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Dedication

With the help of Allah the almighty, I was able to carry out this work which i dedicate to:

- My mother firstly, secondly and thirdly. To my father and my young brother HOUSSAM.

- To my friends: RADJA, CHIRAZ, CHOUROUCK, ZAINEB and their families.

- To my family in NBQ–tebessa- team and my old friends in ELAKADEMIA POST team.

- To all people who are led by science to faith.

vii. The analysis part

COX: Cyclooxygenase

DHI: 5,6-dihydroxyindole

DHICA: 3,6-dihydroxyindole-2-carboxylicacid .

DHN: 1,8-Dihydroxynaphthalene.

DMSO: Dimethyl sulfoxide

DMSO-d6: Deuterated dimethyl sulfoxide

DOPA: O-dihydroxyphenylalanine

DPPH: 2,2-diphenyl-1-picrylhydrazyl

HGA: Homogentistate acid

HMC: Homogentistate

HPQ: Hexahydroxyperylenequinone

IC100: The concentration needed for complete 100% inhibition

IC50: The concentration needed for 50% inhibition

IQ: Indolquinone .

ISP2: International Streptomyces Project 2 (Yeast malt agar)

ISP4: International Streptomyces Project 4 (Inorganic salts starch agar)

ISP6: International Streptomyces Projec (Peptone Yeast Extract Iron agar)

LB: Lysogeny broth.

MHA: Muller Hinton agar

MRI: Magnetic resonance imaging

OECT: Organic electrochemical transistors

PET: Positron emission tomography

NOS: Nitric oxide synthase

QI1: Tautomers quinone -methide

QI2: Quinone –imine

Rpm: Revolutions per minute

Sp: A single unnamed species

SPF: Sun protection factor

SS: *Salmonella Shigella* agar

TBB: Tyrosine basal broth

THN: 1,3,6,8-tetrahydroxynaphthalene

UV: Ultra violet

UVB: Ultra violet Type B

YE-ME: Yeast extract - malt extract agar

Introduction

Pigments, being naturally synthetic chemical compounds, play a crucial role as secondary metabolites with multiple biological functions. They are responsible for the remarkable array of colors observed in the nature, including colors displayed by plants, animals, and microorganisms **(Sinha *et al*., 2017; Kiki, 2023).** The ubiquity of pigments is really fascinating, as they can be found in diverse environments, ranging from marine and soil ecosystems to extreme environments like salt regions and deserts **(Azman, 2018).** In addition to its aesthetic appeal, they have several functions, such as the virulence of an organism and its protection against rays of visible and near ultra violet light **(Sinha**, **2017; Azman**, **2018).**

Bacteria in particular, such as *Acromobacter* and *Bacillus,* can produce pigments **(Mukherjee, 2017).** Including carotenoids (canthaxanthin, zechinenone and astaxanthin), staphyloxanthin, xanthomonadins, flexirubin, prodigiosin, violacein, pyocyanin and melanin **(Azman, 2018)**. Unlike synthetic pigments, the naturally synthesized pigments are considered as eco-friendly, non-toxic, non-carcinogenic, and bio degradable alternatives **(Venil *et al*., 2013).**

The exploration of pigments continues to be an active area of research and development. In recent decades, studies are extensively conducted to explore the potential of pigments from several sources and to find strategies for their production and applications in different industrial sectors. Among them melanins **(Tuli *et al*., 2015).**

In this research**,** we aimed to study melanins from several bacteria. Our work is divided into:

- **Theoretical part,** that contains melanin definition, structure, proprieties and types, as well as the bacterial biosynthesis mechanism, and the physiochemical roles of bacterial melanins and their biotechnological applications.
- **Practical part**: describes the methods used to collect several scientific papers about melanins.
- **Analysis part**: contains the analysis of 12 scientific papers on bacterial melanins.
- **Conclusion:** contains the important findings about bacterial melanins from the different scientific papers analyzed.

Theoretical part

1/ Definition, chemical structure, and characteristics of melanin

The word "Melanin" comes from the Greek word "melanos" which means dark. It was first used by a Swedish scientist Berzelis in 1840, when he extracted a dark pigment from eye membranes. "Melanin" is now a general term for a group of heterogeneous, polymeric pigments with diverse structures, functions and applications. They can be classified based on their source (animal, plant, microbial and fungal, or their physical and chemical properties **(Solano, 2014; Cordero, 2019).**

Most melanins are formed from phenolic compounds by polymerization via quinone **(Huang *et al*., 2018).** Microbial melanin consists of an aromatic ring and a nitrogen-containing functional group. The aromatic ring is composed of two types of monomers, indole and pyrrole, while the nitrogen-containing functional group can be either a carboxyl or quinone group **(figure 01) (Guo, 2023).**

Melanin is insoluble in most organic and inorganic solvents but it can be dissolved in alkaline water and dimethyl sulfoxide (DMSO) and precipitate under acid conditions. Its solubility depends on many factors like: source, purity, polymerization state and pH value. Melanin can be oxidized by oxidants such as: potassium permanganate or hydrogen peroxide **(Guo, 2023).**

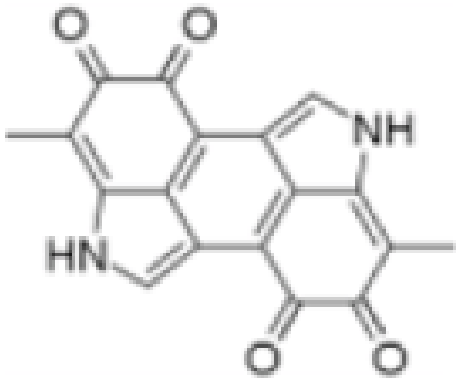

Figure 01: Chemical structure of melanin pigment (Tsouko, 2023).

2/ Melanin types

There are 5 types of melanins: eumelanin, pyomelanin, neuromelanin, pheomelanin, and allomelanins.

2.1/ Eumelanin

It is the most common type of melanin, which is a black/brown pigment. It is synthesized via o-dihydroxyphenylalanine (DOPA) and dopaquinone, which are formed via oxidation of tyrosine or phenylalanine **(table 01).** Substances resulting from this oxidation, undergo cyclization to 5,6-dihydroxyindole (DHI) or 3,6-dihydroxyindole-2-carboxylicacid (DHICA) **(Plonka & Grabacka, 2006).** Eumelanin is basically formed of DHI and DHICA as building blocks **(figure 2).** It also contains their oxidized forms such as tautomers of quinone -methide (QI1), quinone –imine (QI2), indolquinone (IQ), that are all related together between C6 and C7 atoms, by covalent bounds.

Eumelanin has a complex structure and it is difficult to analyze it due to the fact that indoles are able to take more than one bounding configurations, as it has been suggested that the oligomerization process sometimes occurs at positions 3,4 and 7, and most often at positions 4 and 2. The smallest unit that can be formed consists of 4 monomers, while larger than that will never be stable and it is hard to form **(Meredith & Sarna, 2006).**

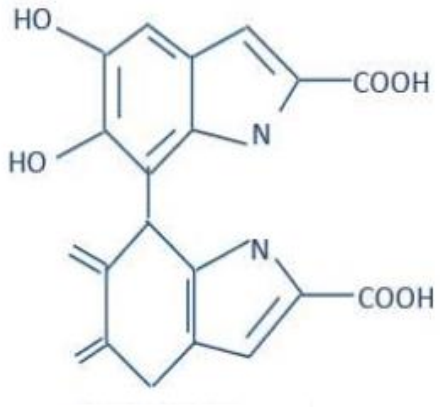

Figure 02: The chemical structure of eumelanin (Pandey *et al*., 2023).

2.2/ Pyomelanin

It is a brown-black pigment, produced generally by some bacteria under stress conditions **(Plonka & Grabaka, 2006**). Pyomelanin is produced through L-tyrosine pathway by oxidation of homogentisate acid (HGA) **(table 01) (Lorquin *et al*., 2022).** Pyomelanin has quinones in the polymer structure, which gives it the ability to interact with iron-containing particle **(Styczynski, 2022) (figure 03).** Pyomelanin production was reported in Pseudomonas *aeruginosa ,Hyphomonas* sp., and *Shewanella clowelliana* **(Shivprasad & Page, 1989).**

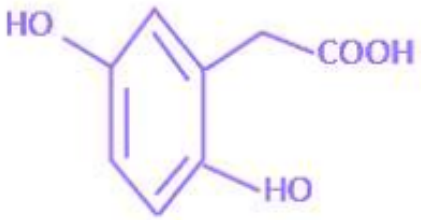

Figure 03: The chemical structure of pyomelanin (Pandey *et al*., 2023).

2.3/ Pheomelanin

It is a less common form of melanin that is responsible for red and yellow pigmentation. It is formed from tyrosine by the oxidation of cysteine and the subsequent polymerization of the resulting intermediates. Although pheomelanin is similar to eumelanin regarding the same pathway, pheomelanin is smaller and more regular polymer than eumelanin **(figure 04)**. It is alkali-soluble and contains a variety of sulfur-containing functional groups **(Kurian & Bhat, 2014** ; **Ito & Wakamatsu, 2018).**

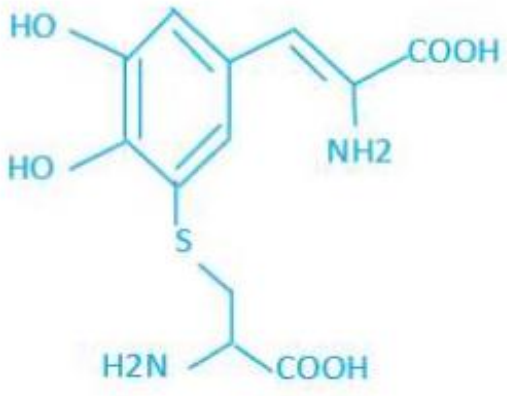

Figure 04: The chemical structure of pheomelanin (Pandey *et al*., 2023).

2.4/ Neuromelanin

Neuromelanin is a brown-black melanin, which is found in the Substantia nigra, in the nervous system. It is primarily localized in discrete granules inside neuron cell bodies. Neuromelanin biosynthesis is not enzymatically controlled due to the absence of the enzyme tyrosinase in the brain. It is produced from 5-S-cysteinyl-dopamine and dopaminochrome as precursors, which are derived from dopamine metabolism. Its shape is described as core-shell, as its core is similar to pheomelanin, while its cover is similar to pheomelanin **(figure 05) (Cao *et al*., 2021).**

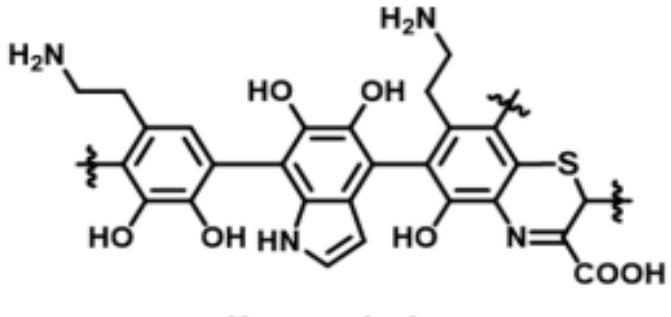

Figure 05: Chemical structure of neuromelanin (Cao *et al*., 2021)

2.5/ Allomelanins

They are the most heterogeneous group of melanin. They contain 3 forms: catechol melanin, Hexahydroxyperylenequinone (HPQ) melanin, and 1,8-Dihydroxynaphthalene (DHN) melanin **(table 01).**

2.5.1/ Catechol melanin

It is a brown insoluble pigment that can be found in plant seeds, and *Aspergillus niger*. It is synthesized in the presence of polyphenols through the oxidation of catechol to produce benzoquinones as an intermediate. Catechol melanin is formed from two initial molecules: hydroxyquinone and o-benzoquinone as well as other components such as carboxyl groups and phenolic hydroxy-groups **(figure 06),** which result from the breakdown of other molecules **(table 01) (Zhou *et al*., 2019).**

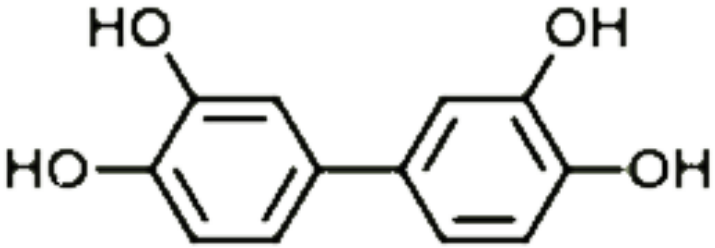

Figure 06: The chemical structure of catechol melanin (Cao *et al*., 2021).

2.5.2/ HPQ melanin

It is a dark brown to black pigment of high weight, formed from malonyl-coA as a precursor**,** through the DHN pathway, using the enzyme III polyketide synthase RppA and a member of the cytochrome P450 family. HPQ melanin is composed of 1,4,6,7,9,12-hexahydroxyperylene- 3,10-quinone and HPQ **(figure 07) (Funa *et al*., 2005)**.

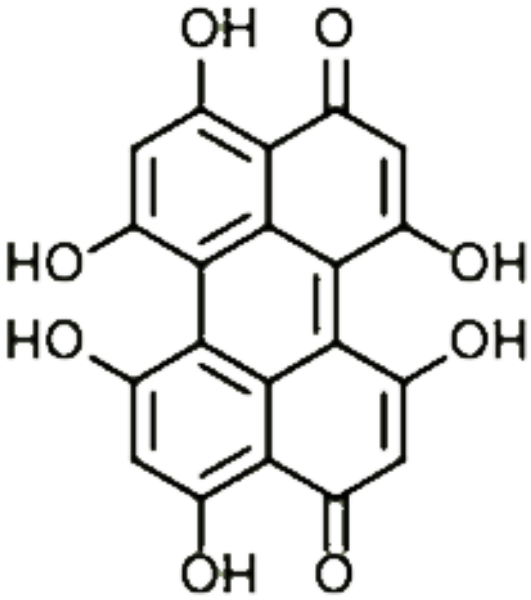

Figure 07: The chemical structure of HPQ (Cao *et al*.,2021).

2.5.3/ DHN melanin

DHN melanin is a specific type of melanin found in fungi **(table 01).** It is derived from the oxidative polymerization of nitrogen-free precursors, specifically DHN **(Cao, 2021).**

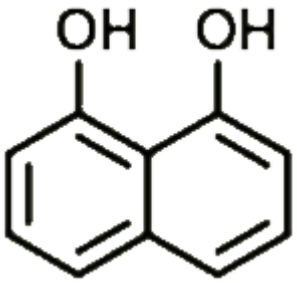

Figure 08: Chemical structure of DHN (Cao *et al*.,2021).

Table 01: Sources and chemical precursors of melanin types (Roy & Rhim, 2021).

Melanin type		Precursor	Source
Eumelanin (DOPA-melanin)		Tyrosine, 5,6-dihydroxyindoles	Animals, bacteria, fungi
Pyomelanin		Homogentisic acid	Fungi, bacteria
Pheomelanin		Tyrosine, 5,6-dihydroxyindoles	Animals
Neuromelanin		5-S-cysteinyl-dopamine,dopaminochrome	Human (brain)
Allomelanins	HPQ melanin	1,8-dihydroxynaphthalene, phenolic precursors	Bacteria
	Catechol melanin		Plants
	DHN melanin		Fungi

3/ Bacterial melanin biosynthesis

Bacterial melanins are synthesized through two different pathways: tyrosine (DOPA-pathway) or malonyl-coenzyme A (DHN-pathway) **(figure 09).**

3.1/ DOPA pathway

In this pathway, bacteria depend on the oxidation of tyrosine (monohydoxydated form) into diphenol (dihydroxylated form), and through some reaction L-DOPA (l-3,4-

dihydroxyphenylalanine) or homogentisate (2,5-dihydroxyphenylacetate), or homoprotocatechuate (3,4-dihydroxyphenylacetate), are generated and will be later oxidized spontaneously, or through an enzymatic activity into dopaquinones or benzoquinones. Finally melanin is formed via polymerization of thesecompounds **(figure 09) (Tran-Ly *et al*., 2010; Pavan *et al*., 2019).**

3.1.1/ DOPA melanin

Tyrosinases (EC 1.14.18.1) are the enzymes involved in this process. They are polyphenol oxidases which contain copper and are capable of hydroxylating tyrosine to L-DOPA, which is then oxidized to o-dopaquinone, after that, the latter undergoes cyclization to form indole quinone. Finally**,** the indole quinone is polymerized to form DOPA melanin, a brown to black pigment**.** Alternatively, in the presence of cysteine, L-DOPA can undergo cysteinylation prior to polymerization, leading to the production of yellow to red melanins that contain sulfur: pheomelanin **(figure 09) (Tran-Ly *et al.*, 2010; Pavan *et al*., 2019).**

3.1.2 / Homogentisate melanin

The process begins with the deamination of homogentisate (HMC) by aromatic amino acid aminotransferase into 4-hydroxyphenyl pyruvate. Then, the hydroxyphenyl pyruvate dioxygenase (EC 1.13.11.27) generates the homogentisate in a complex reaction that contains oxygen. The enzyme homogentisate 1,2-dioxygenase degrades the homogentisate. At final, melanin is formed by accumulation, polymerization, and spontaneous oxidation of homogentisate **(Tran-Ly *et al*., 2010; Pavan *et al*., 2019).**

3.1.3/ Homoprotocatechuate melanin

The synthesis of the dark brown pigment is thought to be linked to the activity of 4-hydroxyphenylacetate 3-monooxygenase (EC 1.14.14.9). This enzyme can catalyze the hydroxylation of 4-hydroxyphenylacetate to produce 3,4-dihydroxyphenylacetate, also known as homoprotocatechuate, which is an isomer of homogentisate. Therefore, it has been suggested that the formation of the pigment is associated with the accumulation of this compound **(Tran-Ly *et al.*, 2010; Pavan *et al*., 2019).**

3.2 / DHN-pathway

This pathway of melanin begins with the decarboxylative condensation of 5 molecules of malonyl-CoA in a sequential manner, catalyzed by RppA, a homodimeric Type III polyketide synthase. This process leads to the production of 1,3,6,8-tetrahydroxynaphthalene (THN). Another enzyme, belonging to the cytochrome P450 family, is co-transcribed with RppA and facilitates the oxidative dimerization of two THN molecules, resulting in the formation of hexahydroxyperylenequinone (HPQ), which leads to the formation of HPQ, an allomelanin **(figure 09) (Tran-Ly *et al*., 2010 ; Pavan *et al*., 2019).**

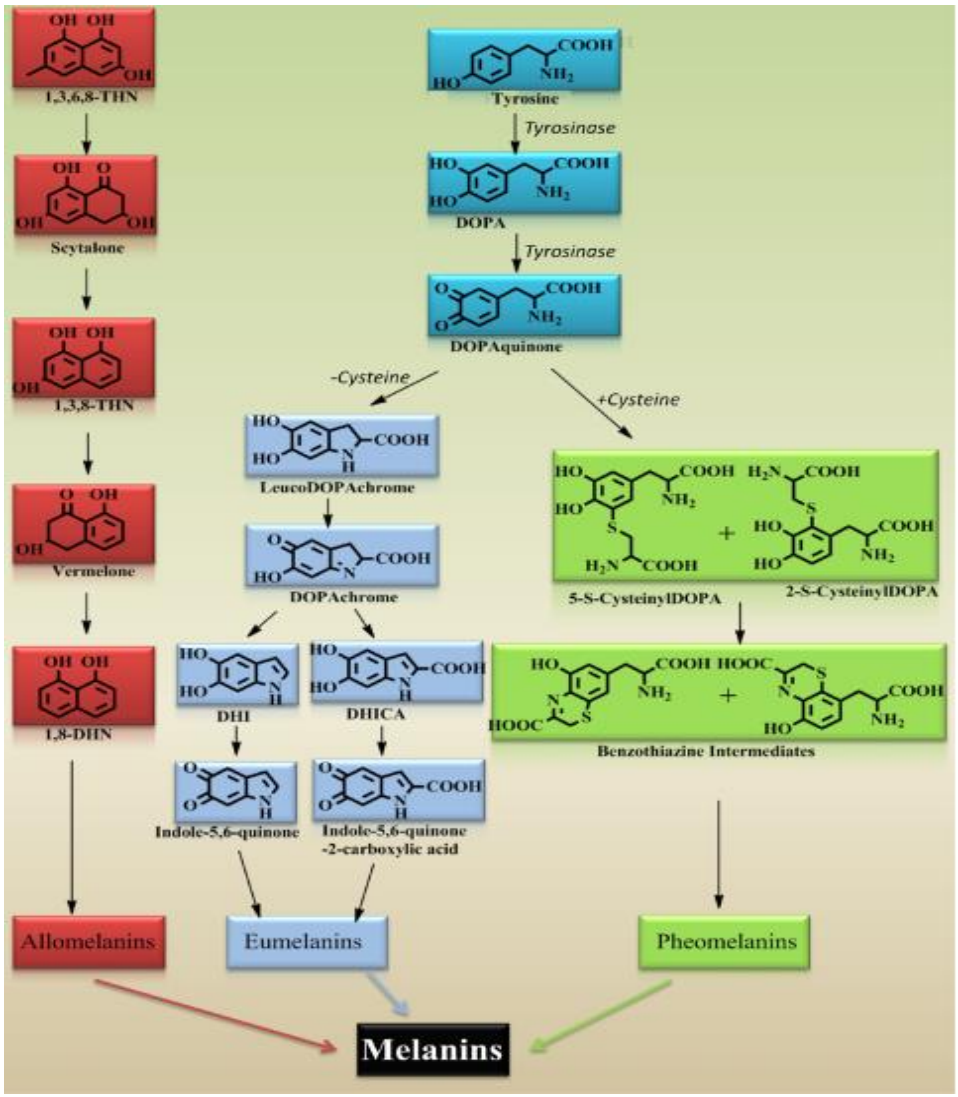

Figure 09: Melanin biosynthesis via DOPA-pathway and DHN-pathway in bacteria (Huang *et al*., 2018).

4/ Bacteria producing melanin

4.1/ Phylum Firmicutes

4.1.1/ *Bacillus*

- **Taxonomy**

Bacillus genus belongs to the domain: Bacteria, phylum: Firmicutes, class: Bacilli, order: Bacillales, and family: Bacillaceae, with around 142 named species **(Vos *et al*., 2011).**

- **Microscopic characteristics**

Bacillus are Gram positive rod-shaped bacteria, straight or slightly curved**,** occurimg singly or in pairs. The genus is able to form endospores and move using peretrichous flagella **(Vos *et al*., 2011).**

- **Macroscopic characteristics on culture media**

Bacillus exhibits a wide range of morphologies. The shape of colonies can be irregular or circular. The textures can be matt, smooth, granular, butrious, moist, mucoid, dry, or glossy. The colors range from buff or creamy-gray to off-white **(Vos *et al*., 2011).**

- **Growth and nutritional characteristics**

Bacillus is mostly chemo-organotrophic, aerobic or facultative anaerobic, mesophilic with optimal growth temperature ranges between 25°C and 40°C, and it can grow on routine media such as nutrient agar and blood agar. It can use a variety of carbon sources. Most species use glucose or other carbohydrates and a variety of nitrogen sources as ammonium sulfate, urea, amino acids, and inorganic nitrogen sources as ammonium ions. *Bacillus* do not have strict vitamin requirements, but it can use yeast extract which contain several vitamins to stimulate its growth **(Vos *et al*., 2011).**

- **Habitat**

Bacillus members can be found in soils, water and several foods as milk, honey bee, cocoa, coffee, vanilla **(Vos *et al*., 2011).**

- **Melanin producing species**

Bacillus species which can produce melanins are: *Bacillus altitudinus, B. cereus, B. hayensii, B. licheniformis, B. megaterium, B. safensis, B. subtilis, B. thuringiensis* and *B. weihenstephanensi* **(Gosset, 2023).**

4.1.2/ *Salinococcus*

- **Taxonomy**

Salinococcus genus belongs to the domain: Bacteria, phylum: Firmicutes, class: Bacilli, order: Bacillales, and family: Staphylococcaceae **(Vos *et al*., 2011).**

- **Microscopic characteristics**

Salinococcus are Gram positive bacteria, spherical cells with 0.5 - 2.5 μm in diameter. They occur singly, in pairs, tetrads and clumps. They are non-motile and non-spore forming bacteria **(Vos *et al*., 2011).**

- **Macroscopic characteristics on culture media**

Salinococcus colonies are round, smooth, pink, red or orange. They do not exhibit a diffusible pigment **(Vos *et al*., 2011).**

- **Growth and nutritional characteristics**

Salinococcus are strictly aerobic, moderately halophilic, which grow in media with 0.25% NaCl. They are mesophilic bacteria that require an optimum temperature between 30-37°C and pH between 7 to 9.5 **(Vos *et al*., 2011).**

- **Habitat**

Salinococcus members are widely distributed in different high salt habitats such as: salterns, saline soils, soda lakes and salted materials **(Vos *et al*., 2011).**

- **Melanin producing species**

One species can produce melanins: *Salinococcus roseus* **(Gosset, 2023).**

4.1.3/ *Planococcus*

- **Taxonomy**

Planococcus genus belongs to the domain: Bacteria, phylum: Firmicutes, class: Bacilli, order: Bacillales, and family: Caryophanaceae **(Vos *et al*., 2011).**

- **Microscopic characteristics**

Planococcus bacteria are Gram positive to Gram variable spherical bacteria, occurring singly, in pairs, in groups of three, or as tetrads, or clumps. They are 1.0-1.2 μm in diameter and motile using one or two flagella **(Vos *et al*., 2011).**

- **Macroscopic characteristics on culture media**

Planococcus colonies are circular, slightly convex, smooth, glistening and yellow-orange in color) **Vos *et al*., 2011).**

- **Growth and nutritional characteristics**

Planococcus are chemo-organotrophic, aerobic, catalase positive, halophilic which can tolerate in 1-17% NaCl. They can be either psychrophilic and mesophilic depending on the specie **(Vos *et al*., 2011)**.

- **Habitat**

Planococcus members can be found in various environments, including sea water, marine calms, shrimp, fish, cyanobacterial mats, prawns, freshwater ponds, glacial soils and sulfur springs **(Vos *et al*., 2011).**

- **Melanin producing species**

One species can produce melanins: *Planococcus maritimu* **(Gosset, 2023).**

4.2/ Phylum Actinobacteria

4.2.1/ *Nocardiopsis*

- **Taxonomy**

Nocardiopsis genus belong to domain: Bacteria, phylum: Actinobacteria, class: Actinobacteria, order: Streptosporangiales, family: Nocardiopsaceae **(Gosset, 2023).**

- **Microscopic characteristics**

Nocardiopsis are Gram positive, non-motile and filamentous actinomycetes. Their substrate mycelium is well developed and their hyphae are long and densely branched **(Bennur *et al*., 2015; Hozzein & Trujillo, 2015)**

- **Macroscopic characteristics on culture media**

Nocardiopsis colonies are coarsely, winkled or folded **(Bennur *et al*., 2015).**

- **Growth and nutritional characteristics**

Nocardiopsis are catalase positive, non-acid fast, which can survive under different and hostile environment such as alkaline and saline conditions. It can grow using yeast extract. Its optimal temperature ranges between 28°C to 30°C **(Yassin *et al*., 1997**; **Bennur *et al*., 2015).**

- **Habitat**

Nocardiopsis members are distributed in multi ecosystems including deserts, deep oceans, marine corals, and sponges **(Bennur *et al*., 2015).**

- **Melanin producing species**

The species producing melanin are*: Nocardiopsis alba* and *Nocardiopsis dassonvillei* **(Gosset, 2023).**

4.2.2/ *Streptomyces*

- **Taxonomy**

Streptomyces genus belong to domain: Bacteria, phylum: Actinobacteria, class: Actinomycetia, order: Streptomycetales, family: Streptomycetaceae **(Gosset, 2023).**

- **Microscopic characteristics**

Streptomyces are Gram positive, non-motile bacteria, growing as mycelium of branching hyphal filamentous of 0.5 to 1.0 μm in diameter, that form chains of three to many spores **(Komaki, 2023).**

- **Macroscopic characteristics on culture media**

Streptomyces colonies can be wrinkled, rough, irregular, dry and white aerial mycelia on ISP2 agar medium **(Cheepurupalli *et al*., 2017)**.

- **Growth and nutritional characteristics**

Streptomyces are aerobic, non-acid-fast, and can grow on various media agar plates supplemented with organic compounds, including peptone-yeast extract iron, YE-ME, oatmeal, and glycerol -asparagine media. *Streptomyces* uses different carbon and nitrogen sources as glutamate, alanine, galactose, soluble starch, and potato starch, sodium nitrate and glycine. The optimal temperature is generally 30°C and the optimum pH is 7 **(Pandey *et al*., 2001; Voelker & Altaba, 2001).**

- **Habitat**

Streptomyces members can be found in almost all environments from deep sea to high mountains, and it is primarily found in soil **(Komaki, 2023).**

- **Melanin-producing species**

The species that can produce melanins are*: Streptomyces avermitilis, S. castaneoglobisporus, S. cyaneofuscatus, S.glaucescens, S.griseus,* S.*kathirae,* S. *lavendulae, S. michiganensis* and *S. parvus* **(Gosset, 2023).**

4.2.3/ *Frankia*

- **Taxonomy**

Frankia genus belong to the domain: bacteria, phylum: Actinobacteria, class: Actinobacteria, order: Actinomycetales, family: Frankiaceae **(Gosset, 2023).**

- **Microscopic characteristics**

Frankia bacteria are gram positive, non-motile filamentous bacteria, that grow in hyphal form. They produce 3 cell types: sporangiospores, hyphae, and diazo-vesicules **(Narayanasamy *et al*., 2020).**

- **Macroscopic characteristics on culture media**

Frankia colonies exhibit polymorphism on solid media ranging from diffuse, starfish, or compact shapes. They can be colorless or pigmented with several colors as: white, grey, black, dark red, brown, green or yellow, and they are mucilaginous in center **(Narayanasamy *et al*., 2020).**

- **Growth and nutritional characteristics**

Frankia are chemo-organotrophic, catalase positive, mesophilic which can grow at temperatures ranges between 28°C and 37°C. They use ammonium chloride as the best nitrogen source, and propionic acid and succinate as best carbon sources. **(Narayanasamy *et al*., 2020).**

- **Habitat**

Frankia strains live in the soil and root nodules of plants, and can be inoculated into the soil even after the host plant has disappeared. **(Benson *et al*., 1993).**

- **Melanin producing species**

The species that produces melanin is *Frankia* sp. **(Gosset, 2023).**

4.2.4/ *Dietzia*

- **Taxonomy**

Dietzia genus belongs to domain: Bacteria, phylum: Actinobacteria, class: Actinobacteria, order: Mycobacteriales, family: Dietziaceae **(Gosset, 2023).**

- **Microscopic characteristics**

Dietzia bacteria are Gram-positive. They appear as cocci and later develop into short rods or rod-shaped cells **(Koerner *et al*., 2009).**

- **Macroscopic characteristics on culture media**

Dietzia form colonies with a rounded, raised or convex shape with a glossy appearance. These colonies range in color from orange to coral red and have smooth edges **(Koerner *et al*., 2009).**

- **Growth and nutritional characteristics**

They are chemeo-organotrophic, and have an oxidative metabolism. They show optimal growth at temperatures below 37°C **(Koerner *et al*., 2009).**

- **Habitat**

Dietzia members are widely distributed throughout the environment **(Koerner *et al*., 2009).**

- **Melanin producing species**

The species that produces melanin is Dietzia *schimae* **(Gosset, 2023).**

4.3/ Phylum Proteobacteria

4.3.1/ *Shewanella*

- **Taxonomy**

Shewanella genus belongs to domain: Bacteria, phylum: Proteobacteria, class: Gamma proteobacteria, order: Alteromonadales, family: Shewanellacea **(Gosset, 2023).**

- **Microscopic characteristics**

Shewanella bacteria are Gram negative, straight or curved rods, motile using a single polar flagellum **(Satomi, 2014).**

- **Macroscopic characteristics on culture media**

Shewanella form light brown, round, smooth, medium sized and raised colonies with intact margins on nutrient agar **(Satomi, 2014).**

- **Growth and nutritional characteristics**

Shewanella are aerobic or facultative anaerobic, catalase positive, psychotropic and can use a range of carbon and nitrogen sources such as glucose, lactate, acetate, pyruvate succinate and citrate **(Satomi, 2014).**

- **Habitat**

Shewanella members are ubiquitous in natural environment, including fresh water, ocean sediments, lakes, marine environment and oil fields, and can be isolated from clinical samples **(Satomi, 2014).**

- **Melanin producing species**

The species producing melanin are *Shewanella algae*, *S.colwelliana*, and *S.oneidensis* **(Gosset, 2023).**

4.3.2/ *Aeromonas*

- **Taxonomy**

Aeromonas genus belongs to the domain: Bacteria, phylum: Proteobacteria, class: Gammaproteobacteria, order: Aeromonadales, family: Aeromonadaceae **(Gosset, 2023).**

- **Microscopic characteristics**

Aeromonas are Gram negative, non-spore forming, rod-shaped bacteria, with 1-3μm in length. *Aeromonas* are divided into 2 groups, non-motile bacteria, and motile bacteria with single polar flagellum **(Pessoa, 2023).**

- **Macroscopic characteristics on culture media**

The first group of *Aeromonas* bacteria, exhibits circular, and convex colonies with diameter of 1-2 mm, while the second group exhibits circular, convex, and translucent colonies with diameter of 1-3 mm diameter **(Pessoa, 2023).**

- **Growth and nutritional characteristics**

Aeromonas are oxidase positive bacteria with ability to ferment glucose. They use glucose, chitin, ammonium salts, and several amino acids as carbon and nitrogen sources. The first group of *Aeromonas* contains psychrophilic bacteria with good growth in temperature range between 22 and 25°C, and the second group contains mesophilic bacteria with good growth in temperature range between 35 and 37°C **(Pessoa, 2023).**

- **Habitat**

Aeromonas members can be found in different ecosystems including aquatic environments, animal, clinical samples, and foods as vegetables, dairy products and beef **(Pessoa, 2023).**

- **Melanin producing species**

The species producing melanin are*: Aeromonas media*, *A. salmonicida subsp .pectinolytica*, and *A. salmonicida subsp. Salmonicida* **(Gosset, 2023).**

4.3.3/ *Vibrio*

- **Taxonomy**

Vibrio genus belongs to domain: Bacteria, phylum: Proteobacteria, class: Gammaproteobacteria, order: Vibrionales, family: Vibrionaceae **(Gosset, 2023).**

- **Microscopic characteristics**

Vibrio bacteria are Gram negative, which are small, straight, slightly curved, curved, or comma-shaped rods, measuring approximately 0.5–0.8 × 1.4–2.6 μm. They are motile using monotrichous or multitrichous polar flagella. Some species have some lateral flagella **(Farmer Iii *et al*., 2015).**

- **Macroscopic characteristics on culture media**

Vibrio exhibits smooth, buff to cream-colored colonies of 5-5 mm diameter. Some species produce grayish colonies on blood agar **(Farmer Iii *et al*., 2015).**

- **Growth and nutritional characteristics**

Vibrio are chemo-organotrophic, catalase positive, can reduce nitrate to nitrite, and capable of fermentative and respiratory metabolism. Most of *Vibrio* members can grow well on different culture media, including protein-based agar and marine and sea water agar. The optimal temperature range of most of species is 18-22°C, while some species can

grow at temperatures ranges between 0 and 4°C, and other can grow in temperature up to 45°C. The optimal pH range is 7-8)**Farmer Iii *et al*., 2015).**

- **Habitat**

Vibrio members can be found primarily in water, both fresh water and marine environments, and its associated plants, animals, and microorganisms **(Farmer Iii *et al*., 2015).**

- **Melanin producing species**

The species producing melanin are: *Vibrio alginolyticus, V. antiquarius, V. cholerae, V. harveyi, V. nigripulchritudo* and *V. rotiferianus* **(Gosset, 2023).**

4.3.4/ *Klebsiella*

- **Taxonomy**

Klebsiella genus belongs to the domain: Bacteria, phylum: Proteobacteria, class: Gamma proteobacteria, order: Enterobacteriales, and family: Enterobacteriaceae **(Gosset, 2023).**

- **Microscopic characteristic**

Klebsiella bacteria are Gram negative, non-motile, straight rods of 0.3-1× 0.6-6 μm which are arranged singly, in pairs, or in short chains. They are often surrounded by capsule (a large polysaccharide capsule) **(Grimont & Grimont, 2015*).***

- **Macroscopic characteristics on culture media**

Klebsiella colonies exhibit dome-shaped glistening of varying degrees of stickness depending on the medium composition **(Grimont & Grimont, 2015).**

- **Growth and nutritional characteristics**

Klebsiella are oxidase positive, facultative anaerobic, capable of fermentative and respiratory metabolism. They grow on meat extract media, and use myo-inositol, L-rhamnose and sucrose as sole carbon sources **(Grimont & Grimont, 2015*).***

- **Habitat**

Klebsiella members can be found in several habitats including: aquatic environments, industrial wastewater, living trees, root surfaces, plants and food with high concentration of sugars and acids **(Grimont& Grimont, 2015*).***

- **Melanin producing species**

The species producing melanin*: Klebsiella pneumoniae* and *K.*sp **(Gosset, 2023).**

4.4/ Phylum Bacteroidetes

4.4.1/ *Flavobacterium*

- **Taxonomy**

Flavobacterium genus belongs to the domain: Bacteria, phylum: Bacteroidota, class: Flavobacteriia, order: Flavobacteriales, family: Flavobacteriaceae **(Gosset, 2023).**

- **Microscopic characteristic**

Flavobacterium bacteria are Gram negative, which occur as single rods with slightly or rounded ends. They measure about 0.5 - 0.5 μm in diameter, and 2 - 5 μm in length. They can be either non-motile or motile by grinding flagella **(Bernardet & Bowman, 2015)**.

- **Macroscopic characteristics on culture media**

Flavobacterium colonies on nutrient rich agar are typically circular, low convex, to convex, smooth and shiny. They range in color from pale to bright yellow **(Bernardet & Bowman, 2015)**.

- **Growth and nutritional characteristics**

Flavobacterium are mostly chemeo-organotrophic, catalase and oxidase positive, obligatory aerobic, with strictly respiratory type of metabolism. About half of the species can reduce nitrates to nitrites. They mostly grow on nutrient, and tryptic soy agar with optimal temperature ranges between 20°C and 30°C, and 2-4% NaCl **(Bernardet & Bowman, 2015).**

- **Habitat**

Flavobacterium members can be found in several niches including soil, fresh water, marine, saline habitats and other different niches **(Bernardet & Bowman, 2015)**.

Melanin producing species

The species that produces melanin is *Flavobacterium kingsejongi* **(Gosset, 2023).**

5/ Industrial production of bacterial melanins

The production of melanin depends highly on the regulation of melanin synthesis enzymes, which is driven by multiple nutritional factors and physicochemical conditions. Tyrosine, peptone, glucose, starch and yeast extract are widely used as carbon and nitrogen sources **(table 02).** Recent studies have also exploited agricultural residues, such as fruit waste extract, corn steep liquor and wheat bran extract, to lower the production cost while ensuring the high yield of production. Copper is an important element for melanin production because of its role as a cofactor for tyrosinases. Besides copper, other metals can also enhance melanin formation **(table 02).** A recent study showed a strong increase in tyrosinase activity and melanin production driven by the addition of iron and nickel **(table 02).** On the other hand, the presence of metals may induce stress responses in bacteria, resulting in melanin formation. In other cases, melanin synthesis is promoted by different kinds of stress such high temperature, nutrient-poor growth media, hyperosmotic pressure, etc. Because of the multiple and diverse factors that affect melanin biosynthesis, there is no universal culture media or cultivation condition for growing melanogenic bacteria. Instead, the composition and ratio of each component should be identified depending on the bacterium **(Tran-Ly *et al*., 2020).**

Table 02: Nutritional factors for some bacterial melanins production (Tran-Ly *et al*., 2020)

Bacteria	Melanin type	Tyrosine added	Metal ion added	substrates
Actinoalloteichus sp. MA-32	DOPA	Yes	Fe, Mg	Glycerol
Bacillus safensis	Not defined	None	None	Fruit waste extract
Brevundimonas sp. SGJ	DOPA	Yes	Cu	Tryptone
Nocardiopsis alba MSA10	Not defined	Yes	Not defined	Sucrose
Pseudomonas sp. WH001 55	Not defined	Yes	None	Starch, yeast extract
Pseudomonas stutzeri HMGM-7	DOPA	Yes	None	Nutrient broth in sea water
Streptomyces glaucescens NEAE-H	DOPA	Yes	Fe	Protease peptone
Streptomyces kathirae SC-1	DOPA	Yes	Cu	Amylodextrine, yeast extract
Streptomyces lusitanus DMZ-3	Not defined	Yes	Cu	Beef extract
Streptomyces sp. ZL-24	DOPA	None	Fe, Ni	Soy peptone

6/ Physiological roles of melanin in the bacterial cell

Melanin pigment plays several physiological roles in the bacterial cell such as:

6.1/ Electron acceptor

Melanin has the property of donating and receiving electrons. It acts as a final electron acceptor rather than dioxygen in electron exchange reactions during anaerobic respiration in bacteria. It also participates in oxidation and reduction processes involving iron compounds by reducing insoluble ferric compounds to the ferrous state, allowing bacteria to utilize them as electron acceptors **(Plonka & Grabacka, 2006*).***

6.2/ Nitrogen fixator

Some bacteria such as *Azotobacter* use melanin to help them fix nitrogen. Melanin enhance the utilization of oxygen by the bacteria in order to maintain low conditions for the bacteria to be able to fix nitrogen for plant use **(Banerjee *et al*., 2014).**

6.3/ Virulence factor

When pathogenic bacteria are exposed to environmental pressure or lack of food, they produce melanin, which in turn helps them increase their virulence in two ways. The first is to weaken the immune response of the host, as it protects the bacteria from the process of phagocytosis by disrupting the effect of reactive oxygen species (ROS) that are highly reactive chemicals formed from diatomic oxygen, water, and hydrogen peroxide. Some prominent ROS are hydroperoxide, superoxide which are harmful to bacteria. The second role can be in the production of pili to allow the bacteria to bind to target cells, and may also promote toxin production. Examples of bacteria that use melanin in their virulence include *Vibrio cholerae*, *Burkholderia cepacia*, and *Klebsiella pneumonia* **(Kurian and Pate, 2014; Singh *et al*., 2023).**

6.4/ Chelating metal ion

The melanin contains in its structure diverse functional groups such as amine, hydroxyl and carboxyl groups, which are characterized by their ability to interact and bind to heavy metals (copper, lead and mercury), which are known to be insoluble. Melanins are capable in protecting the cell itself from in vivo and in vitro sources of oxidative stress **(Azman *et al*., 2018; Guo *et al*., 2023).**

7/ Applications of bacterial melanin

7.1/ Medical and pharmaceutical applications

7.1.1/ Medical imaging

Melanin has been recognized as a potential alternative in various medical imaging techniques, revolutionizing operations such as magnetic resonance imaging (MRI) and positron emission tomography (PET). Melanin is characterized by its high ability to absorb various waves, including electric, light, or magnetic waves. Consequently, melanin can be combined with certain materials for use in medical imaging, producing high-contrast and accurate images capable of detecting tumors and other abnormalities with high efficiency. Melanin's capabilities also extend to optical imaging technology, which relies on the absorption of light energy by the body's organs. This absorption generates sound waves that are tracked to create detailed images of the anatomical structure. The integration of melanin in medical imaging helps enhance the quality of diagnosis and patient care **(Caldas *et al*., 2020).**

7.1.2 / Cancer therapy

Duo to its UV protection property, melanin showed positive results in the activity of inhibiting tumor growth at a concentration of 50 µg/ml, and its effects could vary depending on the type of the cancer. Melanin also has the ability to strengthen the immune system especially for immunocompromised patients due to the ability of melanin nanoparticles to load cancer antigens into lymphatic tissues efficiently, so it can be used in cancer vaccines **(Cuzzubbo & Carpentier , 2021; Guo *et al*., 2023).**

Phototherapy technique is a new technique specialized in the treatment of some types of primary cancers. This treatment includes directing a near-infrared laser on the desired tissues, which leads to a local increase in temperature that eventually kills the tumor cells. Carbon nanoparticles have been used previously in this treatment, but they left side effects. Melanin nanoparticles are considered a better alternative, and this is due to their high ability to absorb energy, as they have been experimentally proven to be able to kill cancer cells in a short time and at a temperature of 50 °C in just 5 min **(Caldas *et al*., 2020).**

7.1.3/ Use in medical transistors

The conductivity characteristic of melanin opens up interesting prospects for creating new medical treatments and devices that can communicate with signals inside the body. One of

these technologies is the use of melanin in thin-film organic electrochemical transistors (OECTs) that operate with the movement of protons within its channels. The conductivity of melanin allows the transmission of electrical signals, and this makes it perfectly suitable for integration with biological systems. By integrating melanin into a transistor, devices capable of sensing and modifying electrical signals in the body can be created, which helps in finding early treatments **(Liu *et al*., 2020).**

7.1.4/ Drug delivery

Due to the biocompatibility of melanin and its biodegradability, melanin nanoparticles are used as a component of a drug delivery system by combining them with the drug through reactions to form a water-soluble nanocomposite. These drugs are injected into the patient's body to be transported to the affected organ and treat it efficiently without side effects **(Caldas *et al*., 2020; Liu *et al*., 2020).**

7.1.5 / Antioxidant activity

Melanin nanoparticles have shown promising results in treating diseases caused by the accumulation of harmful molecules in our bodies, due to their antioxidant properties. Melanin has the ability to bind to metal ions and can inactivate excited molecules and free radicals, giving nanoscale results similar to the effect of ascorbic acid **(Caldas *et al*., 2020, Roy & Rhim, 2021).**

7.2/ Cosmetics applications

7.2.1/ Sunscreens

Various types of UV rays can penetrate the skin, damage DNA, and cause skin cancer. So safer and more effective sunscreens are needed. Melanin is a natural substance that filters UV rays and scavenges reactive oxygen species, making it a promising ingredient in sunscreens, especially since melanin is originally produced as the skin's response to UV rays. In vivo studies have shown that bacterial melanin can provide photoprotection against UV rays and increase the sun protection factor in commercially available sunscreens **(Nacht, 1991; Singh *et al*., 2021).**

7.2.2/ Skin creams

The anti-aging ability of melanin makes it a valuable ingredient in skin creams and skin care products. Just like vitamins E and C, melanin helps protect the skin from premature aging and flare-ups. By incorporating melanin into skin care products, it contributes to

preventing the oxidation process and slowing down aging. In addition, it reduces scars and inflammation caused by some chemicals **(Liberti *et al*., 2020, Guo *et al*., 2023).**

7.3/ Industrial applications

7.3.1/ Textile industry

In the textile industry, the use of environmentally harmful dyes has been a concern for all manufacturers. Melanin is considered a suitable solution as it is a natural and environmentally friendly alternative. Melanin-based pigments have several advantages over industrial dyes. First, they are of high quality so that they can be used to produce more quality fabrics, and more luster, which increases its aesthetic value. Secondly, it can contribute to maintain the cleanliness of fabrics by protecting them from bacteria and inhibiting their growth **(Singh *et al*., 2021).**

7.3.2/ Food industry

The properties of melanin ensure the quality and the safety of food products as well as the extension of their shelf-life. Thanks to its anti-radiation and anti-oxidation properties, melanin can be utilized to protect foods from damage and stress, which would otherwise affect their flavor and nutritional value. Melanin is commonly incorporated into packaging materials for this purpose. Its ability to reduce microbial activity allows melanin to safeguard foods by inhibiting the growth of microbes, thus protecting them from spoilage and infection. Moreover, melanin provides protection against ultraviolet radiation, which can contribute to the deterioration of food quality **(Guo *et al*., 2023).**

7.4/ Environmental applications

Melanin has the ability to interact and bind with heavy metals, such as zinc, copper and magnesium. This unique property makes melanin an effective element in the process of picking up and cleaning minerals from polluted water. Scientists have discovered that *Azotobacter crococcum* uses melanin for providing nitrogen to plants and protecting them. These bacteria act as a good partner for plants in the presence of an environment contaminated with heavy metals, by detoxifying the soil and sequestering heavy metals from it **(Singh *et al*., 2021).**

Analysis part

Using Google Scholar website, 12 scientific papers from the period 2013-2023 related to the isolation and characterization of melanin pigment produced by different bacterial strains were selected for analysis.

The analysis was performed on the basis of:

- The ecological site, and country of the isolated bacterium producing melanins.
- The taxonomy of the bacterium producing melanins (Gram, phyla …etc).
- The media used for melanin production (solid and liquid).
- The chemical characteristics of melanins (solubility, color and nature).
- Biochemical characteristics of melanins) (absorption of UV/Vis, scavenging property and sun protection factor).
- The different benefits and bioactivities of bacterial melanin.

Finally, the results obtained from the comparative analysis were discussed.

1/ The papers used for analysis

The twelve scientific papers used in current study are mentioned in the table below **(table 03).**

Table 03: The papers used for analysis.

N°	Title	References
1	Isolation and characterization of biologically active melanin from *Actinoalloteichus* sp. MA-32.	**Manivasagan *et al.*, 2013**
2	Antioxidant and antimicrobial activities of melanin produced by a *Pseudomonas balearica* strain.	**Zerrad *et al.*, 2014**
3	Food, cosmetic and biological applications of characterized DOPA-melanin from *Vibrio alginolyticus* strain BTKKS3	**Buriak & Bhat, 2018**
4	Biosynthesis, characterization and antagonistic applications of extracellular melanin pigment from marine *Nocardiopsis* Species	**Kararudheen *et al*., 2019**
5	Bioproduction, structure elucidation and in vitro antiproliferative effect of eumelanin pigment from *Streptomyces parvus* BSB49	**Bayram *et al*., 2020**
6	Natural melanin produced by the endophytic *Bacillus subtilis* 4NP-BL associated with the halophyte *Salicornia brachiata*	**Ghadge *et al*., 2020**
7	Production, purification, and characterization of *Streptomyces* sp. strain MPPS2 extracellular pyomelanin pigment	**Bayram, 2021**
8	Biocompatibility and radioprotection by newly characterized melanin pigment and its production from *Dietzia schimae* NM3 in optimized whey medium by response surface methodology	**Eskandari & Etemadifar, 2021a**
9	Melanin biopolymers from newly isolated *Pseudomonas koreensis* strain UIS 19 with potential for cosmetics application, and optimization on molasses waste medium	**Eskandari & Etemadifar, 2021b**
10	Melanin: Production from cheese bacteria, chemical characterization, and biological activities.	**Farraz *et al*., 2021**
11	Characterization of brown-black pigment isolated from soil bacteria, *Beijerinckia fluminensis*	**Joshi *et al. ;* 2021**
12	Bacterial melanin with immense cosmetic potential produced by marine bacteria *Bacillus pumilus* MIN3	**Jinga & Gordhanbhai, 2022**

2/ The origin of bacteria producing melanin

The bacteria producing melanins were isolated from different ecological niches and geographical sites. *Actinoalliteichus* sp. MA-32 was isolated from marine sediments of Tuticorin coast from India **(Manivasagan *et al*., 2013).** *Pseudomonas balearica* U7 from the marine green algae *Ulva lactuca* from a beach in the north west of the Atlantic coast of Morocco **(Zerrad *et al.*, 2014).** Marine *Nocardiopsis* species (*Nocardiopsis* sp. and *Nocardiopsis dassonvillei*) from 2 different marine sediment soil samples from the Aleppey beach, Alappuzha, India **(Buriak & Bhat, 2018).** *Vibrio alginolyticus* BTKKS3 from a marine sediment from Kanyakumari coast of South India **(Kararudheen *et al*., 2019).** *Streptomyces parvus* BSB49 from different soil sample**s**, from Bayburt providence, Turkey **(Bayram *et al*., 2020)**, *Bacillus subtilis* 4NP-BL from the plant *S. brachiata* from

Nava bandar, Bhavangar distinct, Gujarat, India **(Ghadge *et al.*, 2020)**, *Dietzia schimae* NM3 from desert soil sample, from Iran **(Eskandari & Etemadifar, 2021a)**, *Pseudomonas koreensis* UIS 19 from soil sample from Isfahan, Iran **(Eskandari & Etemadifar, 2021b)**, *Beijerinckia fluminensis* UQM from iron ore mine soil from the Tumsar region in the Bhandara district of Maharashtra, in India **(Joshi *et al.* ; 2021)**, *Pseudomonas putida* ESACB 191 from goat's cheese rind with brownish surface from Portugal **(Farraz *et al.*, 2021)**, *Bacillus pumilus* MIN3 from sea water from Naryan sarovar kutch, Gujarat, India **(Jinga & gordhanbhai, 2022)** .

The ecological niche of *Streptomyces* sp. MPPS2 origin was not mentioned (**Bayram, 2021).**

In total, there were five melanin producing bacteria isolated from the marine ecosystem, four from soil, one from plant and one from food. An ecological niche was not mentioned **(figure 10).**

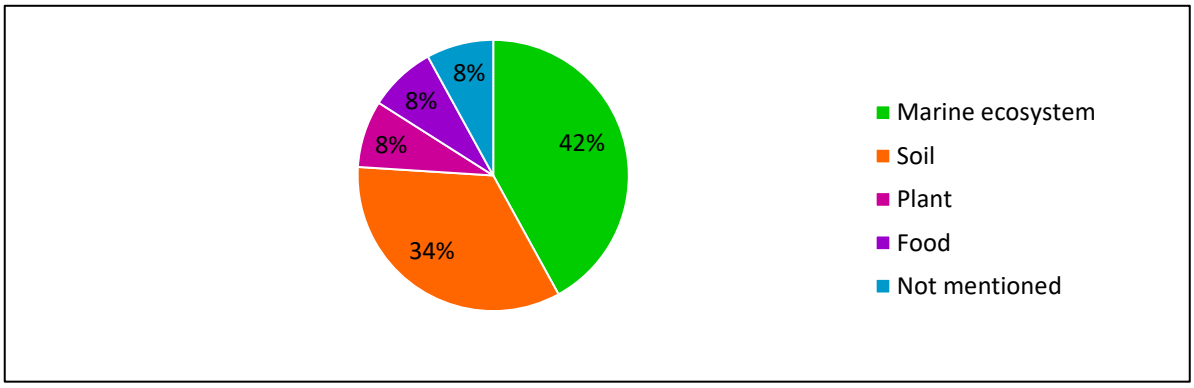

Figure 10: Repartition of bacteria producing melanin on the basis of their ecological niches.

Among the 12 studied bacteria, **7** belong to **Gram positive** and **5** to **Gram negative (figure 11).**

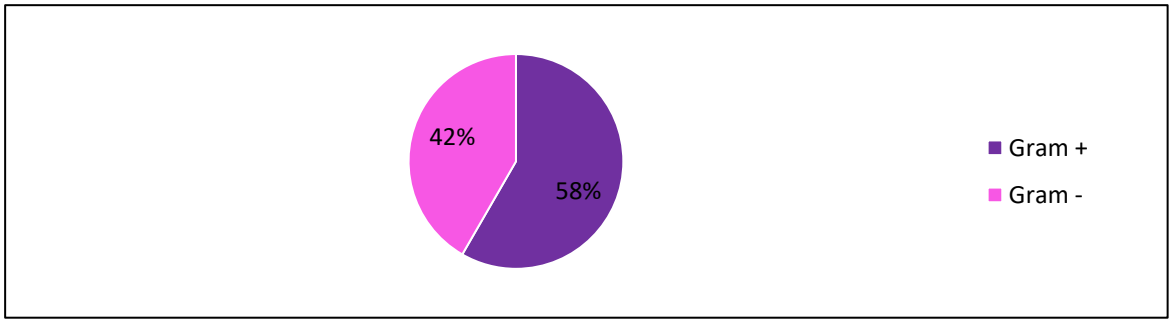

Figure 11: Repartition of bacteria producing melanin on the basis of their Gram type.

The 12 studied bacteria belong to **4 phyla** as the following:

- **Five to the phylum Actinobacteria:** *Actinoalliteichus* sp. MA-32, *Nocardiopsis* species, *Streptomyces parvus* BSB49, *Dietzia schimae* NM3 and *Streptomyces* sp. MPPS2**.**
- **Five to the phylum Proteobacteria:** *Pseudomonas balearica* U7, *Vibrio alginolyticus* BTKKS3, *Pseudomonas koorensis* UIS 19, *Beijerinckia fluminesis* UQM and *Pseudomonas putida* ESACB 191.
- **Two to the phylum Firmicutes:** *Bacillus pumilus* MIN3 and *Bacillus subtilis* 4NP-BL **(figure 12).**

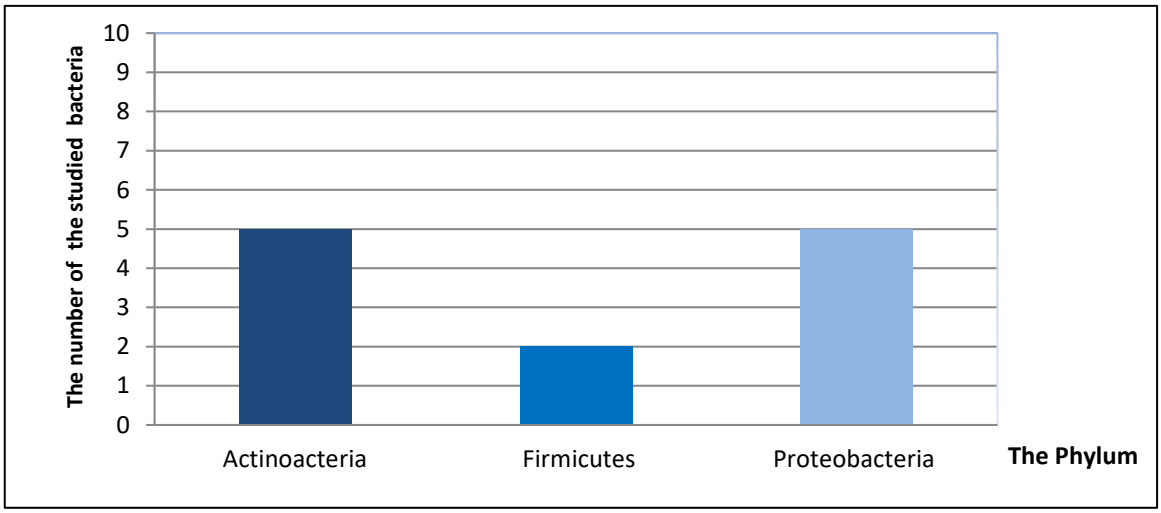

Figure 12: Phyla of bacteria producing melanin

3/ Media used for melanin production

Melanin-producing bacteria were determined using screening media, followed by fermentation assays using liquid media.

3.1/ Screening media

Several culture media were used to screen melanin-producing bacteria. Most of them contained the amino acid L-tyrosine as substrate **(62%)** which were: tyrosine agar in case of *Actinoalloteichus* sp. MA-32 **(Manivasagan *et al.*, 2013)**, M9 minimal medium in case of *Pseudomonas balearica* U7 **(Zerrad *et al.*, 2014)**, ISP7 medium in case of *Nocardiopsis* species **(Buriak & Bhat, 2018)**, tyrosine basal broth in case of *Vibrio alginolyticus* BTKKS3 **(Kararudheen *et al.*, 2019),** nutrient agar in case of *Dietzia schimae* NM3 and *Pseudomonas koreensis* UIS 19 **(Eskandari & Etemadifar, 2021a;**

Eskandari & Etemadifar, 2021b), Ashby's glucose agar in case of *Beijerinckia fluminensis* UQM **(Joshi *et al.*, 2021),** tyrosine basal broth (TBB) in case of *Bacillus pumilus* MIN3 **(Jinga & Gordhanbhai, 2022)**.

Whereas other media did not contain any tyrosine in their composition **(38%)** which were: tryptone–yeast extract agar in case of *Actinoalloteichus* sp. MA-32 **(Manivasagan *et al.*, 2013),** ISP2 agar and ISP4 in case of *Streptomyces parvus* BSB49 **(Bayram *et al.*, 2020),** ISP2 medium in case of *Streptomyces* sp. MPPS2 **(Bayram, 2021*)*** and ISP6 in case of *Nocardiopsis* species **(Buriak & Bhat, 2018) (figure 13).**

Two studies did not use screening media **(Ghadge *et al.*, 2020*;* Farraz *et al.*, 2021).**

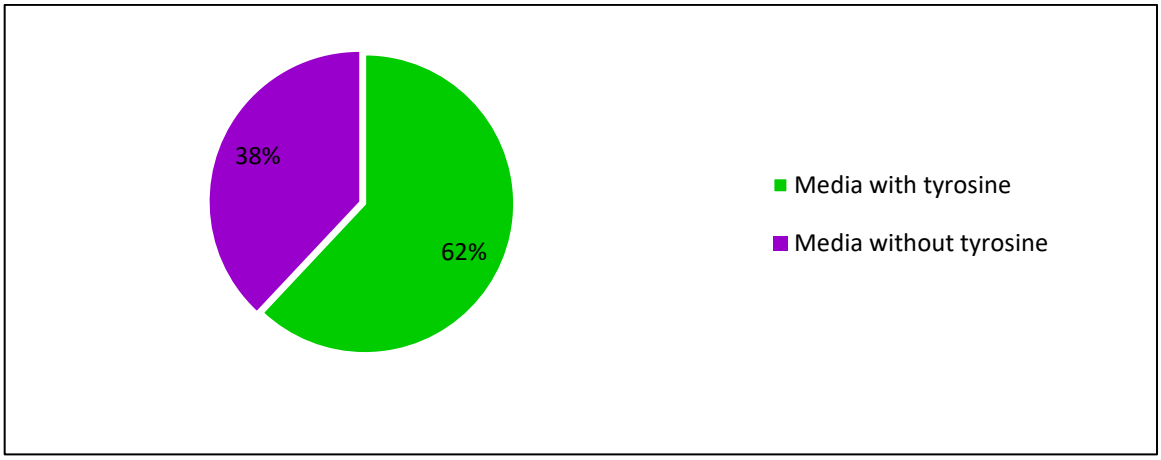

Figure 13 : The use of L-tyrosine substrate in different screening media

Regarding the media containing L-tyrosine, it was supplemented with different concentrations : **0.05%, 25%** in tyrosine agar in case of *Actinoalloteichus* sp. MA-32 and in ISP7 in case of *Nocardiopsis* species **(Manivasagan *et al.*, 2013 ; Buriak & Bhat, 2018) , 0.1% (12%)** in M9 minimal medium in case of *Pseudomonas balearica* U7 **(Zerrad *et al.*, 2014), 0.2% (63%)** in tyrosine basal broth (TBB) medium used for *Bacillus pumilus* MIN3 and *Vibrio alginolyticus* BTKKS3 **(Kararudheen *et al.*, 2019 *;* Jinga & Gordhanbhai, 2022***)***, in nutrient agar in case of *Dietzia schimae* NM3 and *Pseudomonas koreensis* UIS 19 **(Eskandari & Etemadifar, 2021a *;* Eskandari & Etemadifar, 2021b)**, in Ashby's glucose agar in case of *Beijerinckia fluminensis* UQM **(Joshi *et al.*, 2021).**

It seems that **0.2%** was the most used percentage of L-tyrosine in the screening media **(63%),** followed by the percentage **0.05%, 25%** and finally the percentage **0.1% (12%) (figure 14).**

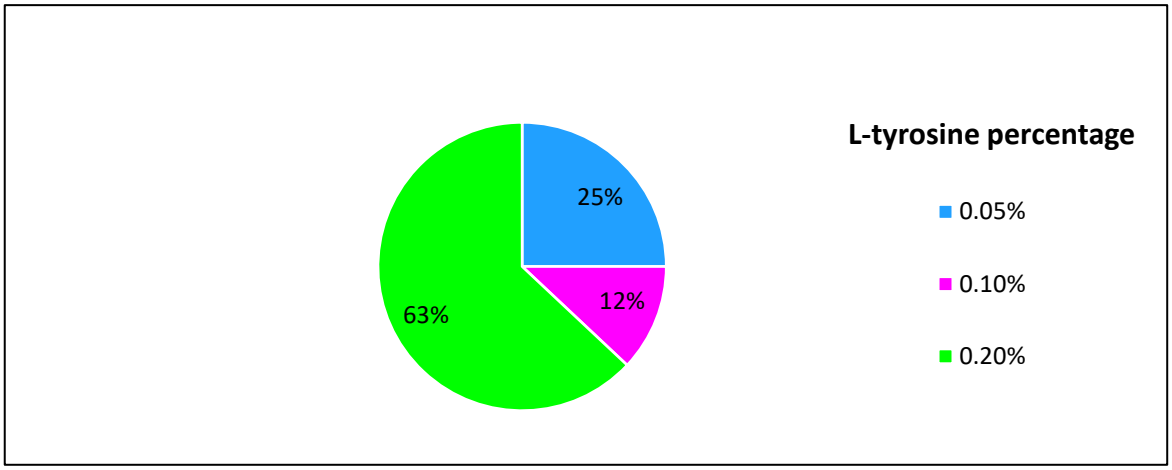

Figure 14: Percentage of L-tyrosine supplemented in different screening media for melanin production.

Regarding the temperature, media used for melanin production were incubated in temperature ranging from 28°C to 37°C **(table 04).** 28°C (17 %), 30°C (33 %), 35°C (8%) and 37°C (17%), whereas three studies did not mention the incubation temperature of the media (25 %) **(figure 15).**

Table 04: Temperature used for bacterial melanin production.

The bacterium	The temperature of incubation (°C)
Actinoalloteichus sp. MA-32	28
Pseudomonas balearica U7	37
Nocardiopsis species	Not mentioned
Vibrio alginolyticus BTKKS3	37
Streptomyces parvus BSB49	30
Bacillus subtilis 4NP-BL	28
Streptomyces sp. MPPS2	30
Dietzia schimae NM3	35
Pseudomonas koreensis UIS 19	30
Beijerinckia fluminensis UQM	30
Pseudomonas putida ESACB 191	Not mentioned
Bacillus pumilus MIN3	Not mentioned

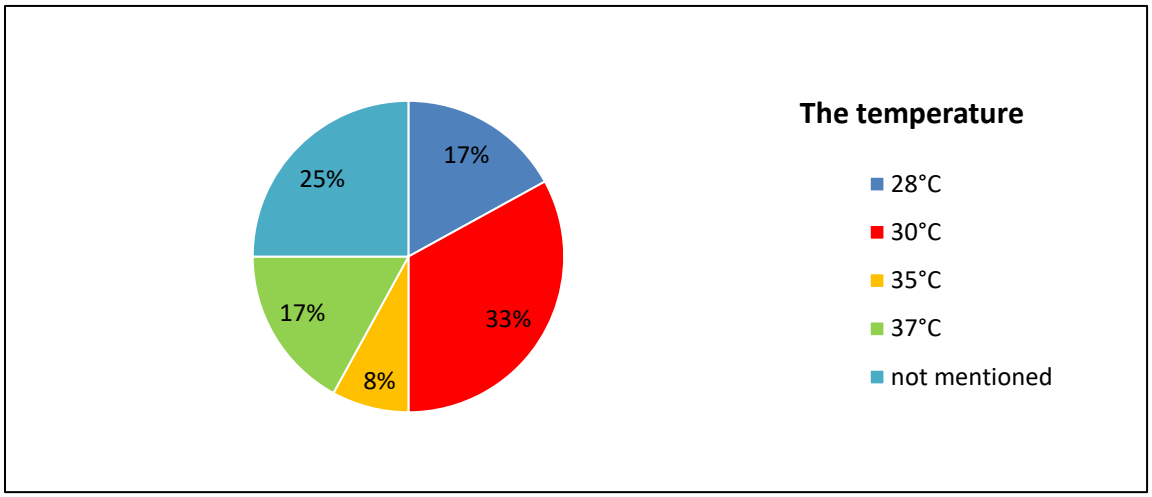

Figure 15: Incubation temperature used for bacterial melanin production.

We can deduce that the researchers used **mesophilic conditions** for bacterial melanin production.

Regarding the pH, media used for melanin production were incubated at pH ranging between 7 to 8 **(table 05).** The percentage 8% for 7, 7.2, 7.4 and 8 consecutively whereas eight studies did not mention the incubation pH of the media (68%) **(figure 16).**

Table 05 : pH used for bacterial melanin production.

The bacterium	The pH of incubation
Actinoalloteichus sp. MA-32	7
Pseudomonas balearica U7	7.4
Nocardiopsis species	Not mentioned
Vibrio alginolyticus BTKKS3	8
Streptomyces parvus BSB49	Not mentioned
Bacillus subtilis 4NP-BL	7.2
Streptomyces sp. MPPS2	Not mentioned
Dietzia schimae NM3	Not mentioned
Pseudomonas koreensis UIS 19	Not mentioned
Beijerinckia fluminensis UQM	Not mentioned
Pseudomonas putida ESACB 191	Not mentioned
Bacillus pumilus MIN3	Not mentioned

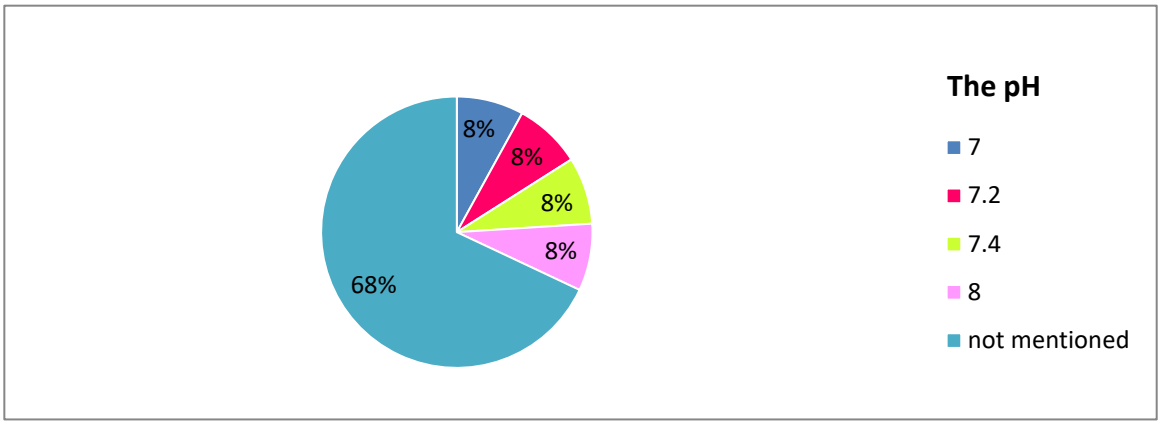

Figure 16 : pH used for bacterial melanin production.

We can deduce that the researchers used **neutral** to **slightly alkaline** conditions for bacterial melanin production **(figure 16).**

Regarding the incubation period, media used for melanin production were incubated for a period ranging from 2 days to 14 days. 2 days (17%), 5days (08%), 7 days (25%), 10 days (08%), and 14 days (08%). Whereas four studies not mentioned the incubation period (34%) **(figure 17).**

Table 06: Incubation period used for bacterial melanin production.

The bacterium	The period of incubation (days)
Actinoalloteichus sp. MA-32	07
Pseudomonas balearica U7	Not mentioned
Nocardiopsis species	14
Vibrio alginolyticus BTKKS3	Not mentioned
Streptomyces parvus BSB49	7
Bacillus subtilis 4NP-BL	Not mentioned
Streptomyces sp. MPPS2	5
Dietzia schimae NM3	2
Pseudomonas koreensis UIS 19	2
Beijerinckia fluminensis UQM	7
Pseudomonas putida ESACB 191	Not mentioned
Bacillus pumilus MIN3	10

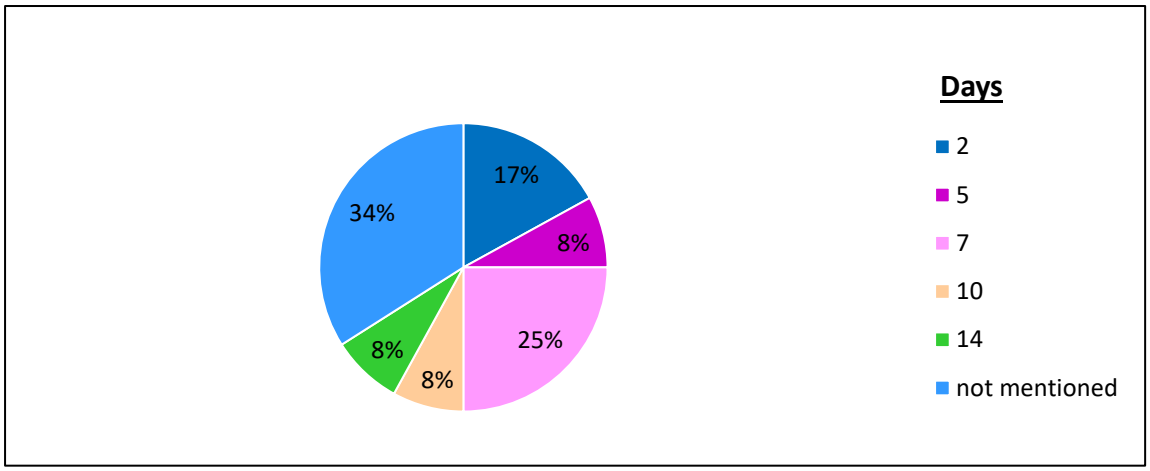

Figure 17: Incubation periods used for bacterial melanin production.

3.2/ Fermentation media

The media used in the fermentation process were either identical in term of components to the ones used in the screening process for melanin production or totally different.

The similar media were tyrosine basal broth in case of *Actinoalloteichus* sp**.** MA-32, *Vibrio alginolyticus* BTKKS3 and *Bacillus pumilus* MIN3 **(Manivasagan *et al*., 2013 ; Kamarudheen *et al*., 2019 ; Jinga & Gordhanbhai, 2022),** M9 minimal medium broth in case of *Pseudomonas balearica* U7 **(Zerrad et al., 2014),** nutrient broth in case of both of *Dietzia schimae* NM3 and *Pseudomonas koreensis* UIS 19 **(Eskandari & Etemadifar, 2021a ; Eskandari & Etemadifar, 2021b),** modified Ashby's glucose broth in case of *Beijerinckia fluminensis* UQM **(Joshi *et al*., 2021).** They represent **58%.**

The different media were nutrient broth in case of *Streptomyces parvus* BSB49 and *Streptomyces* sp. MPPS2 **(Bayram *et al*., 2020; Bayram, 2021),** and SS broth in case of *Nocardiopsis* species **(Kurian & Bhat, 2018),** with a percentage of **25%.**

Whereas, the screening media were not mentioned in two studies **(17%) (Farraz *et al*., 2021; Ghadge *et al*., 2020).**

We can deduce that most researchers used the medium used in the screening part as liquid in the fermentation process part **(58%).** Whereas only **25%** used **a totally different medium. 17% not mentioned (figure 18).**

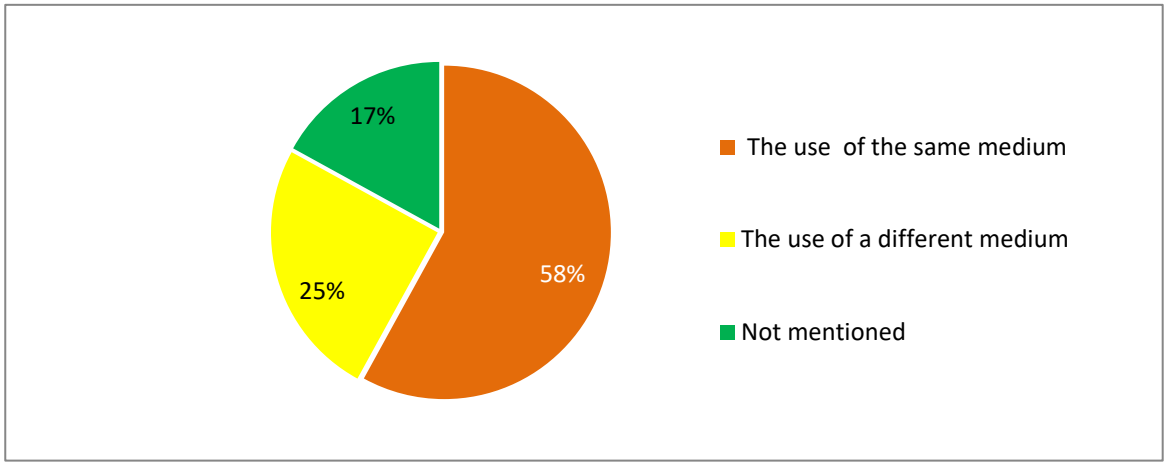

Figure 18: Similarity and difference between screening media and fermentation media in term of composition.

Regarding L-tyrosine, it was supplemented with different concentrations to the media: 0.05% of L-tyrosine in tyrosine broth in case of *Actinoalloteichus* sp**.** MA-32 and in Müeller-Hinton broth in case of *Pseudomonas putida* ESACB 191 **(Manivasagan *et al*., 2013 ; Farraz *et al*., 2021),** 0.1% in M9 broth in case of *Pseudomonas balearica* U7 and in ISP-4 broth in case of *Bacillus subtilis* 4NP-BL **(Zerrad *et al*., 2014 ; Ghadge *et al*., 2020),** 0.2% in nutrient broth in case of *Dietzia schimae* NM3 and *Pseudomonas koreensis* UIS 19, in tyrosine basal broth in case of *Bacillus pumilus* and *Vibrio alginolyticus* BTKKS3 and in Ashby's glucose broth in case of *Beijerinckia fluminensis* UQM **(Kamarudheen *et al*., 2019 ; Eskandari & Etemadifar, 2021a ; Eskandari & Etemadifar, 2021b ; Joshi *et al*., 2021 ; Jinga & Gordhanbhai, 2022).**

While in case of *Nocardiopsis* species, *Streptomyces parvus* BSB49 and *Streptomyces* sp. MPPS2, L-tyrosine was not added to the screening media **(Buriak & Bhat, 2018 ; Bayram *et al*., 2020 ; Bayram, 2021).**

It seems that **0.2%** was the most used percentage of L-tyrosine in the screening media **(43%),** followed by the percentage **0.1% (16%)**, **0.25% (8%)** then **0.05% (8%)** and finally the percentage **0.1% (12%)**. Whereas **25%** did not add tyrosine **(figure 19).**

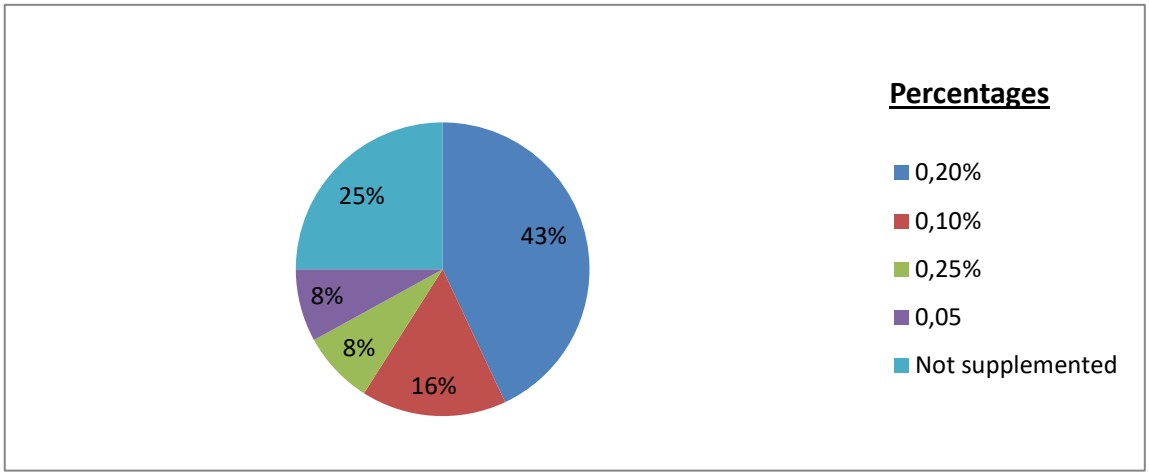

Figure 19: Percentage of L-tyrosine supplemented in different fermentation media for melanin production.

Most of the studies **(67%)** used the same percentage of L-tyrosine that was used in screening protocols, **(8%)** used a fermentation broth with different percentage of L-tyrosine. Finally, in the remaining studies, L-tyrosine was not added to the screening media **(25%) (figure 20).**

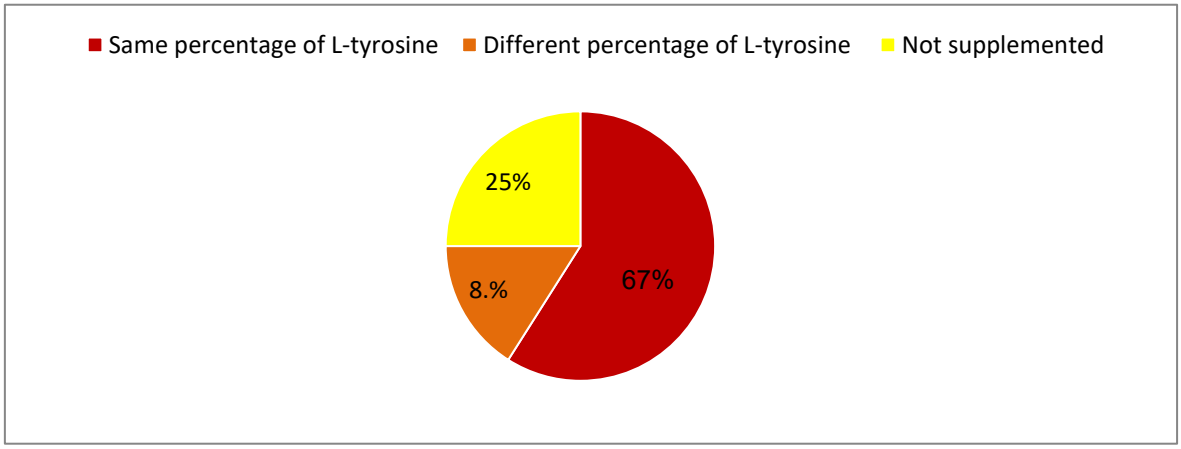

Figure 20: Similarity and difference between screening media and fermentation media in term of L-tyrosine percentage.

Regarding the temperature, the media used for melanin fermentation were incubated at different temperatures. Tyrosine broth in case of *Actinoalloteichus* sp.MA-32 and ISP-4 broth in case of *Bacillus subtilis* 4NP-BL were incubated at 28°C **(Manivasagan *et al.*, 2013 ; Ghadge *et al.*, 2020 ;)** M9 minimal broth in case of *Pseudomonas balearica* U7 and

tyrosine basal broth in case of *Vibrio alginolyticus* BTKKS3 were incubated at 37° **(Zerrad et al., 2014 ; Kararudheen *et al*., 2019),** nutrient broth in case of *Streptomyces parvus* BSB49 , *Streptomyces* sp. MPPS2 and *Dietzia schimae* NM3 were incubated at 35° **(Bayram *et al*., 2020 ; Bayram, 2021 ; Eskandari & Etemadifar, 2021a),** nutrient agar in case of *Pseudomonas koreensis* UIS 19, modified Ashby's glucose broth in case of *Beijerinckia fluminensis* UQM and Müeller-Hinton broth in case of *Pseudomonas putida* ESACB 191 were incubated at 30° **(Eskandari & Etemadifar, 2021b ; Farraz *et al*., 2021 ; Joshi *et al*., 2021).**

While in case of *Bacillus pumilus* MIN3 and *Nocardiopsis* species the temperature of incubation was not mentioned **(Buriak & Bhat, 2018 ; Jinga & Gordhanbhai, 2022)**

The fermentation media used for melanin production were incubated in temperature ranging between 28°C and 37°C **(table 04).** 28°C **(17 %),** 30°C **(25%)**, 35°C **(25%)** and 37°C **(17%),** whereas three studies did not mention the incubation temperature of the media **(16%) (figure 21).**

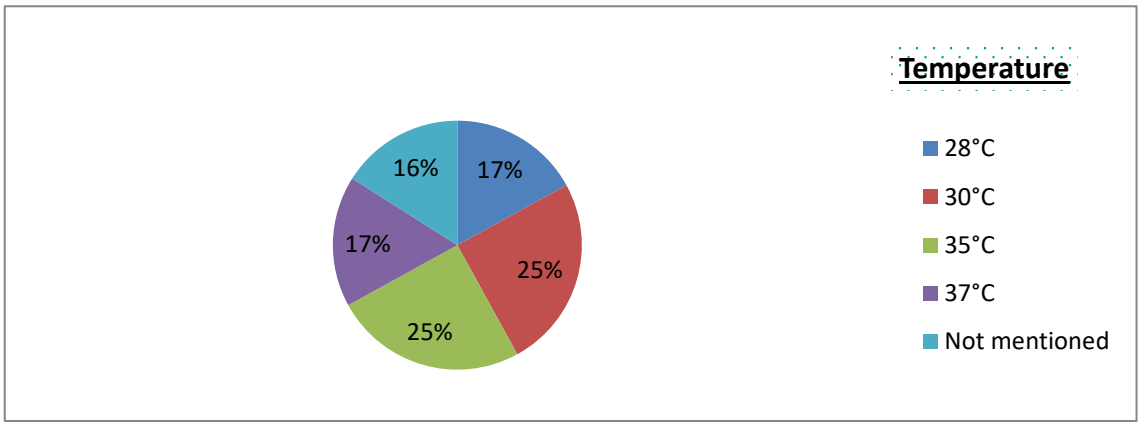

Figure 21: Incubation temperature used in fermentation process for melanin production.

It seems that 59% of studies working on melanin from bacterium used the same temperature used in the screening part of their protocol. Whereas 16% used a different temperature, 16% of studies did not mention the temperature used in the screening part. Finally, 9% of studies did not mention the temperature used in melanin fermentation **(figure 22).**

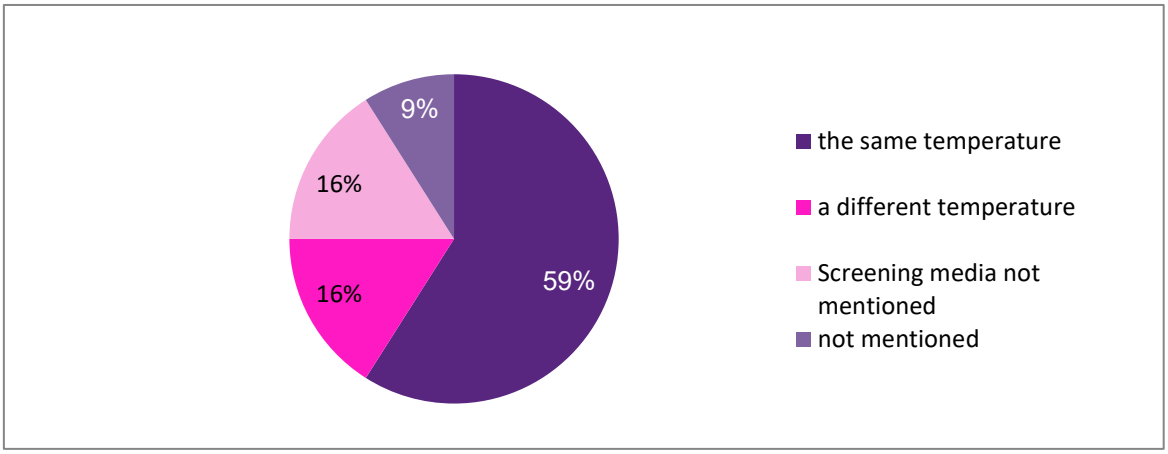

Figure 22: Similarity and difference between screening media and fermentation media in term of temperature of incubation.

Regarding the pH, the fermentation media were incubated at several pH: tyrosine broth at pH=7 in case of *Actinoalloteichus sp*.MA-32 **(Manivasagan *et al*., 2013),** M9 minimal medium broth at pH=7.4 in case of *Pseudomonas balearica* U7 **(Zerrad et al., 2014),** Tyrosine basal broth at PH= 8, in case of *Vibrio alginolyticus* BTKKS3 **(Kararudheen *et al*., 2019),** ISP-4 broth at pH=7.2 in case of *Bacillus subtilis* 4NP-BL **(Ghadge *et al*., 2020).** Which were the same pH values used in screening process. Müeller-Hinton Broth was incubated at pH= 7.3 in case of *Pseudomonas putida* ESACB 191, while the pH used in screening process was not mentioned **(Farraz *et al*., 2021)**.

In case of *Nocardiopsis* species, *Streptomyces* sp. MPPS2, *Streptomyces parvus BSB49*, *Dietzia schimae* NM3, *Pseudomonas koreensis* UIS19, *Beijerinckia fluminensis*, and *Bacillus pumilus* MIN3, pH values were not mentioned **(Buriak & Bhat, 2018; Bayram *et al*., 2020 ; Bayram, 2021 ; Eskandari & Etemadifar, 2021a ; Eskandari & Etemadifar, 2021b ; Joshi *et al*., 2021 ; Jinga & Gordhanbhai, 2022).**

The media used for melanin production were incubated at pH ranging between 7 and 8 **(table 05).** 8% for 7, 7.2, 7.3, 7.4 and 8 consecutively. Whereas seven studies did not mention the incubation pH of the media (60%) **(figure 23).**

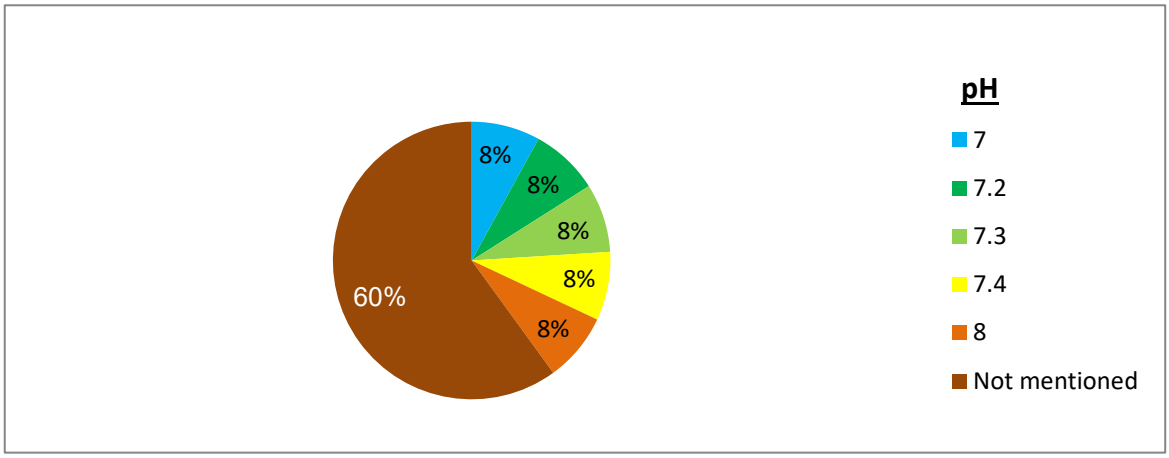

Figure 23: pH values used in fermentation process for melanin production.

25% of researchers working on melanin from bacterium used the same pH used in the screening part of their protocol. Whereas 16% of studies did not mentioned the pH values in the screening protocol, and 59% did not mention the pH values **(figure 24).**

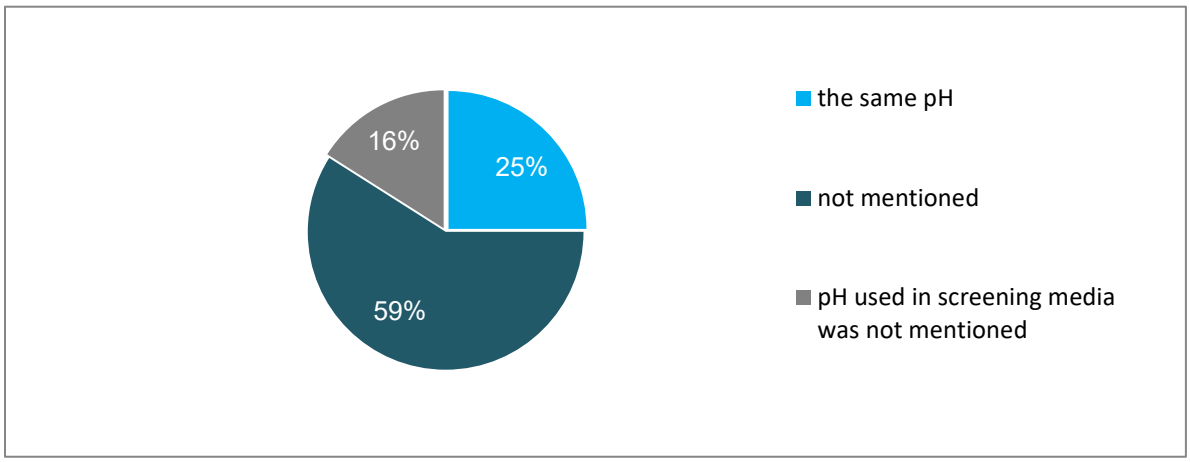

Figure 24: Similarity and difference between screening media and fermentation media in term of pH of incubation

Regarding to incubation time: tyrosine broth in case of *Actinoalloteichus* sp. MA-32, nutrient broth in case of *Streptomyces parvus* BSB49 and *Streptomyces* sp. MPPS2 were incubated for 1 week **(Manivasagan *et al.*, 2013 ; Bayram *et al.*, 2020 ; Bayram, 2021),** SS broth was incubated for 14 days in case of *Nocardiopsis* species **(Buriak & Bhat, 2018),** tyrosine basal broth was incubated for 7.5 days in case of *Vibrio alginolyticus* BTKKS3 **(Karmarudheen *et al.*, 2019**Nutrient broth was incubated for 4 days in case of *Dietzia schimae* NM3 **(Eskandari & Etemadifar, 2021a),** tyrosine basal broth (TBB) was incubated for 10 days in case of *Bacillus pumilus* MIN3 **(Jinga & Gordhanbhai, 2022).**

In case of *Pseudomonas balearica* U7, *Bacillus subtilis* 4NP-BL, *Pseudomonas koreensis* UIS 19, *Beijerinckia fluminensis* UQM, and *Pseudomonas putida* ESACB 191, incubation periods were not mentioned**. (Zerrad et al., 2014 ; Ghadge *et al.*, 2020 ; Eskandari & Etemadifar, 2021b ; Farraz *et al.*, 2021 ; Joshi *et al.* ; 2021).**

The media used for melanin production were incubated for a period ranging from 180 h to 14 days. 7.5 days **(8%)**, 4 days **(08%),** 7 days **(25%),** 10 days **(08%),** and 14 days **(08%),** 10 days (8%) Whereas four studies not mentioned the incubation period **(43%) (figure 25).**

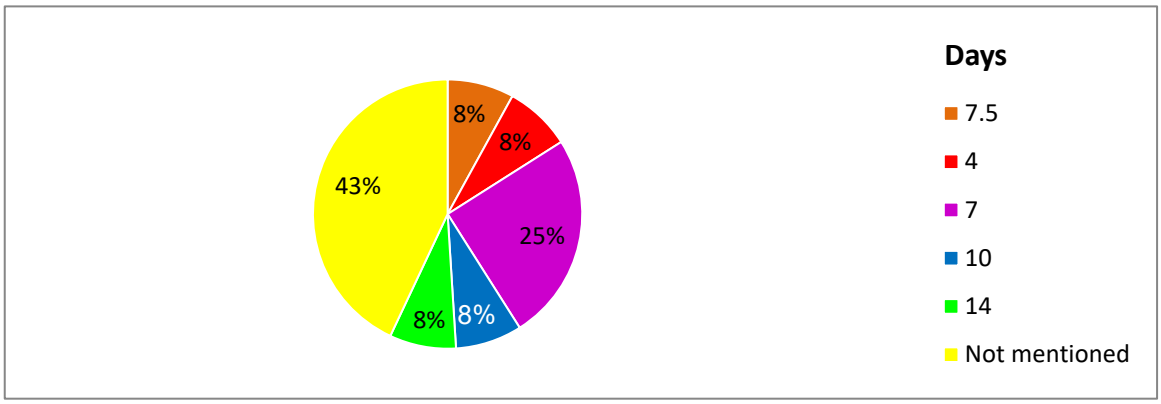

Figure 25: Incubation periods used in fermentation process for melanin production.

33% of media were incubated in **the same period** used in the screening part, **17%** used **different periods**, while **50%** are **not mentioned (figure 26).**

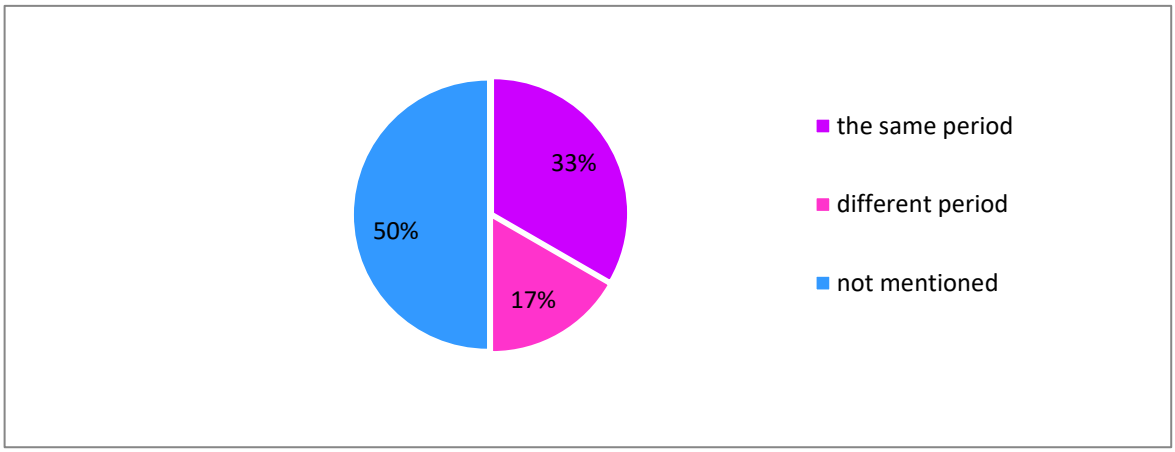

Figure 26: Similarity and difference between screening media and fermentation media in term of incubation periods

Regarding the agitation conditions, several speeds were used in the fermentation process:

180 rpm in case of *Actinoalloteichus* sp. MA-32 **(Manivasagan *et al.*, 2013),** 140 rpm in case of *Pseudomonas balearica* U7 and *Vibrio alginolyticus* BTKKS3 **(Zerrad et al., 2014 ; Karmarudheen *et al.*, 2019** 250 rpm in case of *Nocardiopsis* species **(Buriak & Bhat, 2018),** 200 rpm in case of *Streptomyces parvus* BSB49 **(Bayram *et al.*, 2020),** 200 rpm in case of *Streptomyces* sp. MPPS2 **(Bayram, 2021),** 160 rpm in case of *Dietzia schimae* MN3 and Pseudomonas koreensis UIS19 **(Eskandari & Etemadifar, 2021a ; Eskandari & Etemadifar, 2021b).**

In case of *Bacillus subtilis* 4NP-BL, *Beijerinckia fluminensis* UQM, *Pseudomonas putida* ESACB 191, and Bacillus *pumilus* MIN3, agitation conditions were not mentioned **(Ghadge *et al.*, 2020; Joshi *et al.*; 2021**; **Farraz *et al.*, 2021; Jinga & Gordhanbhai, 2022).**

It seems that **17%** of researchers used **200 rpm, 17 %** used **160 rpm, 17%** used **140 rpm,** while **8 %** used **250 rpm** and **8%** used **180 rpm (figure 27).**

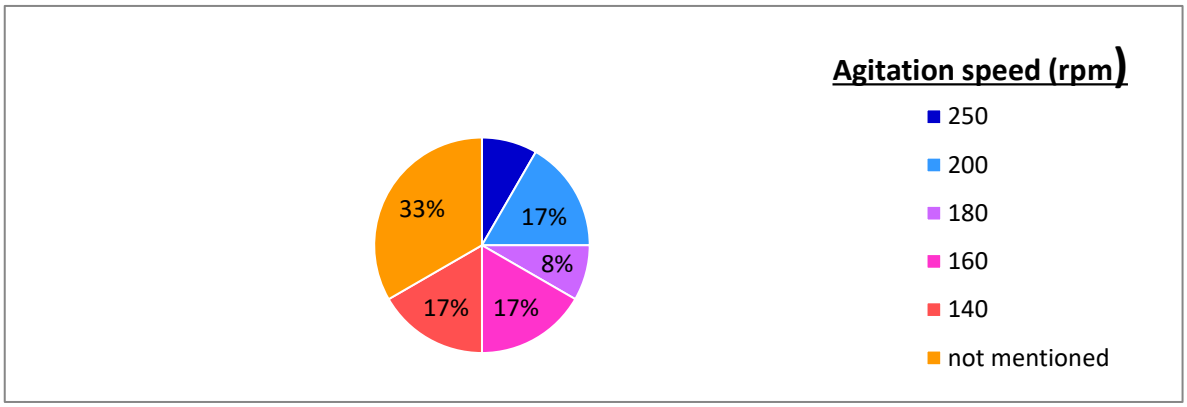

Figure 27: Agitation conditions used in fermentation process for melanin production

4/ Chemical characteristics

Melanins exhibited different chemical characteristics:

4.1/ Solubility

Pseudomonas balearica U7 melanin was soluble in hot / cold water, NaOH, and KOH, sparingly soluble in methanol and ethanol, but insoluble in HCL, chloroform, acetone, hexane, ethyl-acetate, acetic acid, ether and DMSO (Dimethyl Sulfoxide) **(Zerrad *et al.*, 2014).** *Nocardiopsis* species melanins were soluble in DMSO, alkaline water (pH=10), and phosphate buffer saline, but were insoluble in ethyl acetate, and chloroform **(Buriak &**

Bhat, 2018). *Vibrio alginolyticus* BTKKS3 melanin was soluble only in alkaline solvents and DMSO **(Kararudheen *et al*., 2019).** *Streptomyces parvus BSB49 and Streptomyces* sp. MPPS2 melanins were soluble in DMSO-d6 **(Bayram, 2020; Bayram, 2021).** *Bacillus subtilis* 4NP-BL melanin was dissolved in alkaline solutions and water **(Ghadge *et al*., 2020).** *Streptomyces* sp. MPPS2 melanin was soluble in DMSO-d 6**(Bayram, 2021).** *Dietzia schimae* MN3 melanin was soluble in distilled water **(Eskandari & Etemadifar, 2021a).** For *Beijerinckia fluminensis* UQM, melanin showed high solubility in strong alkalis like sodium hydroxide and potassium hydroxide (**Joshi *et al*., 2021).** *Bacillus pumilus* MIN3 melanin was insoluble in water and dissolved in alkaline solutions **(Jinga & Gordhanbhai, 2022).**

While is cases of *Pseudomonas putida* ESACB 191, *Pseudomonas koreensis* UIS19, *Actinoalloteichus sp***.** MA-32, melanin solubility was not determined **(Manivasagan *et al*., 2013 *;* Eskandari & Etemadifar, 2021b *;* Farraz *et al*., 2021).**

4.2/ Color

Three terms were used to describe melanins color: dark, black and brown.

These pigments were dark in case of *Streptomyces parvus* BSB49**,** *Pseudomonas balearica* U7 and *Dietzia schimae* MN3 **(Zerrad *et al*., 2014; Bayram *et al*., 2020; Eskandari & Etemadifar, 2021a).** Black in case of *Actinoalloteichus* sp. MA-32, *Nocardiopsis* species, *Vibrio alginolyticus* BTKKS3, *Bacillus pumilus* MIN3 **(Manivasagan *et al*., 2013; Buriak & Bhat, 2018 ; Kamarudheen *et al*., 2019 ; Jinga & Gordhanbhai, 2022).** Initially black that can turn brown in high pH levels in case of *Bacillus subtilis* 4NP-BL **(Ghadge *et al*., 2020).** Brown in case of *Pseudomonas putida* ESACB **191 (Farraz *et al*., 2021).** Brownish-black in case of *Streptomyces* sp. MPPS2 and *Beijerinckia fluminensis* **(Bayram, 2021; Joshi *et al*., 2021).** Either black or brown in case of *Pseudomonas koreensis* UIS19 **(Eskandari & Etemadifar, 2021b).**

4.3/ Nature and type of the produced melanins

The melanins extracted from *Actinoalloteichus* sp. MA-32, *Streptomyces parvus* BSB49, *Bacillus subtilis* 4NP-BL, *Pseudomonas koreensis* UIS 19, *Dietzia schimae* NM3 and *Pseudomonas putida* ESACB 191 were identified as eumelanin **(Manivasagan *et al*., 2013 *;* Bayram *et al*., 2020 *;* Ghadge *et al*., 2020 *;* Eskandari & Etemadifar, 2021b *;* Farraz *et al*., 2021 *;* Eskandari & Etemadifar, 2021a).** The melanin extracted from *Streptomyces* sp. MPPS2 was identified as pyomelanin **(Bayram, 2021).** Whereas, *Vibrio alginolyticus*

BTKKS3 and *Bacillus pumilus* MIN3 produced both eumelanin and pyomelanin **(Kararudheen *et al*., 2019; Jinga & Gordhanbhai, 2022).**

The nature of melanins produced by *Beijerinckia fluminensis* UQM, *Pseudomonas balearica* U7 and *Nocardiopsis* species were non-defined **(Zerrad *et al*., 2014; Kurian & Bhat, 2018 ; Joshi *et al*., 2021).**

6 bacteria produced **eumelanin, 2** bacteria produced **both eumelanin and pyomelanin** and finally **1** bacterium produced **pyomelanin alone (figure 28).**

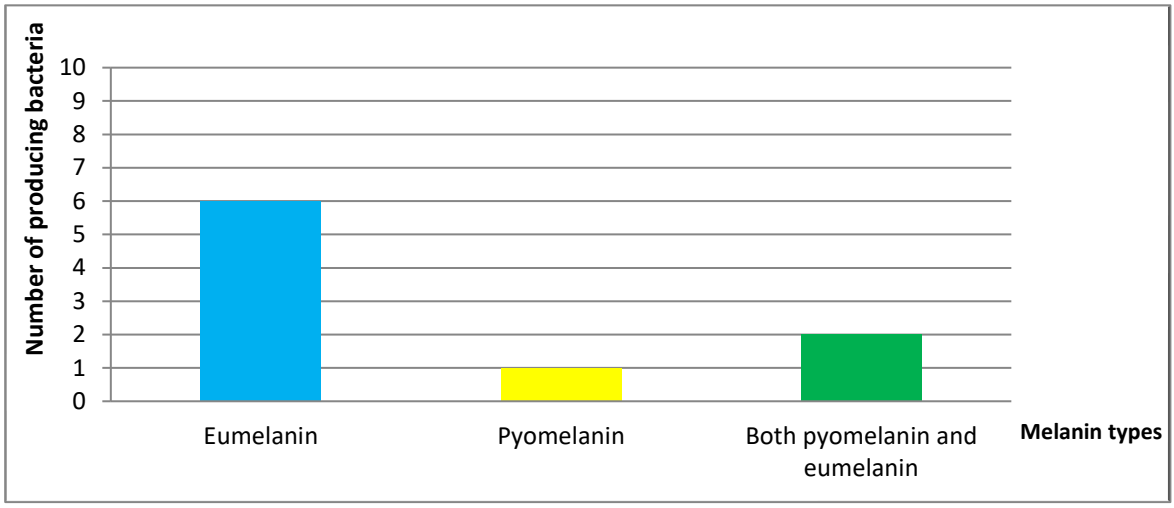

Figure 28: Types of melanin produced by different bacteria

5/ Biochemical characteristics

5.1/ Absorption and transmission of UV/VIS

UV/VIS is a technique used to study the absorption and transmission of ultraviolet (UV) and visible light by molecules. It helps elucidate their structure **(Sun, 2004).**

The absorption peaks of melanins of the studied bacteria appeared as the following: *Actinoalloteichus* sp**.** MA-32 at 300 nm **(Manivasagan *et al*., 2013),** *Pseudomonas balearica* U7 at 280 nm - 335 nm **(Zerrad *et al*., 2014),** *Nocardiopsis* species at 350 nm **(Buriak & Bhat, 2018),** *Streptomyces parvus* BSB49 at 230 nm **(Bayram *et al*., 2020,.** *Bacillus subtilis* 4NP-BL at 210 nm **(Ghadge *et al*., 2020),** *Streptomyces* sp. MPPS2 and *Beijerinckia fluminensis* UQM at 280 nm **(Bayram, 2021; Joshi *et al*., 2021),** *Dietzia schimae* NM3 at (200-300nm) **(Eskandari & Etemadifar, 2021a).**

While the absorption results of melanins produced by *Pseudomonas putida* ESACB 191, *Vibrio alginolyticus* BTKKS3, *Bacillus pumilus* MIN3 and *Pseudomonas koreensis* UIS19

melanins were not determined **(Kararudheen *et al.*, 2019 *;* Eskandari & Etemadifar, 2021b *;* Farraz *et al.*, 2021 *;* Jinga & Gordhanbhai, 2022).**

5.2/ Radical scavenging property

Radical scavenging is a process in which some chemicals, known as free radical scavengers or antioxidants, remove highly reactive and unstable compounds called free radicals from the body **(Hatwalne, 2015).** DPPH is a simple test system that uses 2,2-diphenyl-1-picrylhydrazyl to evaluate the radical scavenging potential of a test compound. The DPPH test relies on the elimination of DPPH, a stabilized free radical **(Gerhäuser, 2009; Mfotie Njoya, 2021).**

IC50 value is the amount or the concentration of melanin necessary to decrease the initial DPPH radical concentration by 50%. The concentration needed for complete inhibition is called IC100 **(Swinney, 2011).**

The radical scavenging values of bacterial melanins were as the follows: IC50= 85000 µg/mL for the melanin produced by *Actinoalloteichus* sp. MA-32 **(Manivasagan *et al.*, 2013).** IC100=6230 µg/mL for the melanin produced by *Pseudomonas balearica* U7 **(Zerrad *et al.*, 2014).** IC50=74.2 µg/mL for the melanin produced by *Pseudomonas putida* ESACB 191 **(Farraz *et al.*, 2021).** Radical scavenging values estimated by: 68.66% for 100 µg/mL of the melanin produced by *Vibrio alginolyticus* BTKKS3 **(Kamarudheen *et al.*, 2019).** 45.93% for 100 µg/mL of the melanin produced by *Streptomyces parvus* BSB49 **(Bayram *et al.*, 2020).** 94.47% for 100 µg/ml of the melanin produced by *Bacillus subtilis* 4NP-BL **(Ghadge *et al.*, 2020).** 188.9% for 10 µg/mL of the melanin produced by *Dietzia schimae* NM3 **(Eskandari & Etemadifar, 2021a).** > 92.42% for 20000 µg/mL of the melanin produced by *Pseudomonas koreensis* strain UIS 19 melanin **(Eskandari & Etemadifar, 2021b).** In case of *Bacillus pumilus* MIN3, melanin exhibited several values of radical scavenging activity with different concentrations of melanin produced. The concentrations 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml showed 59.46%, 59.79%, 60.8%, 61.81%, and 63.71% respectively **(Jinga & Gordhanbhai, 2022).**

While in case of *Nocardiopsis* Species, *Streptomyces* sp and *Beijerinckia fluminensis* UQM, the radical scavenging potential was not determined **(Buriak & Bhat, 2018 *;* Bayram, 2021 *;* Joshi *et al.*, 2021).**

5.3/ Sun protection factor (SPF)

SPF is a relative measurement that indicates the level of protection against UVB rays. It helps determine how long skin can be subjected to the sun without getting burned **(Lohnes & Kate, 2017).**

The melanins SPF was determined in three studies only. The melanin produced by *Dietzia schimae* NM3 exhibited sun protection factor estimated by 20.22 with following absorptions: 0.005595, 0.02737, 0.08737, 0.087523, 0.043804, 0.021814, and 0.004464, at the wavelengths: 290 nm, 295 nm, 300 nm, 305 nm, 310 nm, 315 nm, 320 nm respectively **(Eskandari & Etemadifar, 2021a).** The melanin produced by *Pseudomonas koreensis* UIS 19 exhibited sun protection factor estimated by 61.55 with the following absorptions: 0.912, 0.892, 0.873, 0.856, 0.841, 0.897, and 0.884, at the wavelengths: 290 nm, 295 nm, 300 nm, 305 nm, 310 nm, 315 nm, 320 nm respectively **(Eskandari & Etemadifar, 2021b).** Finally, the melanin produced by *Vibrio alginolyticus* BTKKS3 was able to increase sun protection factor (SPF) of commercial sunscreens by an average of 3.42 units **(Kamarudheen *et al*., 2019).**

6/ Biological activities

6.1/ Antimicrobial activity

Three methods were used to detect the antimicrobial potential of purified melanin from the diverse bacteria: the 96 well microtiter plate, the well diffusion and the disc diffusion method. The results showed that the melanin produced by *Actinoalloteichus* sp. MA-32 had antibacterial activity against *Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Shigella shiga, Shigella dysenteriae,* and *Shigella boydii,* with inhibition zones equal of 18mm,13mm,10mm, 14mm ,11mm, 20mm, 12mm, 13mm,11mm, and 14mm respectively with 30g/disc of melanin concentration and 25mm, 20mm,14mm, 22mm,15mm,29mm,16mm, 21mm,23mm, 20mm with 50 g/disc of melanin. While the minimum inhibitory concentrations (MIC) were: 10g/mL, 15g/mL, 18g/mL, 16g/mL, 19g/mL, 13g/mL, 16g/mL, 20g/mL, 18g/mL, 23g/mL respectively *(***Manivasagan *et al*., 2013).** *Pseudomonas balearica* U7 showed a strong anti-bacterial activity against bacteria tested with inhibition diameters as follows: *Staphylococcus aureus* (DI = 41 mm) than the *Escherichia coli* (DI= 30 mm), phytopathogenic strains *Erwinia carotovora* (DI = 30 mm), and *Erwinia chrysanthemi* (DI = 28 mm) **(Zerrad *et al*., 2014).** *Nocardiopsis* species

melanins showed antibacterial activities against several pathogens: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Proteus* sp., *Salmonella typhi, Klebsiella* sp, and *Bacillus subtilis* at highest activity of 61.28% against *Listeria monocytogenes*, and 68.08% against *Bacillus* sp at 150 µg/mL, exerted by the specie JN2 **(Kurian & Bhat, 2018).** *Bacillus subtilis* 4NP-BL melanin displayed antimicrobial activity against: *Mycobacterium smegmatis* MTCC 6, *Escherichia coli* MCC 2412, *Xanthomonas campestris* NCIM 5028, *Alteromonas macleodii* MCC 2815, *Enterobacter aerogenes* MCC 3092, and *Klebsiella. pneumoniae* MCC 2451 (data not shown) **(Ghadge *et al*., 2020).** *Bacillus pumilus* MIN3 melanin exhibited significant antibacterial activity with minimum inhibitory concentrations of 40µg/mL against *Bacillus* sp., 20µg/mL against *Escherichia. coli, Salmonella* sp., and *Shigella* sp., and 60µg/mL against *Staphylococcus aureus* **(Jinga & Gordhanbhai, 2022).**

6.2/ Anti-biofilm activity

Anti-biofilm activity refers to the ability of certain substances or microorganisms to prevent or disrupt the formation of biofilms. It provides a better understanding of how to control an infection **(Miquel *et al*., 2016).**

The anti-biofilm activity was detected using 96-well microtiter plate in 2 melanin-producing bacteria: *Nocardiopsis* species and *Vibrio alginolyticus* BTKKS3 **(Kurian & Bhat, 2018; Kamarudheen *et al*., 2019).**

Nocardiopsis species melanins showed highest activities against biofilm estimated by 64.20% and 65.99% respectively against *Staphylococcus* sp., 61.28 % and 68.08 % against *Listeria monocytogenes* and *Bacillus* sp. respectively at 150 µg/mL of melanin exerted by *Nocardiopsis dassonvillei* **(Buriak & Bhat, 2018).** While the melanin from *Vibrio alginolyticus* BTKKS3 inhibited 38.31%, 79.81%, 79.99% and 58.02%, of biofilm formed with *Bacillus altitudinus* BTMW1, *Pseudomonas aeruginos*a BTRY1, *Staphylococcus warneri* BTDF2 and *Bacillus* sp. BTSD1 respectively **(Kamarudheen *et al*., 2019).**

6.3/ Cytotoxic activity

Different methods were performed to detect the cytotoxicity of bacterial melanins:

- The 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay**,** where different concentrations of melanin from *Vibrio alginolyticus* BTKKS3 were tested

against L929 cells which are a fibroblast cell line derived from adipose tissue and normal subcutaneous areolar of a male C3H/An mouse **(Heap *et al.*, 2021).**

- The MTT viability method where *Pseudomonas putida* ESACB 191 melanin was tested against different cancer cell lines such as A375, HEPG2, Caco2, and HeLa Kyoto responsible of human melanoma**,** human hepatoma (human liver cancer), colon carcinoma and cervical cancer respectively **(Donato *et al*., 2015; Lea, 2015; Avram *et al*., 2017; Hu *et al*., 2019)**.
- The 96-well plate method containing RPMI 1640 medium, supplemented with fetal bovine serum, and antibiotics where *Dietzia schimae* NM3 melanin was tested against HFB cells (human fibroblast cells).

Results showed that melanin produced from *Vibrio alginolyticus* BTKKS3 inhibited 37.04% of L929 cells at the concentration 100 µg /mL, and its IC50 was estimated by 134.98 µg/mL. **(Kararudheen *et al*., 2019).** *Dietzia schimae* NM3 melanin exhibited toxicity on the normal cells at the concentrations up to 250 µg ml-1 **(Eskandari & Etemadifar, 2021a).** Finally, the IC50 of cytotoxicity of melanin produced by *Pseudomonas putida* ESACB against cancer cell lines tested were: as following: 1.77 mg/mL against A375, 2.51 mg/mL against HeLa Kyoto, 0.89 mg/mL against HepG2 and 1.08 mg/mL against Caco2 **(Farraz *et al*., 2021).**

6.4/ Anti-inflammatory activity

The anti-inflammatory activity represents the ability of substances to reduce the production or the activities of pro-inflammatory cytokines. The importance of anti-inflammatory activity lies in its potential to alleviate symptoms, reduce the immune response and tissue damage **(Arulselvan *et al.*, 2016).**

A unique study mentioned the anti-inflammatory activity of bacterial melanins. In that study LPS-stimulated Raw cells were treated with different concentrations of melanin with different concentrations of melanin, and diclofenac sodium .The anti-inflammatory potential was evaluated by assessing the activity of 04 inflammatory enzymes: cyclooxygenase (COX), lipoxygenase (LOX), myeloperoxidase (MPO), and nitric oxide synthase (NOS) **(Kamarudheen *et al*., 2019).** It was shown that a concentration of 100 µg/mL of melanin produced by *Vibrio alginolyticus* BTKKS3 reduced COX and LOX activity to 45.80% and 63.67%, respectively. Also it reduced the cellular nitrite levels to

648.05 µg/mL 648.05 µg/mL of cell lysate at the highest dose (100 lg/mL) and it reduced the MPO activity at 0.0014U/mL of cell lysate **(Kamarudheen *et al*., 2019).**

6.5/ Anti-quorum sensing activity

The anti-quorum sensing activity represents the ability of certain compounds to inhibit the quorum sensing mechanism used by bacteria to coordinate their behavior and gene expression depending on cell-to-cell signaling. It helps in the development of antimicrobial agents which can inhibit bacterial communication and virulence **(Abudoleh & Mahasneh, 2017).**

A unique study mentioned the anti-quorum sensing of bacterial melanins. Using agar well diffusion method, quorum sensing bacterium *Chromobacterium violaceum* (MTCC 2656) was treated with several concentrations of melanin produced by two *Nocardiopsis* species at 10, 20,40, 60,80,100 and 150µg/mL. The zones of inhibition were measured to detect the anti-quorum sensing potential of melanin extracted.

Results confirmed that both *Nocardiopsis* species melanins had the ability to inhibit the growth of quorum sensing bacteria *Chromobacterium violaceum* MTCC 2656, with highest zones of inhibition of 13 mm by *Nocardiopsis* sp, and 8mm by *Nocardiopsis dassonvillei at* 150 µg/mL of melanin concentration **(Buriak & Bhat, 2018).**

Conclusion

Melanins were produced by different bacteria. Five isolated from the marine ecosystem, four from soil, one from plant and one from food. Among the 12 studied bacteria, 7 belong to Gram positive and 5 to Gram negative. Also five belong to the phylum Actinobacteria, five to the phylum Proteobacteria and two to the phylum Firmicutes.

The bacteria were screened for producing melanin in different solid media. Most of these media contained the amino acid L-tyrosine as substrate (62%), with 0.2% as the most used percentage of it (63%).

Regarding the temperature, media were incubated in temperature ranging between 28°C and 37°C, which represent mesophilic conditions. Regarding the pH, media used for melanin production were incubated at pH ranging between 7 and 8, which represent that the researchers used neutral conditions to slightly alkaline conditions. Finally, the screening media were incubated for a period ranging from 2 days to 14 days.

Regarding the fermentation broths, most studies maintained the same media which were used in the screening part of their protocol (58%). The media were incubated at temperature ranging between 28°C and 37°C, at a pH ranging between 7 and 8, and a period ranging from 7.5 to 14 days.

Six bacteria produced eumelanin, 2 bacteria produced both eumelanin and pyomelanin and finally 1 bacterium produced pyomelanin alone, which were ranging in color from black to brown and vary in terms of solubility. They were characterized by their ability to scavenge free radicals and prevent oxidation, as well as to absorb ultraviolet rays and protect from harmful sunlight.

They showed antimicrobial, cytotoxic, anti-inflammatory, antibiofilm and anti-quorum sensing activities which allow them to be used as promising alternatives in various medical and pharmaceutical applications.

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Summary

The aim of this work was to study melanins, based on the analysis of 12 research papers about melanins produced by diverse bacteria.

The results showed that bacteria isolated from different ecological niches and belonging to different phyla were able to produce mainly two types of melanin: eumelanin and pyomelanin, which ranged in color between black and brown.

Bacterial melanins were produced through screening and fermentation protocols, mostly in mesophilic and neutral to alkaline conditions and a period from 2 to 14 days. The used substrate was mainly the amino-acid L-tyrosine.

Bacterial melanins were soluble Bacterial melanins in different solutions as : water, methanol, ethanol, DMSO, and alkaline solvents, and possessed the property to scavenge free radicals and prevent oxidation, as well to absorb ultraviolet rays and protect from harmful sunlight. They possessed also several activities such antibacterial, antifungal , anti-inflammatory, antibiofilm activities which makes them promising alternative in the industrial fields, especially cosmetic, medical and food industries.

Key words: melanin, L-tyrosine, pigment, bacteria, industrial applications.

Résumé

Le but de ce travail etait d'étudier les mélanines bactériennes, à partir de l'analyse de 12 articles de recherche sur les mélanines produites par diverses bactéries.

Les résultats ont montré que les bactéries isolées de différentes niches écologiques et appartenant à différents phylums étaient capables de produire principalement deux types de mélanine : l'eumélanine et la pyomélanine, dont la couleur varie entre le noir et le brun.

Les mélanines bactériennes ont été produites en utilisant des protocoles de criblage et de fermentation. Le substrat utilisé était principalement l'acide aminé L-tyrosine. Principalement mésophile et conditions neutres à alcalines, de 2 à 14 jours.

Ils étaient solubles dans différentes solutions telles que : l'eau, le méthanol, l'éthanol, le DMSO et les solvants alcalins, et possédaient la propriété de piéger les radicaux libres et de prévenir l'oxydation, ainsi que d'absorber les rayons ultraviolets et de protéger des rayons nocifs du soleil. Ils possédaient également plusieurs activités telles que des activités antibactériennes, antifongiques, anti-inflammatoires, anti biofilm. Ce qui en fait une alternative prometteuse dans les domaines industriels, notamment cosmétiques, médicaux et alimentaires.

Mots clés : mélanine, L-tyrosine, pigment, bactéries, applications industrielles.

الملخص

كان الهدف من هذا العمل هو دراسة الميلانينات، بناءً على تحليل 12 ورقة بحثية حول الميلانينات التي تنتجها البكتيريا المتنوعة.

وأظهرت النتائج أن البكتيريا المعزولة من بيئات بيئية مختلفة والتي تنتمي إلى شعب مختلفة كانت قادرة على إنتاج نوعين رئيسيين من الميلانين: يوميلانين وبيوميلانين، والذي تراوح لونه بين الأسود والبني.

تم إنتاج الميلانين البكتيري من خلال بروتوكولات الفحص والتخمير، معظمها في ظروف متوسطة ومحايدة إلى قلوية ولمدة تتراوح من 2 إلى 14 يومًا. كانت الركيزة المستخدمة بشكل أساسي هي الحمض الأميني التيروزين.

الميلانين البكتيري قابل للذوبان في محاليل مختلفة مثل: الماء، الميثانول، الإيثانول، DMSO، والمذيبات القلوية، وتمتلك خاصية تطهير الجذور الحرة ومنع الأكسدة، وكذلك امتصاص الأشعة فوق البنفسجية والحماية من أشعة الشمس الضارة. كما تمتلك العديد من الأنشطة مثل الأنشطة المضادة للبكتيريا والفطريات والمضادة للالتهابات والمضادة للأغشية الحيوية مما يجعلها بديلاً واعداً في المجالات الصناعية وخاصة الصناعات التجميلية والطبية والغذائية.

الكلمات المفتاحية: الميلانين، التيروزين، الصباغ، البكتيريا، التطبيقات الصناعية.

Thesis submitted for the award of the Master's degree

Field: Microbiology
Specialty : Microbial Ecology

Title: bacterial melanins

Summary:

The aim of this work was to study melanins, based on the analysis of 12 research papers about melanins produced by diverse bacteria.

The results showed that bacteria isolated from different ecological niches and belonging to different phyla were able to produce mainly two types of melanin: eumelanin and pyomelanin, which ranged in color between black and brown.

Bacterial melanins were produced through screening and fermentation protocols, mostly in mesophilic and neutral to alkaline conditions and a period from 2 to 14 days. The used substrate was mainly the amino-acid L-tyrosine.

Bacterial melanins were soluble Bacterial melanins in different solutions as : water, methanol, ethanol, DMSO, and alkaline solvents, and possessed the property to scavenge free radicals and prevent oxidation, as well to absorb ultraviolet rays and protect from harmful sunlight. They possessed also several activities such antibacterial, antifungal , anti-inflammatory, antibiofilm activities which makes them promising alternative in the industrial fields, especially cosmetic, medical and food industries.

Key words: melanin, L-tyrosine, pigment, bacteria, industrial applications.

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