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Microbial Pigments: Types, Production Methods, and Diverse Applications in Various Industries: A Review

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Abstract

Microbial pigments represent a class of natural pigments with significant potential across diverse industries including food, textiles, dairy, cosmetics, marine applications, and medicine. These pigments are considered valuable due to their abundant resources, high production yield, low cost, ease of cultivation, adaptability to various environments, optimization, and stability. Additionally, they are eco-friendly, biodegradable, and free of harmful side effects. Microbial pigments have shown promise in treating various diseases due to their bioactive properties, such as antibacterial, anticancer, and immunosuppressive activities. This review explores the various types of microbial pigments, their sources, characteristics, production and their applications in various fields.

Keywords: Microbial pigment, Riboflavin, Food industry

Introduction:

Pigments are organic molecules that absorb specific wavelengths of light while reflecting others, giving rise to the colors we perceive. Microbial pigments, produced by microorganisms like bacteria, fungi, and algae, have become of particular interest in recent years due to their broad applicability in various industries. These pigments are not merely aesthetic; they also possess functional properties, such as antioxidant, antimicrobial, and anticancer activities. In recent decades, researchers have increasingly focused on microbial pigments for use in food, textiles, cosmetics, pharmaceuticals, and even environmental applications[1].

Synthetic pigments, which have long been used in these industries, are now under scrutiny due to concerns over their safety and environmental impact[2]. The growing demand for natural products has led to a shift toward microbial pigments as a safer, more sustainable alternative. The potential benefits of microbial pigments include their high yield, sustainability, and the ability to produce them year-round regardless of seasonal and geographical constraints[3]. Furthermore, many microbial pigments are produced through fermentation processes, making their production scalable and controllable.

This review delves into the different types of microbial pigments, their mechanisms of production, factors influencing their synthesis, and their diverse applications in various fields.

1. Microbial pigments:

Microbial pigments are produced by a variety of microorganisms, including bacteria, fungi, and algae. These pigments are of great interest due to their natural origins, making them preferable to synthetic dyes, especially for consumers looking for eco-friendly alternatives. Microbial pigments not only offer visual appeal but also serve important biological functions such as protection against UV radiation, oxidative stress, and pathogen invasion.

The production of microbial pigments can occur intracellularly or extracellularly, and it is often regulated by various environmental factors such as light, temperature, pH, and nutrient availability[4]. The most commonly studied microbial pigments include carotenoids, anthocyanins, and flavonoids, each with distinct chemical properties and biological activities. [5].

2. Classification of Microbial Pigments

Microbial pigments are classified based on their chemical structure and biological origin. The primary categories include:

2.1 Riboflavin (Vitamin B2):

Riboflavin is a yellow, water-soluble pigment synthesized by several microorganisms, including Aspergillus, Candida, and Bacillus species. It is commonly used in food products such as breakfast cereals, processed cheese, and dairy products. Riboflavin is essential for cellular metabolism and has applications in human nutrition.

2.2 Canthaxanthin:

This orange-red carotenoid pigment is produced by various bacteria, including Bradyrhizobium species. Canthaxanthin is known for its strong antioxidant properties, helping prevent lipid oxidation in various food and cosmetic products[6].

2.3 Prodigiosin:

A red pigment produced by Serratia marcescens, prodigiosin has demonstrated significant therapeutic potential, particularly as an anticancer and immunosuppressive agent. It also possesses antibacterial, antifungal, and antimalarial activities, making it useful in pharmaceutical applications.

2.4 Violacein:

A purple pigment produced by Chromobacterium species, violacein has antibacterial properties and is used in both medical and industrial applications. It has potential as a natural UV protectant due to its UV absorbance properties[7].

2.5 Phycocyanin:

This blue pigment is produced by cyanobacteria such as Spirulina and Aphanizomenon flos-aquae. Phycocyanin is used as a natural food coloring agent and in various health supplements due to its antioxidant and anti-inflammatory properties.

2.6 Beta-Carotene:

A well-known carotenoid, beta-carotene is produced by Blakeslea trispora and other microorganisms. It is a precursor of vitamin A and is commonly used in the food industry for coloring products like dairy and confectionery.

2.7 Lycopene:

A bright red carotenoid pigment, lycopene is produced by microorganisms such as Fusarium and Blakeslea trispora. It is widely used in the food industry to color meats and tomato-based products and is also associated with health benefits, including potential anticancer effects.

2.8 Melanin:

Melanin pigments are produced by several microorganisms, including Pseudomonas and Aspergillus species. These pigments have antioxidant properties and are widely used in cosmetics, particularly in sunscreen formulations.

2.9 Astaxanthin:

A red-orange pigment found in microalgae, astaxanthin has gained attention due to its potent antioxidant properties, which make it beneficial in both cosmetic and therapeutic applications[8].

Table .1 Types of microbial pigments [8,23]

Microorganisms	Pigments/Molecule	Colour/Appearance
Baceria		
Flavobacterium specie, Paracoccus zeaxanthinifaciens	Zeaxanthin	Yellow
Achromobacter	Zeaxanthin	Creamy
Brevibacterium specie	Zeaxanthin	Orange Yellow
Corynebacterium michigannise	Zeaxanthin	Greyish to Creamish
Corynebacterium insidiosum	Indigoidine	Blue

Rugamonas rubra, Streptoverticillium rubrireticuli, Vibrio gaogenes, Alteromonas rubra	Prodigiosin	Bluish-Red
Xanthophyllomyces dendrorhous	Astaxanthin	Pink-Red
Haloferax alexandrinus	Canthaxanthin	Dark Red
Agrobacterium aurantiacum	Astaxanthin	Pink-red
Staphylococcus aureus	Staphyloxanthin, Zeaxanthin	Golden Yellow
Chromobacterium violaceum	Violacein	Purple
Serratia marcescens, Serratia rubidaea,	Prodigiosin	Red
Pseudomonas aeruginosa	Pyocyanin	Blue-Green
Xanthomonas oryzae	Xanthomonadin	Yellow
Janthinobacterium lividum	Violacein	Purple
Bradyrhizobium species	Canthaxanthin	Dark- red
Bacillus	Zeaxanthin	Brown
Fungi		
Monascus spp.	Monascorubramin	Red
Talaromyces atroroseus	Azaphilones	Red
Pseudoalteromonas denitrificans	Cycloprodigiosin	Red
Fusarium Sporotrichioides, Blakeslea trispora	Lycopene	Red
Cordyceps unilateralis	Naphtoquinone	Deep blood red
Ashbya gossypi	Riboflavin	Yellow
Monascus spp.	Rubropunctatin	Orange
Xanthomonas oryzae	Xanthomonadin	Yellow
Blakeslea trispora, Fusarium sporotrichioides, Mucor, circinelloides, Blakesleeanus	B- carotene	Yellow-orange
Monascus spp.	Canthaxanthin	Orange, pink
Algae		
Haematococcus pluvialis Microalgae	Astaxanthin	Pink-red
Dunaliella salina Microalgae	B- carotene	Orange
Chlorella and others Microalgae	Lutein	Yellow

Porphyridium cruentum and many other microalgae and cyanobacteria Algae, Cyanobacteria	Phycoerythrin	Red
Arthrospira sp. [formerly Spirulina sp.] and many other microalgae and cyanobacteria Algae, Cyanobacteria	Phycocyanin	Blue

3. Microbial pigment processing:

Microbial pigments are often classified into two types: soluble pigments, which quickly infiltrate the surrounding media and are referred to as extracellular pigments, and insoluble pigments, which are limited to the interior of the cell and are referred to as intracellular pigments. Extracellular pigments can be isolated directly from liquid cultures using chromatography. Intracellular pigments, on the other hand, require the culture to be sonicated, which cracks the cell open and releases the pigment into the surrounding fluid.

Different bacterial species produce different colors depending on their particular growth requirements. The absorbance of the purified pigment can be accurately examined to determine the percentage yield on a laboratory scale; the higher the absorbance, the more efficiently the pigment is manufactured. By standardizing the incubation conditions and adjusting the media, it is simple to obtain the best pigment production and quality. Without the right conditions, bacterial culture will not be able to overcome the lag; as a result, the quantity and quality of pigment produced will be lower. By developing high-yielding strains that increase pigment production per unit mass while reducing the expense of maintaining rigorous fermentation conditions, molecular cloning can enhance pigment production[20].

4. Purification of pigment

The downstream purification of the pigment from the fermentation media has a significant impact on the type of pigment being purified. Consequently, pigment purification necessitates an appropriate purification plan. Bacterial pigments are selectively soluble in organic solvents like methanol, ethanol, acetone, ethyl acetate, or hexane because the majority of them are insoluble in water. The organic solvent is allowed to evaporate after the pigment has been produced in it. Our pure pigment is the powdery residue that remains, and it can be applied to functional analysis[20].

5. Characterization of extracted pigments:

Thin-layer chromatography [TLC] and high-performance liquid chromatography [HPLC] are used to characterize the pigments. After extraction, the pigments were put through TLC and HPLC to determine their constituent parts. Using HPLC, the pigments' chemical purity was evaluated.The pigments were observed on a TLC plate with methanol as the mobile phase. Following the run, the TLC plate was dried at room temperature and placed in an iodine chamber to generate spots.

UV-visible and Fourier Transform Infrared [FTIR] examination, the dried pigments were diluted in 1ml of 5% methanol solution [1 mg/ml], and their UV-visible absorption range was evaluated using a UV-Visible spectrophotometer. To forecast their structural conformation and functional group, the extracted pigments [1 mg/ml of pigment dissolved in methanol] were examined using FTIR spectroscopy in the 4000 cm-1 to 400 cm-1 range[21].

6. Factors Affecting Pigment Production

The production of pigments by microorganisms is influenced by a variety of physicochemical conditions that can either promote or hinder the formation of different metabolites. The synthesis of pigments can occur both intracellularly and extracellularly, and is affected by factors such as light, pH, temperature, and the composition of the growth medium. Additionally, factors like time, space, seasonal variations [such as alluvial, nival, and pluvial conditions], sampling locations, habitats, and specific laboratory cultivation conditions play significant roles in pigment production[9].

6.1 Light:

Light plays a key role in inducing pigment [such as carotenoid] production in certain non-photosynthetic species, including genera like Mycobacterium, Flavobacterium dehydrogenans, and Myxococcus. The presence or absence of light, the wavelength of light, and light intensity all significantly affect pigment production[12]. For instance, optimizing light intensity in Synechocystis growth with 620-nm LEDs, which target phycocyanin [PC], resulted in six times more efficient pigment production compared to broad-spectrum light, with PC being the most abundant pigment by mass[13].

6.2 Temperature:

Several studies have shown that even slight variations in temperature, whether an increase or decrease, can impact pigment synthesis. For example, raising the temperature to 30°C led to increased production of red pigments in Monascus purpureus LPB 97 at 500 nm, while a higher temperature of 40°C resulted in the production of more yellow pigments absorbing at 400 nm. An optimal temperature is essential for maximizing pigment production[14].

6.3 pH:

The pH of the growth medium significantly affects microbial proliferation and pigment synthesis. For instance, the optimal pH ranges for Monascus sp. and Rhodotorula are 5.5-6.5 and 4.0-4.5, respectively [15]. Lycopene production is favored by a neutral to slightly alkaline pH, while an acidic pH promotes the synthesis of β-carotene. Additionally, the production of ankaflavin is preferred at a low pH of around 4.0[11].

6.4Media composition:

To enhance pigment production, the growth medium should include carbon sources such as glucose, maltose, lactose, and mannose, as well as nitrogen sources like ammonium chloride, ammonium nitrate, and potassium nitrate[11]. Additionally, the inclusion of essential minerals can further boost pigment yield. For instance, a variety of carbon and nitrogen sources have been found to positively impact pigment production and development in Streptomyces species[16].

6.4 Aeration:

The rate of aeration significantly influences pigment production, as seen in the red pigment synthesis of M. ruber. Altering the aeration rate during the stationary phase impacted red pigment production without affecting microbial growth. This suggests that the oxygen supply plays a crucial role in the synthesis of red pigments[12].

6.5 Type of fermentation:

Pigmented bacteria, fungi, and yeast are highly sensitive to various physicochemical factors. Consequently, these microorganisms require a diverse range of in vitro cultivation conditions to produce different pigments, whether in solid-state or submerged fermentation. It is essential to identify the optimal culture conditions for each species or strain during fermentation[18] Generally, pigment production is higher in solid-state fermentation compared to submerged fermentation[11].

7. Applications and Potential of Microbial Pigments

7.1 Food industry:

Microbial pigments play a vital role in food fermentation, offering a superior alternative to synthetic food colorants and plant-based pigments. They are favored due to their availability, non-seasonal production, scalability, higher yield per hectare, and efficient downstream processing. Pigments such as Monascus, Arpink Red [natural red] from Penicillium oxalicum, β-carotene from Blakeslea trispora, and Astaxanthin from various bacteria are already widely used in food coloring. Extensive research has been conducted to reduce the manufacturing and processing costs of these natural colors, improving their stability, shelf life, and ability to compete with synthetic alternatives[8].

7.2 Cosmetics:

Cosmetics such as lipstick, nail polish, and other products often feature vibrant colors. However, many industrial pigments are made using chemical reagents like benzene and toluene, which are considered unsafe and unsuitable by many. In contrast, natural pigments, known for their stability and safety, are increasingly used in cosmetic formulations. Research has highlighted microbes as valuable sources of biological pigments that are not only safe and innovative but also stable and cost-effective. For instance, phycocyanin has been studied for its antioxidant, immune-boosting, and anti-inflammatory properties, and its stability makes it a key ingredient in cosmetics like eyeliners, eyeshadows, and lipsticks[7].

7.3 Marine ecosystem:

Heavy metal-based antifouling chemicals are harmful to the environment, highlighting the need for eco-friendly alternatives. Researchers have explored pigment-based antifouling compounds, such as prodigiosin from Serratia species, to address marine fouling by bacteria like Gallionella and Alteromonas species. Prodigiosin has also been shown to prevent the attachment of Cyanobacterium species to glass surfaces. Additionally, melanin pigment from Pseudomonas stutzeri has demonstrated the ability to effectively absorb heavy metals like lead, copper, mercury, arsenic, and chromium, likely due to the presence of carboxyl [COOH], phenolic [OH], and amino [NH] groups. The highest efficiency of metal absorption was observed at moderately acidic to neutral pH levels[10].

7.3 Dairy industry:

The dairy industry utilizes harmless pigments produced by Monascus species as food colorants, flavor enhancers, and preservatives. Monascus ruber is employed to make flavor-infused milk by metabolizing rice carbohydrates and producing pigments as secondary metabolites. When rice undergoes solid-state fermentation, it results in the production of red, orange, and yellow pigments[11].

7.4 Textile industry:

The textile industry relies heavily on pigments, particularly synthetic ones. While synthetic dyes are readily available, affordable, and offer a wide range of colors, they pose risks such as skin allergies and other harmful effects on human health. Their synthesis can lead to chemical hazards, toxicity, and the release of toxic or unwanted compounds. As a result, there is growing consumer demand for natural or microbiological pigments, often referred to as natural dyeing, due to the harmful effects of synthetic pigments. Natural pigments are non-carcinogenic and environmentally friendly, making them a preferable choice in the textile industry[10].

7.5 Therapeutic uses:

Pigmented secondary metabolites produced by microorganisms have proven to be effective in treating various diseases, exhibiting antibacterial, anticancer, and immunosuppressive properties. Research into bioactive compounds from bacteria has made significant progress and continues to expand. For instance, anthocyanins are known for their biological benefits, including cancer risk reduction, inflammation reduction, and immune response modulation. Bacteria such as Streptomyces and Serratia can produce red compounds like prodigiosin, metacycloprodigiosin, desmethoxy prodigiosin, and prodigiosin 25-C at 25°C. These compounds, particularly prodigiosin 25^{0}C, possess antibacterial and antimalarial properties, as well as immunosuppressive effects[18].

8. Challenges faced in using microbial pigments:

Although there are various types of natural pigments derived from microorganisms, the development of new compounds for use in the food and pharmaceutical industries faces significant regulatory challenges. The cost of using natural colors is five times higher than that of synthetic alternatives, and in the case of confections, the cost can be as much as 20 times greater [8]. Additionally, factors such as the type of bioreactor and its design (e.g., conventional bioreactors, stirred-tank reactors, air-lift reactors, or trickle-bed reactors), the fermentation method (batch, feed-batch, or continuous), and the physicochemical and biological conditions during fermentation must all be carefully considered to optimize the productivity of microbial pigments[1].

Carotenoids, chlorophyll, anthocyanins, and other major microbial pigments face similar challenges when used in the food industry. Carotenoids, which are strongly pigmented isoprenoid plant compounds, are highly conjugated and unstable when exposed to light or oxygen. Similarly, chlorophyll degrades quickly under enzymatic processes or environmental conditions such as light, oxygen, heat, or acid, leading to the formation of chlorophyll derivatives. Formulating these natural colors can be challenging, though methods like micro-encapsulation can help enhance stability and, in some cases, solubility. Many fungal pigments, however, are not suitable for use as natural colorants due to the presence of mycotoxins. Therefore, selecting non-toxic, non-pathogenic strains for natural pigment extraction is essential [8]. While pigments are utilized in the cosmetic industry, ongoing research is focused on gaining a deeper understanding of their safety for use [7].

Conclusion and future perspective:

The growing concern for environmental preservation and human safety has led to a renewed interest in natural pigment sources [19]. Compared to synthetic chemicals, natural pigments derived from microorganisms such as bacteria, fungi, and microalgae are gaining increased value and demand [1]. These microbial pigments are widely used in the food, cosmetics, and pharmaceutical industries. In the pharmaceutical sector, they show promise due to their effectiveness against various mammalian cancer cell lines and pathogenic bacteria. Moreover, these pigments possess antioxidant properties, which enable them to scavenge free radicals and support proper cellular function. In the food industry, microbial pigments are commonly used as colorants to enhance the appearance of products. Their antioxidant benefits also make them popular in cosmetics, especially in anti-aging formulations [3]. Given their therapeutic properties, microbial pigments hold potential as bioindicators, antioxidants, and anticancer agents, making them an increasingly important focus in biomedical research [19]. Furthermore, studying microbial pigments can offer insights into the evolutionary origins of life and the distribution of chromophore-based traits across all lineages. Unlike non-pigmented microorganisms, which require more time and effort for isolation and characterization, microbial pigments are easier to extract and analyze. This focus on microbial pigments can drive growth in research, development, and economic demand across multiple industries [3].

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