of skin cancer related deaths. Malignant melanoma of skin accounts for 1,60,000 new cases annually and in the United States, about one in 150 people will develop a malignant melanoma during their lifetime (Parkin et al., 2005; Jemal et al., 2007). The lymphocytes of melanoma patients can be restimulated in vitro with autologous tumor cells to generate antitumor cytolytic T lymphocytes (CTL) such antitumor CTL clones which appear to recognize melanin as antigen. Also when blood lymphocytes of melanoma patients are stimulated in vitro with tumor cells of the same patient, one often observes the proliferation of T lymphocytes that exert cytolytic activity on the autologous melanoma cells (Brichard et al., 1993). The antibody response to fungal melanin was showed that melanin can be immunogenic, and the humoral immune response is T cell independent. The melanin antigen may therefore constitute a useful target for specific immunotherapy of melanoma. This clearly indicates that melanin can be used as vaccine against the human melanocyte cancer (Nosanchuk et al., 1998).
2.6.4 Anti-HIV property

The melanin in solution inhibits the HIV (Human immunodeficiency virus) by blocking syncytium formation between viral surface glycoprotein gp120 and surface of uninfected cell. Also the anti-HIV property was found due to the gp120-CD4 binding, thus melanin could prove to be a new class of pharmacologically active substances with possible utility as anti-HIV therapeutics (Montefiori and Zhou 1991).

2.6.5 Bioremediation of radioactive waste

Microbial melanin production by bacteria was used to increased uranium immobilization in uranium contaminated soil. In order to develop an in situ, uranium bio-immobilization technology the one-time addition of tyrosine to soil exploited the ability of indigenous microbes to produce pyomelanin, resulting in uranium immobilization. Thus melanin producing bacteria can be used for bioremediation of radioactive waste (Turick et al., 2008).

2.6.6 Reporter gene

The genes responsible for the melanin synthesis from bacteria were used as a reporter gene to screen the recombination in host bacteria. The production of melanin on tyrosine agar indicates the wild type while the absence of melanin indicates the desired gene was transferred. Thus melanin producing genes can be a best alternative to generally used blue white screening method in *E. coli*. (Tseng et al., 1990, Adham et al., 2003).

2.7 Melanin producing microorganisms

Melanin synthesis ability is generally found in pathogenic strains of bacteria. The melanin synthesis using homogentisic acid as a precursor of was first reported in *Vibrio cholerae*, *Hyphomonas* species and *Shewanella colwelliana* (Kotob et al., 1995). The synthesis of melanin and its characterization such as solubility, free radical nature was initially studied in *Proteus mirabilis* (Agodi et al., 1996). A novel marine bacterium *Alteromonas* strain MMB-1, was isolated from the Mediterranean Sea and its melanin synthesis ability was studied using L-tyrosine as a precursor previously (Solano et al., 1997). The melanin pigment from *Burkholderia cepacia* was formerly
reported for escaping monocyte respiratory burst activity by scavenging superoxide anion (Zughaier et al., 1999).

The extra cellular melanin from *Shewanella algae* BrY was reported previously to serve as the sole terminal electron acceptor. Upon reduction the reduced, soluble melanin reduced insoluble hydrous ferric oxide in the absence of bacteria, and melanin was proved as a soluble Fe (III)-reducing compound (Turick et al. 2002). Melanin production was earlier studied by UV-resistant mutant of *Bacillus thuringiensis* subsp. *Kurstaki* and its UV-protection ability for insecticidal crystals was tested (Saxena et al., 2002). The thermo tolerant strains of *Bacillus thuringiensis* were also reported for melanin production (Ruan et al., 2004). A wild strain of *Bacillus thuringiensis* subsp. *dendrolimus* L-7601 producing melanin was reported and the UV-protection efficacy of melanin on insecticide formulations following UV irradiation was studied formerly (Chen et al., 2004). A hexahydroxy perylenequinone melanin was produced earlier from *Streptomyces griseus* by employing biosynthetic pathway involving a type III polyketide synthase (PKS), RppA, and a cytochrome P-450 enzyme, P-450mel (Funa et al., 2005).

The purification of water-soluble melanin was reported first time from *Bacillus thuringiensis* subsp. *galleriae* strain K1 which was carried out using different sorbants such as activated charcoal, CM cellulose, silica gel C-25I and Dowex (Aghajanyan et al. 2005). The induction of melanin synthesis in *Cryptococcus neoformans* by *Klebsiella aerogenes* was found in which colorless colonies of *C. neoformans* grown near to *K. aerogenes* colonies were observed to produce melanin. This study concluded that precursor for melanin synthesis was produced by *C. neoformans* (Frases et al., 2006). The optimization of physico-chemical parameters for the melanin production was studied formerly in *E. coli* W3110 (Lagunas-Munoz et al., 2006). The melanin synthesis was also reported earlier from *Streptomyces* species (Dastager et al., 2006). The synthesis of pyomelanin was reported previously from *Burkholderia cenocepacia* C5424 by using a homogentisate intermediate and the antioxidant properties of melanin were also studied in this strain (Keith et al., 2007). The high level of melanin was produced earlier from novel species of *Aeromonas media* and offers effective photo protection of a commercial bioinsecticide against UV radiation (Wan et al., 2007). The production of water-soluble melanin was reported recently from recombinant deep-Sea sediment meta-genomic clone of *E. coli*
The production of melanin was reported recently from *Klebsiella* sp. GSK (Shrishailnath et al., 2010).

The pathogenic fungal species reported earlier to produce melanin includes *Cryptococcus neoformans* which indicates that melanin production was virulence associated (Nosanchuk and Casadevall, 1997). The melanin synthesis was detected previously in the dimorphic fungal pathogen *Paracoccidioides brasiliensis* in vitro and during infection (Gomez et al. 2001). The melanin associated with cell wall was first demonstrated and its characterization was carried out in *Pneumocystis carinii* (Icenhour et al., 2003).

The production of melanin in vitro and in vivo was studied previously from *Aspergillus fumigatus* conidia. (Youngchim et al., 2004). The melanin synthesis from homogentisic acid was studied earlier by using *Cryptococcus neoformans* and characterization of melanin was carried out (Frases et al. 2007). A comparative studies of fungal melanin and humin like substances was recently carried out from *Cerrena maxima* 0275 (Koroleva et al., 2007). The synthesis of melanin was recently demonstrated in *Coccidioides posadasii* arthroconidia, spherules, and endospores produce *in vitro* and tissue forms (Nosanchuk et al., 2007). The in vivo melanin biosynthesis in *Madurella mycetomatis* and its effect on susceptibility to itaconazole and ketoconazole were studied formerly in brief (Sande et al., 2007).

### 2.8 L-DOPA production

L-DOPA is an intermediate produced during initial step of melanin synthesis from L-tyrosine. It is an amino acid analogue produced by one step reaction from L-tyrosine. L-DOPA is widely used as drug for the treatment of Parkinson’s disease (PD). The PD is associated with diminished level of dopamine in brain. L-DOPA is a metabolic precursor of dopamine, can easily cross the blood brain barrier and finally converted in to dopamine. L-DOPA can be orally administered to relive symptoms of Parkinson's disease.

#### 2.8.1 Function of L-DOPA in human body

The functions of L-DOPA in humans include a precursor of the catecholamine neurotransmitters dopamine, nor-epinephrine and epinephrine (Figure 6) (Fling and Paul, 2001; Ali and Haq, 2006a). It acts as an important modulator of renal dopamine.
A novel approach for biotransformation of L-tyrosine to melanin by microbial system

The oxidation products of L-DOPA prevent \( \text{H}_2\text{O}_2 \) induced oxidative damage to cellular DNA by enhancing the cellular antioxidant defense mechanisms (Toyama et al., 2003; Ali and Haq, 2006a). Although the chemical synthesis of L-DOPA was reported (Knowles, 2003; Valdes et al., 2004), it is also produced by various biological systems. The biological means includes such as extraction from seeds and by using microbial sources and enzymes.

![Chemical diagram of L-DOPA metabolism](http://en.wikipedia.org)
2.8.2 Plant sources and cell suspension cultures

The L-DOPA is conventionally extracted from the seeds of *Mucuna pruriens* seeds and *Vicia faba* beans (Chattopadhyay et al., 1994, Shetty et al., 2001). The L-DOPA content from *Vicia faba* beans was reported previously to be enhanced by using food grade elicitors, gellan gum, and a polysaccharide from *Pseudomonas elodea* and xanthan gum from *Xanthomonas campestris* (Shetty et al., 2001). The cell suspension culture *Mucuna pruriens* was also studied earlier for L-DOPA production in buffer containing L-tyrosine (Chattopadhyay et al., 1994). The *Portulaca grandiflora* cell suspension culture was formerly reported for L-DOPA production. This callus cultures were induced in MS medium provided with growth regulators such as benzylaminopurine and 2, 4-dichloro-phenoxyacetic acid (Rani et al., 2007). Also banana cell suspension cultures were reported earlier for potential L-DOPA synthesis (Bapat et al., 2000).

2.8.3 Microbial sources

The production of L-DOPA by microbiological methods has been reported by two processes one is production in complex synthetic medium and other is in the reaction mixture containing L-tyrosine and biomass. The two bacteria *Vibrio tyrosinaticus* and *Pseudomonas melanogenum* were reported earlier for producing good yield of L-DOPA in complex medium with intermittent addition of L-tyrosine. These reports also describe the effects of various media components and substrates of L-DOPA (Yoshida et al., 1973; Yoshida et al. 1974).

An actinomycete species was formerly isolated from soil after subjecting to the chemical mutagenesis by N-methyl-N-nitro-N' nitrosoguanidine (NTG), the resultant mutant strain was reported for potential L-DOPA production (Sukumaram et al., 1979).

The novel fungal species *Acremonium rutilum* was reported earlier for optimization of media conditions using potato dextrose broth for L-DOPA production. (Krishnaveni et al., 2009). The Egyptian halophilic black yeast was reported recently for L-DOPA production in a broth containing nitrogen source like ram horn hydrolysate (Mahmoud and Bendary, 2010).
The other method for L-DOPA production was, employing grown biomass in a reaction buffer containing L-tyrosine. This L-DOPA production by using this method first reported by mutant fungal strain Aspergillus oryzae was (Haq et al., 2002). L-DOPA yield was further improved by adding the reaction mixture with specific enhancer cresquinone (Haq et al., 2003). The double mutation of Aspergillus oryzae by using UV radiations and chemical mutagen NTG were reported previously for enhanced production of L-DOPA (Ali et al., 2005). The effect of a clay mineral illite on conversion of L-tyrosine to L-DOPA by Aspergillus oryzae ME2 under acidic reaction conditions was formerly observed (Ali and Haq, 2006a). The enhanced L-DOPA production and kinetic basis of another clay mineral celite was studied earlier using A. oryzae ME2 (Ali and Haq, 2006b). The yeast Yarrowia lipolytica cell mass in tyrosine containing reaction buffer was reported recently for bioconversion of L-tyrosine to L-DOPA with addition of diatomite (Ali et al., 2007). The L-DOPA production from catechol and pyrochatechol in reaction mixture using Erwinia herbicola cell mass was studied (Koyanagi et al., 2005).

2.8.4 Enzyme immobilization

The Escherichia intermedia cells were immobilized previously by entrapment in carrageenan to produce L-DOPA from catechol, pyruvate and ammonia (Para and Baratti, 1988). The production of L-DOPA from mushroom tyrosinase immobilized zeolite was earlier studied by using glutaraldehyde as a cross-linking agent (Seetharam and Saville, 2002). The mushroom tyrosinase was immobilized formerly on modified polystyrene-polyamino styrene (PSNH) and polymethylchloride styrene (PSCL) with glutaraldehyde as an activating agent for L-DOPA production from L-tyrosine (Ho et al., 2003). The production of L-DOPA was studied recently by using tyrosinase immobilized in copper-alginate gels by using batch and packed bed reactors (Ates et al., 2007).
2.10 Research objectives and thesis outline

- Isolation, screening and identification of bacterial species for production of melanin and L-DOPA
- Standardization of nutritional parameters for melanin production from isolated high yielding bacterial species.
- Purification and characterization of melanin by using analytical technique viz. UV-Vis spectroscopy, FTIR and EPR.
- Comparison of melanin yields from isolated bacterial species.
- Microbial transformation of L-tyrosine to melanin by using cells of bacterial species.
- Standardization of nutritional parameters for L-DOPA production from isolated high yielding bacterial species.
- Comparison of L-DOPA yields from isolated bacterial species.
- Bioconversion of L-tyrosine to L-DOPA by using cells of bacterial species.
- Analysis of produced L-DOPA by using techniques viz. HPTLC, HPLC and GCMS.
- Purification and primary characterization of tyrosinase from melanogenic bacterial species.