

Microbial Pigments: A Perspective Towards Biocolors

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ABSTRACT

There is a growing demand for healthy and natural products which has led to the development of bioproducts. The development of natural colors/ bio-colors is a growing research trend and has the potential to alter the use of synthetic colorants in the global market. The discovery of microbial pigments was indispensable, as the continued use of synthetic colorants over the decades has caused immense environmental pollution along with various human health vulnerabilities. Hence, the concept of microbial pigments is getting notable attention from researchers and industries. Moreover, the bioactivities possessed by the microorganism qualify them as a great competitor in the food colorant industry, nutraceuticals, and various pharmaceutical areas. This review accentuates the significance of microbial pigments as bio-color with its applications in the pharma industry.

KEYWORDS: Microbial Pigments, Biocolor, Carotenoids, Pharmacological Activity

Date of Submission: 05-11-2023

Date of Acceptance: 15-11-2023

I. INTRODUCTION

Color is one of the remarkable visual traits and is a paramount element of any product. Colors exert an influence on acceptability and impart an appealing aspect to any marketable product. Since, ancient times, colors have been used as a helping element in the making of numerous products to make them look more presentable and to compensate for the loss of natural colors while processing.¹ Humans associate colors with various characteristics of products viz. freshness, taste, flavors, etc. Since then, colors have been quite the sensory evaluation tool for the quality of the product. There are a number of artificial synthetic colors that are significantly used in several industries like food, cosmetics, textiles, and pharmaceuticals. Though synthetic dyes are reliable and economical in comparison to natural colors, they possess an immense chain of threats to environmental conservation.² As a result of which many synthetic dyes are banned from use because of their toxicological problems.³ These dreadful effects of synthetic dyes have set off enormous research on natural pigments. ³ Thus, a market shift towards the evolution and the creation of bio-colors has been contemplated. This market shift is more infused with the neoteric development of Nutraceutical products as bio-color as they are recognized to be comprised of many beneficial properties.¹

The term Bio-color refers to the colors/pigments that are generally extracted from natural resources like plants and vegetables due to their biological origin. In spite of the fact that there are a number of natural pigments, only a couple of pigments are obtainable in adequate quantities for industrial purposes as they are usually extracted from plant sources.² However, it has been tracked down that the pigments extracted from microorganisms are a great alternative to those bio-colors obtained from plant resources. In a matter of time, microbial pigments are more efficient as compared to plant sources due to their ability to rapidly grow, the vast variety of species of microorganisms found in nature, and the ease of cultivation. Thus, the production of microbial pigments is flexible and controllable. Also, the medicinal properties that microorganisms incorporate add up to the advantages of microbial pigments.

II. CLASSIFICATION OF MICROBIAL PIGMENTS

The pigments obtained from microorganisms can be classified into three major terms.¹

- 1) On the basis of their source they can be classified as Algae source, fungal source, and bacterial source.
- 2) On the basis of color they can be broadly classified by the primary color they possess viz.
 - Yellow pigment (Riboflavin, Carotenoids),
 - Red Pigment (Prodigiosin, Carotenoids) ,
 - Blue Pigment (Indigiodine, Violacein).
- 3) On the basis of solubility they can be classified as water soluble or fat soluble.

III. BIOACTIVITY OF MICROBIAL PIGMENTS

Microbial pigments have been obtained from biological sources and play an important role in boosting the body's health. There are various molecules that are composed of microorganisms, such as antibiotics,

enzymes, vitamins, and many others.⁴ Microbial pigments have great commercial potential due to their pharmacological properties. The majority of microbial pigments comprise polyphenols that are antioxidants.¹ The microbial pigments that hold the potential for antioxidant activity are violacein, carotenoids, anthocyanins, and naphthoquinones.⁵ Research shows that the pigments obtained from an endophytic fungus are influential in comparison to the commercially available antibiotics i.e. streptomycin.⁵ The microbial pigments that incorporate anti-microbial properties can be used to treat various multi-drug resistant microbial strains. There are a number of pigments that possess anticancer properties. Table.01 Shows various species of microorganisms and their respective pharmacological actions.⁶

Sr.No	Microorganism	Pigment	Color	Pharmacological Action
1	<i>Agrobacterium aurantiacum</i>	Astaxanthin	Pink-Red	Antioxidant, Anticancer
2	<i>Bacillus cereus</i>	Azaphenanthrene	Green	Anticancer, Antibacterial
3	<i>Rhodococcus maris</i>	Beta-carotene	Bluish-Red	Used to treat erythropoietic protoporphyria
4	<i>Kocuria sp.</i>	Carotenoids	Yellow	Anticancer
5	<i>Bradyrhizobium sp.</i>	Canthaxanthin	Orange	Antioxidant, Anticancer
6	<i>Streptococcus agalactiae</i>	Granadaene	Orange-Red	Antioxidant, Detoxify ROS
7	<i>Proteobacteria</i>	Heptyl Prodigiosin	Red	Antiplasmodial
8	<i>Pseudomonas guinea</i>	Melanin	Black	Antioxidant
9	<i>Serratia marcescens</i>	Prodigiosin	Red	Anticancer
10	<i>Bacillus sp.</i>	Riboflavin	Yellow	Nutritional supplement
11	<i>Streptomyces echinoruber</i>	Rubrolone	Red	Antimicrobial
12	<i>Cytophaga/Flexibacteria</i>	Tryptanthrin	Light-dark Yellow	Antioxidant, Anticancer
13	<i>Chromobacterium violaceum</i>	Violacein	Purple	Antibacterial, Antifungal
14	<i>Flavobacterium sp.</i>	Zeaxanthin	Yellow	Photo-protectant, Antioxidant

Table.01. List of pigment-producing microorganisms.^{1, 5, 6}

IV. TYPES OF MICROBIAL PIGMENTS

Carotenoids: -

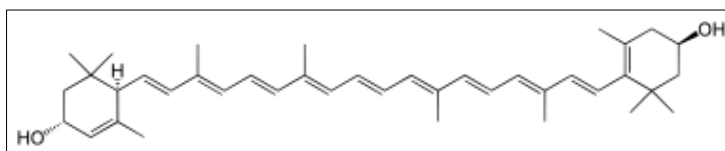


Figure 01. Chemical Structure for Carotenoid

Carotenoids as microbial pigments represent a fascinating area of research, with their diverse colors, functional properties, and industrial applications. Carotenoids are the pigments obtained from the vast diversity of bacteria (*Bradyrhizonium sp.*), algae (*Spirulina*), yeast (*Phaffia rhodozyma*), etc.⁷ The pigment carotenoid varies from red to yellow shades of color.⁸ Carotenoids are tetraterpenoid compounds composed of isoprene units. They have a long-conjugated system of alternating single and double bonds, which gives them their characteristic colors. Carotenoids are synthesized from common metabolic precursors such as isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are derived from the central metabolic pathway known as the mevalonate pathway. These are lipid-soluble pigments however, they are oxygen, heat, and light sensitive.⁹ Carotenoids have the ability to protect against photo-oxidation (oxidation reaction occurring in presence of light energy, it involves excitation of photosensitizer and energy transfer to lipid molecules or oxygen) and therefore possess immense antioxidant properties.¹⁰ Carotenoids also incorporate antimicrobial properties. It has been discovered that the higher concentration of these pigments leads to the lysis of pathogenic bacteria found in human cells. Carotenoid is mainly found in the microorganism *Kocuria sp.*, *Pseudomonas sp.*, *Paracoccus sp.* and *Halobacterium Salinarium*.

Violacein: -

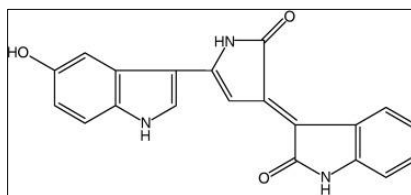


Figure 02. Chemical Structure for Violacein

Violacein is known to be the most versatile pigment that is comprised of a variety of biological properties such as antitumoral, antimicrobial and antiparasitary properties.¹¹ Violacein is found typically in violet-blue color. Violacein is a complex pigment with a distinct chemical structure. It is composed of two molecules of 5-hydroxy indole joined together to form a dimer. This dimeric structure gives violacein its intense violet color, hence the name. The biosynthesis of violacein involves a series of enzymatic reactions. It is primarily produced via the shikimate pathway, a metabolic pathway that generates aromatic amino acids. The key enzyme involved in violacein biosynthesis is VioA, which catalyzes the condensation of two molecules of 5-hydroxyindole to form the dimeric structure. Violacein stands out as a unique and fascinating microbial pigment due to its vibrant color, complex structure, and diverse biological activities. Violacein is considered to be bacterial pigment as it is extracted from various sources of bacteria i.e. *Chromobacterium violaceum*, *Pseudoalteromonas sp.*, etc.¹ Violacein possesses strong antibacterial properties that make it a propitious contender as a natural antibiotic.¹² The major studied bioactivity of violacein is anticancer activity.¹²

Astaxanthin: -

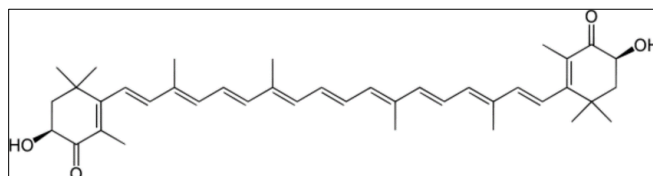


Figure 03. Chemical structure for Astaxanthin

Astaxanthin can be distinguished as xanthophyllus which is a keto carotenoid derivative. Astaxanthin has a unique chemical structure characterized by a long chain of conjugated double bonds and cyclic end groups. This structure gives astaxanthin its distinctive red color. Astaxanthin is a lipophilic pigment that naturally occurs in reddish-orange color.¹ A large amount of astaxanthin is obtained from marine ecosystems. This pigment is a potent antioxidant and a nutritional supplement, and a propitious therapeutic compound.¹³ It possesses antioxidant, anti-inflammatory and anti-apoptotic properties.¹³ Astaxanthin has great potential as a bioactive compound that can be used in the prevention or treatment of malignant cells. The bacterial sources of astaxanthin are *Paracoccus marcusii*, *Paracoccus carotinifaciens*, *Agrobacterium aurantiacum*, etc.

Melanin: -

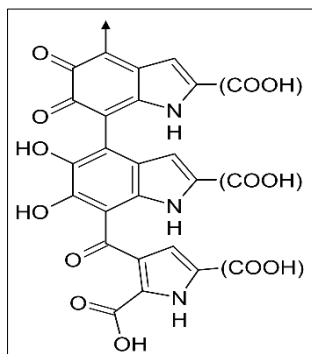


Figure 04. Chemical structure for Melanin

Melanin is a biological pigment that is predominantly found in various biological systems.¹⁴ It is a complex polymer derived from the amino acid tyrosine.¹⁴ It consists of repeating units linked together, forming a three-dimensional network. The precise structure and composition of melanin can vary depending on the organism

producing it. These are dark color pigments that consist of various pharmacological properties. Melanin is typically found in different shades of brown to black color.¹⁵ Melanin serves as an essential microbial pigment with multiple functions, including protection against environmental stresses, antioxidant properties, and potential contributions to microbial virulence.¹⁴ Melanins are well-recognized for their immunoregulatory, photoprotective, and antioxidant properties.¹⁵ In recent studies, it has been brought up that melanin possesses anti-inflammatory, radioprotective, and hypoglycemic benefits.¹³ The various melanin-producing microorganisms are *Vibrio cholera*, *Shewanella colwelliana*, and *Pseudomonas guinea*.¹⁵

Prodigiosin: -

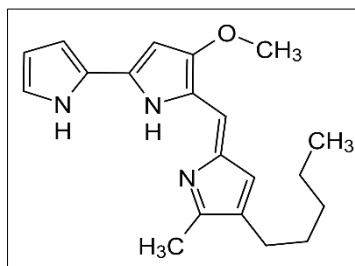


Figure 05. Chemical structure for Prodigiosin

Prodigiosin is a natural pigment with a distinct chemical structure. It belongs to the family of pyrrolopyrrole compounds and consists of a linear chain of pyrrole rings. The specific arrangement of these rings, along with the presence of various substituents, contributes to the red color of prodigiosin. The pigment can range from bright red to deep purple, depending on the specific bacterial strain and environmental conditions.¹⁵ Prodigiosin is a red-colored pigment that is a pharmaceutically significant bioactive compound. It has been documented, this pigment is a secondary metabolic alkaloid that is mostly produced by bacteria. Prodigiosin can be synthesized by both gram-positive and gram-negative bacteria.¹⁶ Prodigiosin and its synthetic derivatives are effective against cancer cell lines and multi-drug resistant cells.¹⁶ Prodigiosin also has great therapeutic potential as an antioxidant.¹ The microbial sources of prodigiosin are *Serratia marcescens*, *Vibrio psychroerythrus*, etc.

V. EXTRACTION OF PIGMENT FROM MICROORGANISMS

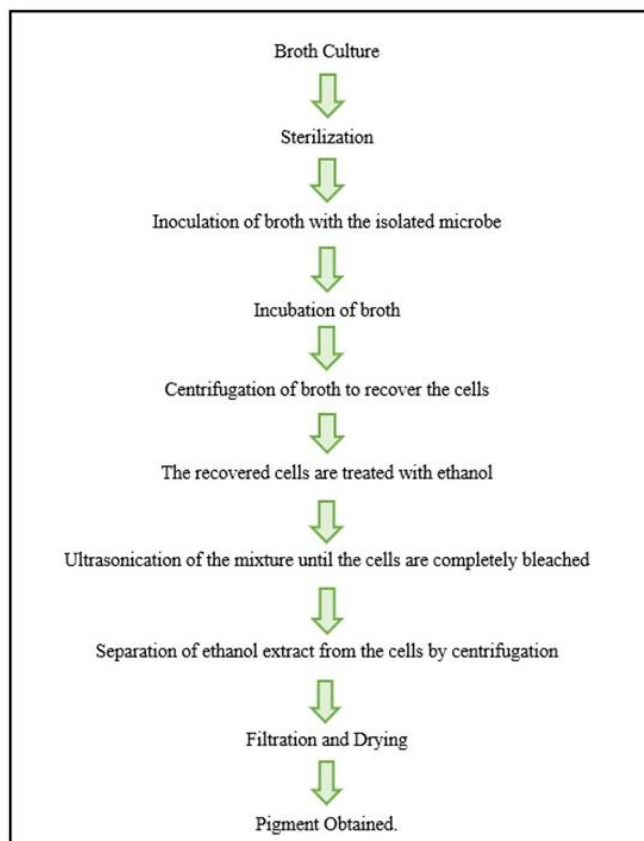


Figure.06 Extraction process of pigments2

The pigment production from microorganisms undergoes various number of processes that include the following steps, Figure.06 shows the step-wise procedure of extraction of pigments.²

VI. FACTORS AFFECTING MICROBIAL PIGMENT PRODUCTION

Nutrient availability:

The availability of essential nutrients in the growth medium is crucial for microbial pigment production. Different pigments may require specific nutrients as precursors or cofactors. For example, some pigments like carotenoids require a carbon source, while others like melanins may require nitrogen or sulfur.²

Light conditions:

Light is an important environmental factor that affects the synthesis and expression of microbial pigments, especially in photosynthetic organisms. Light intensity, wavelength, and duration of exposure can influence pigment production. Certain pigments, such as chlorophylls, phycobilins, and bacteriochlorophylls, are directly involved in light harvesting and photosynthesis.¹⁷

pH and temperature:

The pH and temperature of the growth environment have significant impacts on pigment production. Microorganisms have optimal ranges of pH and temperature for growth and pigment synthesis. Variations outside these ranges can inhibit pigment production or alter the color spectrum produced. Different pigments may exhibit different pH and temperature optima.¹⁸

Oxygen levels:

Oxygen availability can affect microbial pigment production, particularly in anaerobic or facultative anaerobic organisms. Some pigments, such as carotenoids and bacteriochlorophylls, may be synthesized under low oxygen conditions, while others, like melanins, may be stimulated by oxygen exposure.¹⁷

Genetic regulation:

Microbial pigment production is governed by genetic regulation, including the expression of specific pigment biosynthesis genes. These genes are often regulated by various factors, such as transcription factors, signal molecules, or environmental cues. Mutations or alterations in these regulatory mechanisms can affect pigment production.¹⁶

Stress conditions:

Microorganisms often produce pigments as a response to environmental stress. Factors like high salinity, UV radiation, heavy metals, or nutrient limitation can induce the synthesis of specific pigments as protective mechanisms. These stress-induced pigments, such as melanins or carotenoids, act as antioxidants, UV protectants, or metal chelators.¹⁹

VII. CURRENT LIMITATIONS AND FUTURE PERSPECTIVE OF MICROBIAL PIGMENTS

Microbial pigments are one of the promising fields of research in life sciences to validate an enormous number of applications in various industries. Microbial pigments exhibit a wide range of utilization in the food, drug, textile, and cosmetic industries. These pigments can be a substantial alternative to synthetic colorants and have great potential to meet the emerging requirements of colorants in the global market. Microbial pigments indirectly hold a significant role in the conservation of the ecosystem. However, there are certain limitations such as high-cost investment for large-scale production, lower stability, variations in shades of color due to few optional parameters, and lack of specifications in terms of microorganism-producing pigments, technological imperfection leads to delay in progress for industrial production of pigments, are tending to recoil the efforts of researchers to replace the synthetic pigments²⁰. From the perspective of the greater future of microbial pigments, the studies should be more concerned with finding easier methods for the production of microorganisms in order to increase their industrial applications. The development of various biotechnological tools such as metabolic engineering, mutagenesis, and genetic manipulation will have an immense role in the improvement of strains of pigment-producing microorganisms. The research on such pigments would eventually divulge the evolutionary species of origin of life and their characteristics in relation to colorants and various applications. The ease of production processes and cost-effectiveness of microbial pigments leads to a greater perspective of diverse application in the upcoming future. The wide-ranging bioactivities of microbial pigments viz. anti-microbial, anti-cancer, antioxidant, and anti-inflammatory, trigger the potential of microbial pigments in the pharmaceutical field.

VIII. CONCLUSION

Microorganisms being a good source of color and the innumerable sources of microbes in nature leads to a better potential for microbial pigments. Microbial pigments can commercially replace artificial dyes and can have a beneficial impact on humans as well as the environment. Nowadays awareness of the use of biocolors is increasing in the market due to their therapeutic uses and non-toxic nature. The advancement in microbial biotechnology has better scope in the development of microbial pigments and the overall upgradation of biocolors.

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