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Properties, various functions and application of bacterial melanins – some properties and possible application of melanin produced by *Azotobacter chroococcum*

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Abstract. Melanins are a group of macromolecular pigments formed as a result of oxidative polymerization of phenolic and indole compounds. They are considered the most common, heterogeneous, resistant and evolutionarily oldest pigments found in nature. Melanins occurrred very early in the evolution of various groups of organisms. They have already been found in dinosaur, bird and primitive cephalopod fossils. Today, melanins are found in every kingdom of living organisms and play an important role in the processes of reproduction, thermoregulation, chemoprotection and camouflage. In addition to the important functions they perform in organisms, they exhibit a different chemical structure and are characterized by a wide range of colors, from black-brown to yellow-red. Differences in chemical structure have become the criterion for dividing melanins into four groups, i.e. eumelanin, allomelanin, pheomelanin and neuromelanin. Production of melanins is characteristic for many microorganisms, including free-living *Azotobacter* bacteria. The genus *Azotobacter* comprises eight species and only *Azotobacter bryophylli* does not produce pigments. *Azotobacter chroococcum*, the most abundant in soils all over the world, produces a dark brown melanin pigment non-diffusible into the substrate. Melanins synthesized by this species of bacteria increased the growth of some plants and detoxification of soils and waters polluted with heavy metals. In addition, the method of obtaining melanin produced by *A. chroococcum* is simple and relatively cheap compared to the cost of obtaining synthetic melanins, which gives the opportunity to conduct further research on the use of this pigment in biotechnology and molecular biology.
In work it was describe the physicochemical properties, various functions and possible applications of bacterial melanins in various industries. The publication also summarizes the current knowledge on some properties and the possibility of use in bioremediation of soils and waters contaminated with heavy metals melanins synthesized by *Azotobacter chroococcum*.

Key words: bacterial melanin, polyphenol oxidase, stress protection, biosynthesis, *Azotobacter chroococcum*

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INTRODUCTION

Natural pigments are extracted from biological material of plant, animal and microbial origin (Stolarzewicz et al., 2012). Many bacterial species can synthesize pigments. Colorful bacteria outcompete species and mutants that do not produce pigments. In the case of bacteria, the benefits of pigment production are the acquisition of energy and food, the extraction of ferric from the habitat, protection from the harmful effects of high-concentration visible light, ultraviolet radiation, extreme temperatures and compounds with antibacterial effects. It is noteworthy that some pigments have antibiotic activity as well as virulence factors for pathogenic bacteria. The pigmentation of microorganisms is essential in clinical diagnostics, as it facilitates species identification (Wolska et al., 2010).

Melanins are a group of macromolecular, black or brown in color pigments formed as a result of a multi-step oxidative polymerization of phenolic and indole compounds. The most common substrate for the biosynthesis of these compounds is the amino acid tyrosine, dihydroxyindole, dihydroxynaphthalene and catechol. Melanins exhibit specific physical and chemical properties. They reduce many compounds, including silver ions, ferrocyanide, and potassium permanganate, have UV absorption and scattering abilities, exhibit antioxidant properties, and are excellent chelators of metal cations (Saud, Alaubydi, 2016). Production of melanins is characteristic for many microorganisms, mainly soil bacteria, some parasitic bacteria and fungi, and marine algae. Among soil bacteria, melanins are produced by actinomycetes (some species of *Streptomyces* (Paget et al., 1994)), bacterial species belonging to the genus *Azotobacter* (Shivprasad, Page, 1989; Aquilanti et al., 2004a), *Azospirillum* (Sadasivan, Neyra, 1987) and some strains of *Rhizobium* (Hynes et al., 1988). Among marine algae, *Shewanella* (Turick et al., 2008), *Shewanella colwelliana* (Kotob et al., 1995), and *Marinomonas mediterranea* (Lopez-Serrano et al., 2004) possess this ability. On the other hand, among pathogenic bacteria, melanin is most often produced by species, i.e., *Burkholderia cepacia* (Zughaier et al., 1999), *Klebsiella pneumoniae* (Hawkins, Johnston, 1988), *Legionella pneumophila* (Baine et al., 1978), *Mycobacterium leprae* (Prabhakaran et al., 1968), *Proteus mirabilis* (Agodi et al., 1996) and *Vibrio cholerae* (Coyne, Al-Harthi, 1992).

Bacteria belonging to the genus *Azotobacter* are free-living, strict aerobes that require oxygen. Of the species of this genus, *Azotobacter chroococcum* is the most widely distributed in soils worldwide, and it probably also dominates soils in Poland (Döbereiner, 1995; Martyniuk, Martyniuk, 2003; Lenart, 2012). Colonies of these bacteria are large, convex with an irregular shape, mucilaginous, and darkening after several days of culture, which is associated with the production of a dark brown melanin pigment non-diffusible into the substrate. The aspects of melanin production by *Azotobacter chroococcum* have been described in quite some detail in the literature (Thompson, Skerman, 1979; Shivprasad, Page, 1989; Gospodaryov, Lushchak, 2011; Banerjee et al., 2014).

The paper systematizes current reports on the conditions of melanin production by bacteria, including by *Azotobacter chroococcum*, some of its physicochemical properties and possible applications in biotechnology. The work presents, in a synthetic form, the current state of knowledge in this area.

PHYSICOCHEMICAL PROPERTIES OF MELANINS

Melanins are a diverse group of pigments synthesized in living prokaryotic and eukaryotic organisms by hydroxylation and polymerization of organic compounds. Melanin production is one of the universal features of the adaptation of living organisms to changing environmental conditions. The presence of different types of melanin in representatives of almost every major taxon indicates the evolutionary significance of melanogenesis (Plonka, Grabacka, 2006).

All the pigments belonging to the melanin group are characterized by their high molecular weight, irregular and three-dimensional amorphous structure and negative charge. There is no single, strict definition of melanins due to the high heterogeneity of these compounds (color, molecular weight, composition, origin and function). The most commonly used definition is: „heterogeneous polymers formed by the oxidation of phenolic compounds and further polymerization of intermediate compounds and resulting quinones" (Solano, 2014). The diverse and heterogeneous structure of melanins is due to the ubiquitous sources of their origin. In addition, melanin's physicochemical properties make it even more difficult to accurately identify and characterize its structure (Pralea et al., 2019). Among the characteristic qualities of melanins that allow them to be distinguished from other compounds are insolubility in most common solvents, loss of color under oxidizing agents, resistance to degradation under cold and hot acids, ability to directly reduce ammoniacal $AgNO_3$ solution, solubility in alkali solutions, positive reaction to polyphenols (Piattelli et al., 1965; Nicolaus, 1968; Nosanchuk et al., 2015; Pralea et al., 2019). A dark color usually characterizes melanins, but the palette ranges from blackish brown to yellowish red. This variation in color and hue is due to different abilities to absorb and scatter light. According to literature data, the smaller the melanin granules, the lighter their coloration (Prota, 1992).

TYPES OF MELANINS

The melanin-producing ability is widespread among microorganisms. From a chemical point of view, the common feature of microbial melanins is that they are the product of oxidative polymerization of various phenolic substances (Przemyslaw, May, 2006). Melanins are heterogeneous polymers of dihydroxyindole (DHI) and dihydroxyindolecarboxylic acid (DHICA) monomers linked by heterogeneous non-hydrolyzing bonds (Crippa et al., 1989; Cheng et al., 1994). Differences in chemical structure have become the criterion for dividing melanins into four groups (Table 1), i.e.:

– eumelanins – blackish brown insoluble pigments formed by the oxidation of tyrosine (and/or phenylalanine) to 3,4-dihydroxyphenylalanine (DOPA) and dopaquinone, which are further converted to 5,6-dihydroxyindole or 5,6-dihydroxyindole-2-carboxylic acid. Eumelanins are most common in animals and humans (Langfelder et al., 2003; Plonka, Grabacka, 2006),

– allomelanins – are the least studied and the most heterogeneous group of polymers formed by the oxidation of di-hydroxynaphthalene or tetrahydroxynaphthalene to phaeomelanin, γ-glutamine-4-hydroxybenzene, catechol and 4-hydroxyphenylacetic acid. Plants, fungi and microorganisms produce allomelanins. This group includes the following subgroups of melanins: catechol melanin (in plants), DHN melanin and phaeomelanin (in bacteria and fungi) (Nicolaus et al., 1964; Funa et al., 1999; Jacobson, 2000; Plonka, Grabacka, 2006; Cordero, Casadevall, 2017),

– pheomelanins – yellow-red pigments soluble in alkaline solutions. This variety of melanins is formed by biochemical transformations involving a change in eumelanin synthesis. The onset of synthesis of both compounds is similar; however, in the case of pheomelanin production, 3,4-dihydroxyphenylalanine undergoes cysteinylation, either directly or via glutathione. Cysteinyl-3,4-dihydroxyphenylalanine, the final product of the reaction, further polymerizes into various benzothiazine derivatives. This is a type of animal melanin found in red hair, freckles or feathers (Nappi, Ottaviani, 2000; Plonka, Grabacka, 2006),

– neuromelanins – dark pigments synthesized in human neurons by oxidation of dopamine and other catecholamine precursors (Fedorov et al., 2005).

Table 1. Melanins – types, source of origin and precursors (Tran-Ly et al., 2020).

Type of melanin	Source of origin	Precursor
Eumelanin	animals, bacteria, fungi	tyrosine, 3,4-dihydroxy-L-phenylalanine (L-DOPA)
Pheomelanin	animals	5-S-cysteinyl-DOPA
Neuromelanin	humans (brain)	dopamine, 5-S-cysteinyl-dopamine
Catechol melanin	plants	catechol
DHNmelanin	bacteria, fungi	1,8-dihydroxynaphtalene (DHN)
Phaeomelanin	bacteria, fungi	homogentisinic acid

ECOLOGICAL AND PHYSIOLOGICAL ROLE OF BACTERIAL MELANINS

The capability of microorganisms to produce pigments is a trait acquired in the course of evolution, increasing their survival opportunities in the external environment. The pigments are designed to protect microorganisms from the adverse effects of various physicochemical factors (Liu, Nizet, 2009). Due to the multifunctional nature of melanins, they are used as a) antioxidants and radical scavengers (Keith et al., 2007; Ju et al., 2011; Le Na et al., 2019), b) photo protectors that effectively absorb and dissipate solar radiation in the form of heat (d'Ischia et al., 2015), (c) absorbers that chelate metals and bind organic compounds (Banerjee et al., 2014; Karlsson, Lindquist, 2016; Tran-Ly et al., 2020a), and (d) organic semiconductors (Bothma et al., 2008). Besides, these pigments protect cells from hydrolytic enzymes and the enzymes themselves from proteases (Valeru et al., 2009; Saud, Alaubydi, 2016). In addition to performing the earlier functions, melanin is considered a biocompatible and environmentally friendly compound, as it is naturally synthesized by most living organisms (Tran-Ly et al., 2020b). The link between melanin synthesis and increased virulence of pathogenic bacteria has been widely reported in the literature (Nosanchuk, Casadevall, 2003; Plonka, Grabacka, 2006). The pigment contributes to the virulence of microorganisms by decreasing the susceptibility of pathogens to host defense mechanisms and influences the host immune response (Nosanchuk, Casadevall, 2006). Melanins synthesized by water-dwelling *Vibrio cholerae* bacteria protect cells from high temperature and osmotic stress. The protective role of melanins under osmotic stress is related to their ability to absorb K+ and Na+ cations, which prevents cell dehydration (Coyne, Al-Harthi., 1992). A study by Valeru et al. (2009) shows that the color mutant of *Vibrio cholerae* has enhanced infectivity and increased resistance to UV radiation due to the absorption of the energy of this radiation by melanin. On the other hand, Patel et al. (1996) showed that the melanin of *Bacillus thuringiensis* increases the toxicity of this bacterium to insects while protecting it from the adverse effects of UV radiation. In addition, resistance *B. thuringiensis* to UV radiation has been linked to the presence of melanin in the sheaths of endospores, while adsorption or incorporation of melanin into crystals of the toxic protein formed during sporulation increases the stability of the insecticide.

In turn, the ability of melanins to bind heavy metals is associated with numerous carboxyl, phenolic, hydroxyl and amino groups in their structure, which are sites of binding or adsorption for metal ions. This can reduce the effectiveness of antifungal and antibacterial agents containing metal ions in eliminating melanin-producing microorganisms (Patel et al., 1996).

The literature has reported the potent antioxidant properties of melanins and their ability to stabilize free radicals and bind unpaired electrons to increase the virulence of colored strains compared to non-colored strains. The above relation was observed in some colored strains of *Burkholderia cenocepacia* (Keith et al., 2007) and the plant pathogen *Ralstonia solanacearum* (Ahmad et al., 2016). Piñero et al. (2007) found that melanin in the symbiotic bacterium *Rhizobium etli* plays a key role in the first stages of wart formation when the bacteria must deal with reactive oxygen species and phenolic compounds produced by plants. These dyes are also involved in interactions between bacterial biofilms and other organisms. Melanin secreted by *Pseudoalteromonas lipolytica* biofilms prevents their colonization by clam larvae (Zeng et al., 2017) and in the case of a *Vibrio cholerae* biofilm increases the production of reactive oxygen species, thereby protecting the microorganism from predation by the amoeba species *Acanthamoeba castellanii* (Noorian et al., 2017).

Melanins also acquire iron from the environment (Gospodaryov, Lushchak, 2011). Most bacteria obtain this element through the production of siderophores. Chatfield and Cianciotto (2007) described a system of ferric procurement by a bacterium of the *Legionella pneumophila* species with the participation of pyomelanin, associated with ferric reductase activity. Melanins produced by bacteria belonging to the genera: *Azotobacter, Burkholderia, Pseudomonas, Klebsiella, Serratia* and *Vibrio* are also involved in iron acquisition. The mechanism of melanin-mediated ferric oxide reduction has been described in the Gram-negative marine bacteria *Shewanella alge* and the fungus *Cryptococcus neoformans*. Among pathogenic bacteria, *Pseudomonas aeruginosa* can reduce ferric extracellularly with pyomelanin (Chatfield, Cianciotto, 2007). Some microorganisms, as a result of melanin synthesis, are able to survive in extreme environments by adapting to rapidly changing conditions. Examples of such microorganisms include *Streptomyces cyaneofuscatus* isolated from the desert soils of Algeria (Harir et al., 2018), *Bacillus weihenstephanensis* from the soils of northeastern Poland (Drewnowska et al., 2015), *Lysobacter oligotrophicus* obtained from Antarctic sites (Kimura et al., 2015) and *Aeromonas. salmonicida* subsp. *pectinolytica* from heavily polluted waters of the Matanza River in Argentina (Pavan et al., 2000).

SYSTEMATIC AFFILIATION AND PIGMENT PRODUCTION BY SPECIES OF THE GENUS *AZOTOBACTER*

The genus *Azotobacter* was identified by Beijerinck in 1901. Bacteria of the genus *Azotobacter* belongs to the family *Pseudomonadaceae*, included in the subclass γ-Proteobacteria (Tchan, New, 1984; Özen, Ussery, 2012; Rubio et al., 2013; Robson et al., 2015). Currently, 8 species and 4 subspecies are known within the genus *Azotobacter* (De Smedth et al., 1980; Tchan, New, 1984; Aquilanti et al., 2004b; Jin et al., 2020).

- *Azotobacter armeniacus* (Thompson, Skerman, 1979),
- *Azotobacter beijerinckii* (Lipman, 1904),
- *Azotobacter bryophylli* (Liu et al., 2019),
- *Azotobacter chroococcum* (Beijerinck, 1901),
  - *Azotobacter chroococcum* subsp. *chroococcum* (Jin et al., 2020),
  - *Azotobacter chroococcum* subsp. *isscasi* (Jin et al., 2020),
- *Azotobacter nigricans* (Krasilnikov, 1949),
  - *Azotobacter nigricans* subsp. *achromogenes* (Thompson, Skerman, 1979),
  - *Azotobacter nigricans* subsp. *nigricans* (Howey et al., 1990),
- *Azotobacter paspali* (Döbereiner, 1966),
- *Azotobacter salinestris* (Page, Shivprasad, 1991),
- *Azotobacter vinelandii* (Lipman, 1903).

Of the species mentioned above, *Azotobacter bryophylli* is a colorless species, while the other species show the ability to produce melanin pigments of different colors (Table 2).

Table 2. Pigments produced by species belonging to the genus *Azotobacter* (Tchan, New, 1984; Page, Shivprasad, 1991; Aquilanti et al., 2004a; Liu et al., 2019).

Species	Pigment
A. armeniacus	brown-black pigment diffusing into the substrate
A. beijerinckii	yellow to light brown pigment not diffusing into the substrate
A. bryophylli	does not produce any pigment
A. chroococcum	dark brown melanin pigment not diffusing to the substrate
A. nigricans	brownish-black to reddish-purple pigment diffusing into the substrate
A. paspali	yellow fluorescent pigment diffusing into the substrate
A. salinestris	dark brown pigment not diffusing into substrate
A. vinelandii	yellow-green fluorescent pigment diffusing to substrate

PROPERTIES OF MELANIN SYNTHESIZED BY AZOTOBACTER CHROOCOCCUM AND POSSIBLE APPLICATIONS.

Free-living N_2 assimilators of the genus *Azotobacter* have been the subject of much research for decades. They have become model microorganisms in studies of the biochemistry and energetics of N_2 fixation, as well as in studies of the spatial structure and function of nitrogenase and the genetic regulation of biological, atmospheric nitrogen fixation (Paul, Clark, 2000).

The ability of these bacteria to produce melanins is widely reported in the literature (Tchan, New, 1984; Shivprasad, Page, 1989; Gospodaryov, Lushchak, 2011; Banerjee et al., 2014). The compound used by *Azotobacter chroococcum* to produce melanin is catechol. The most significant amounts of this pigment are produced under aerobic conditions because oxygen is one of the substrates of the enzyme polyphenol oxidase (Shivprasad, Page, 1989). Colonies of *A. chroococcum* bacteria darken after several days of culture due to the accumulation of dark brown melanin pigment that does not diffuse into the medium (Tchan, New, 1984; Aquilanti et al., 2004a) (Fig. 1). Melanin produced by this bacterial species belongs to the allomelanins group. Its synthesis does not require the presence of precursor molecules (e.g., tyrosine, dihydroxyphenylalanine, catechol) in the culture medium, as the bacteria produce these as the cells age (Bortels, Henkel, 1968). Melanin is produced after about five days of culture, staining most liquid medium or forming dark halos around bacterial colonies on solid medium. Melanin formation has been studied in several strains of *A. chroococcum* (Shivprasad, Page, 1989; Herter et al., 2011; Banerjee et al., 2014). Gospodaryov, Lushchak (2011) observed increased melanin production on the Ashby substrate with benzoic acid as a carbon source.

On the other hand, Shivprasad, Page (1989) noticed an inhibitory effect of benzoic acid and a stimulating effect of copper and iron ions on melanin production by *Azotobacter chroococcum*. They found that pigment synthesis was significantly enhanced at specific concentrations of copper sulfate in the medium. This is confirmed by a study by Gospodaryov, Lushchak (2011), who noted that copper sulfate at a concentration of 10 μM added to the substrate increased melanin production, while higher concentrations inhibited the process. Excessively high concentrations of copper sulfate can inhibit bacterial growth and reduce the efficiency of this pigment syn-

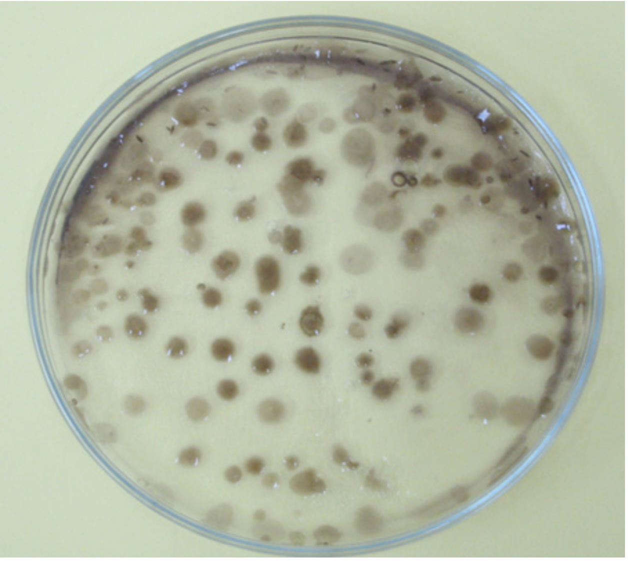

Figure 1. 5-day-old colonies of the reference strain *Azotobacter chroococcum* DSM 281.

thesis. The studies discussed above show that melanogenesis in *Azotobacter chroococcum* is catalyzed by a copper-dependent polyphenol oxidase.

Like all melanins, melanin produced by *A. chroococcum* is soluble in alkaline reagents (Shivprasad, Page, 1989; Lin et al., 2005). Shivprasad and Page (1989) identified the dye extracted from *A. chroococcum* cells as melanin based on the following characteristics: solubility in hot 0.5 M NaOH and 1 M Na_2CO_3, and insolubility in cold water, hot water, ethanol, chloroform, acetone and cold 0.5 M NaOH. Melanin synthesized by this species can bind calcium ions. Alkalization of the medium containing calcium ions results in the formation of black flocs that are a melanin complex with $Ca(OH)_2$. The ability to bind calcium is not a distinguishing feature of *A. chroococcum* melanin, as this feature has also been described for melanins synthesized by many different bacterial species (Bush, Simon, 2007). Microscopic analysis of *A. chroococcum* colonies revealed the presence of calcium crystals of various sizes in brown halo zones around the bacterial colonies, in the colonies themselves, and in the mucus. The crystals were absent or sparse in unpigmented colonies of this bacterial species.

Research conducted by Robson et al. (2015) showed the presence of 4 chromosomal genes (cum) encoding polyphenol oxidases in the genome of *A. chroococcum* strain NCIMB 8003. In addition, they found that the strain mentioned above of *A. chroococcum* is capable of producing carotenes in addition to melanin synthesis. Colonies of this strain on Burk's substrate took on a brown-black color, while on nutrient agar, they took on a yellow-brown color. The chromosome of *A. chroococcum NCIMB 8003* contains 7 genes (crtZ, crtE, crtX, crtB, crtY, crtI, idi) encoding proteins, i.e., CrtE, CrtB, CrtY and CrtI involved in the synthesis of the carotene-like compound. The genes exhibit more than 60% identity with the orthologs of genes present in the genome of *Pseudomonas stutzeri*. At the same time, they were not detected in the genome of strain *A. vinelandii* DJ (Klassen, 2010). Melanins produced by *A. chroococcum* protect cells from hydroxyl radicals formed in the Fenton reaction, whereas carotenes are excellent antioxidants (Shivprasad, Page, 1989; Robson et al., 2015).

Literature data suggest that melanin, especially allomelanin, has properties similar to those of soil humic substances, which promote the growth and yield of plants (Sutton, Sposito, 2005; Plonka, Grabacka, 2006; Muscolo et al., 2007). In their study, Gospodaryov, Lushchak (2011) observed the stimulating effect of purified melanin produced by *A. chroococcum* on such plants as lettuce, tomato, rapeseed and radish. The promotion of plant growth and development by melanin is also related to its ability to maintain water in the soil (Russo, Berlyn, 1990). In addition, the *A. chroococcum* strain is an excellent indicator of the presence of benzoic acid, and its salts in various substrates since precipitation of synthesized melanin occurs only in the presence of benzoic acid. Melanins extracted from *A. chroococcum* cultures, thanks to their affinity for metals and high adsorption capacity, are used in the bioremediation of soils and waters contaminated with heavy metals (Rizvi et al., 2019).

The research results available in the literature can contribute to clarifying the ecological role of melanins produced by soil bacteria of the genus *Azotobacter* and the importance of melanization processes in nature.

Possible application of bacterial melanins in various industries

Numerous bacterial species, including pathogens, are capable of producing melanin. This pigment exerts a variety of functions, always beneficial to the host. Natural sources of melanin are currently being sought, among which melanins synthesized by microorganisms (bacteria, fungi) are the most promising. Their advantage is that they can be manufactured on a large scale, relatively cheaply, compared to the cost of obtaining synthetic melanins (Goncalvez, Pombeiro-Sponchiado, 2005). Melanins have been intensively studied for their properties and wide range of practical applications. Melanins produced by bacteria have been shown to effectively protect fibroblasts from UV radiation, indicating that they could be used as an ingredient in sunscreens (Geng et al., 2008; Kurian, Bhat, 2018). They have also found applications in medical diagnostics as a contrast agent in the optoacoustic tomography (Liopo et al., 2015). The pigments in question exhibit semiconductor properties and conduct electricity, hence the potential for their use in many branches of technology. Synthetic DOPA-melanin has been used for in situ formation of semiconductor coatings (Lee et al., 2007). There are also sunglasses on the market with lenses containing melanin, which absorb ultraviolet radiation, thereby protecting vision (Lopusiewicz, Lisiecki, 2016). Melanin produced by *Shewanella oneidensis* increases electricity production in microbial fuel cells thanks to its ability to transport electrons (Turick et al., 2010). Research in recent years has drawn attention to the possibility of using melanins in innovative nanotechnologies, such as synthesising crystalline, flexible and thermostable nanocomposite films used in biomedicine (Kiran et al., 2017). The applicability of melanins in molecular biology is only feasible if pigment synthesis is regulated by a single gene or operon that serves as a marker for plasmid transfer from one organism to another. This is an excellent alternative to markers such as β-galactosidase or proteins responsible for antibiotic resistance (Tseng et al., 1990). From a medical point of view, melanogenic microorganisms are model microorganisms used to study the effects of various factors on melanin production. The anti-cancer properties of allomelanins are known (Kamei et al., 1997), as well as the inhibitory effect of melanins on HIV proliferation (Montefiori, Zhou, 1991; Sidibe et al., 1996). Melanin extracted from *Pseudomonas balearica* demonstrates antimicrobial activity against *Staphylococcus aureus*, *Escherichia. coli*, *Candida albicans* (Zerrad et al., 2014). Thanks to their affinity for metals and high adsorption capacity, melanogenic bacteria can be used in bioremediation. It is recommended to detoxify soils or waters contaminated with heavy metals using melanin-producing microorganisms such as *Azotobacter chroococcum* (Rizvi et al., 2019) and *Pseudomonas stutzeri* (Thaira et al., 2019; Manirethan et al., 2018). In turn, melanin extracted from *E. coli* cultures has been used successfully for the bioremediation of drug-contaminated wastewater (Gustavsson et al., 2016).

CONCLUSIONS

Melanins are compounds with unique attributes. The ability to produce the pigment group mentioned above is widespread among bacteria, and their role is complex and multifaceted. Melanins protect bacterial cells from external environmental factors, i.e. extreme temperatures, UV radiation, oxidizing agents, heavy metals and antibiotics. Melanins of natural origin synthesized by melanogenic microorganisms (bacteria, fungi) are increasingly being studied and applied. The advantage of natural melanins over synthetic ones is that they can be manufactured on a large scale by relatively simple and inexpensive methods. Melanins are intensively studied for their properties and wide range of practical applications. For example, obtaining high-performance microbial melanins involves selecting suitable microorganisms that can produce melanin extracellularly from an exogenous substrate and improving the metabolic process by adding tyrosine and copper to the culture substrate. Melanins produced by bacteria have found applications in modern medicine, biotechnology, cosmetology and technology. From an agricultural perspective, these pigments can be used to promote plant growth and development and in the bioremediation of soils and waters contaminated with heavy metals. However, these pigments still hold many secrets and given their unique properties and role, they fully deserve continued research into new possibilities for their application.

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