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## CHROMATIC WONDERS: EXPLORING THE FASCINATING REALM OF MICROBIAL PIGMENTS

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### ABSTRACT

Pigments are compounds that are widely used in industries that come in a wide variety of colours. Pigment production is one of the charismatic traits of microbes. Microbial pigments are of great interest owing to the stability of the pigments produced and the availability of cultivation technology. Apparently, microbial pigments are not merely colours, but they possess a mixture of diverse chemical components with multifaceted potential biological activities. Microbial pigments are a promising alternative to other colour additives extracted from vegetables or animals because they are considered as natural, pose no seasonal production problems and show high productivity. These pigments, besides their aesthetic appeal are gaining recognition for their eco-friendly and non-toxic nature, making them ideal for utilization in dyes, foodstuffs, pharmaceuticals, cosmetics and other industrial applications. These microbial pigments have broad area of application, mainly in food industries, pharmaceutical industries and textile industries. Food grade pigments such as  $\beta$ -carotene, arpink red, riboflavin, lycopene and monascus pigments are used in food industry. In pharmaceutical industry pigments like anthocyanin, prodigiosin and violace in are widely used to treat diseases. The advantages of pigment production from microorganisms comprise easy and fast growth in the cheap culture medium, independence from weather conditions and colours of different shades. Moreover, natural pigments produced from biological origin have medicinal importance as been used as antioxidant, antimicrobial, additives, colour intensifiers and anticancer as well as economically friendly. Some of bacteria capable of producing pigment with different varieties of colours are *Agrobacterium aurantiacum*, *Staphylococcus aureus*, *Chromobacterium violaceum*, *Serratia marcescens*, *Bacillus* sp., *Flavobacterium* sp. etc. colours are pink-red, golden yellow, purple, red, creamy and yellow, respectively.

**Key words** : Microbial pigments, Eco-friendly, Colour intensifiers, Antioxidant.

### Introduction

Microbial pigments represent a fascinating aspect of the microbial world, offering a diverse array of colors and functionalities. These pigments are synthesized by various microorganisms such as bacteria, fungi, algae and archaea. They serve a multitude of purposes, including protection against environmental stressors like UV radiation, facilitating nutrient acquisition and serving as signalling molecules. In addition to their biological roles, microbial pigments have garnered significant interest for their potential applications in various fields, including food,

cosmetics, pharmaceuticals and even biotechnology.

Pigments are compounds with characteristics of importance to many industries. In the food industry they are used as additives, color intensifiers, antioxidants, etc. Pigments come in a wide variety of colors, some of which are water-soluble (Tibor, 2006).

The production of microbial pigments can be influenced by various factors, including nutrient availability, pH, temperature and oxygen levels. This dynamic synthesis process makes them intriguing subjects for research and biotechnological exploitation. Scientists are

exploring methods to optimize pigment production through genetic engineering, fermentation techniques and environmental manipulation.

In recent years, there has been growing interest in utilizing microbial pigments as natural alternatives to synthetic dyes and colorants. Their eco-friendly nature, biodegradability and potential health benefits make them attractive candidates for replacing conventional pigments in various industries. Furthermore, their antimicrobial, antioxidant, and anticancer properties have sparked investigations into their therapeutic potential.

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In addition to their industrial and biomedical significance, microbial pigments also offer insights into microbial ecology and evolution. The study of pigment-producing microorganisms provides valuable information about their interactions with the environment and other organisms, as well as their roles in ecosystem dynamics.

#### Microbial pigments that can be used as food grade colors

Some of the major pigments found in microorganisms which are used as food colorants are canthaxanthin, astaxanthin, prodigiosin, phycocyanin, violacein, riboflavin, beta-carotene, melanin and lycopene. Microbial pigments can be either inorganic or organic, although organic pigments tend to be more useful as food colorants.

- i. **Canthaxanthin** : is an orange to deep pink colored carotenoid that is lipid soluble and a potent antioxidant. The chemical formula of canthaxanthin is  $C_{40}H_{52}O_2$ . It is isolated from *Bradyrhizobium* spp., is a trans-carotenoid pigment and is approved as a food colorant and used in a range of foods as well as salmon and poultry feed (Chuyen *et al.*, 2018; Surai *et al.*, 2012; Jaswir *et al.*, 2011).
- ii. **Astaxanthin** : is a red-orange pigment, naturally found in basidiomycetous yeast, microalgae, salmon and crustaceans, red shrimp, cray fish, feathers of some birds and is lipid soluble (Dufosse *et al.*, 2009; Guedes *et al.*, 2011; Zuluaga *et al.*, 2017; Pogorzelska *et al.*, 2018; Gupta *et al.*, 2007). The chemical formula of astaxanthin is  $C_{40}H_{52}O_4$ . It's an approved coloring agent used in fish and animal foods.
- iii. **Prodigiosin** : Many strains of *Serratia marcescens*, produce a red pigment, which shows antibacterial,

antimalarial, antibiotic and antineoplastic activity (Dufosse *et al.*, 2018; Dufosse *et al.*, 2017). The chemical formula of prodigiosin is  $C_{20}H_{25}N_3O$ . It has been successfully applied as coloring agents in yogurt, milk and carbonated drinks (Namazkar *et al.*, 2013).

- iv. **Phycocyanin** : is a blue pigment produced by chlorophyll A containing cyanobacteria. *Aphanizomenon flosaquae* and *Spirulina* produces phycocyanin, which is being used in the food and beverage industry as the natural coloring agent 'Lina Blue' and is also found in sweets and ice cream (Dufosse *et al.*, 2018; Eriksen *et al.*, 2008; Barsanti *et al.*, 2008).
- v. **Violacein** : *Chromobacterium violaceum* is one of the most prominent producers of this purple pigment, other bacterial species also produces the pigment and mostly have a purple hue. The chemical formula of violacein is  $C_{20}H_{13}N_3O_3$ . It exhibits antifungal, antibiotic, antitumor and antibacterial properties. Violacein has shown potential use in food, cosmetic and textile industries (Duran *et al.*, 2012 and Konzen *et al.*, 2006).
- vi. **Riboflavin** : Water soluble vitamin B2, is a yellow colored pigment and produced by various microorganisms. The chemical formula of riboflavin is  $C_{17}H_{20}N_4O_6$ . It is used in diary items, breakfast cereals, baby foods, sauces, fruit drinks and energy drinks (Dufosse *et al.*, 2018; Unagul *et al.*, 2005; Hong *et al.*, 2008; Powers *et al.*, 2003).
- vii. **Beta-carotene** : A red-orange colored organic pigment, mostly extracted from the beta-carotene rich algae, *Dunaliella salina*. The chemical formula of beta-carotene is  $C_{40}H_{56}$ . Production of  $\beta$ -carotene through fermentation of *Blakeslea trispora* produces a pigment equivalent to pigments produced through a chemical process and is an acceptable coloring agent (Terao, 1989; Dufosse, 2009; Dufosse, 2017; Lopes *et al.*, 2009; Cerda-Olmedo, 2001). It is used in a variety of food items ranging from red to yellow in color.
- viii. **Melanin** : Melanins are natural pigments present in animals, plants and in many micro-organisms. The chemical formula of melanin is  $C_{18}H_{10}N_2O_4$ . They are widely used in eye glasses, cosmetic, food items, sunscreen protection creams, pharmaceuticals and food items (Dufosse, 2009; Dufosse, 2017; Vinarov *et al.*, 2003; Eriksen, 2008; Dufossé, 2004; Dufossé, 2016).
- ix. **Lycopene** : Widely present and consumed in tomatoes, a brilliant red pigment consisting of

carotenoid. The chemical formula of lycopene is  $C_{40}H_{56}$ . It has been isolated from microbes like *Fusarium sporotrichioides* and *Blakeslea trispora* and has the potential to attenuate persistent diseases such as some types of cancers and coronary heart disease (Giovannucci *et al.*, 2002). It is used in meat coloring in countries like the USA, Australia and New Zealand.

### Factors affecting Microbial Pigment Production

- 1) **Temperature** : The production of microbial pigments is greatly affected by the temperature of incubation, depending upon the type of microorganism. The growth of *Monascus* sp. requires 25-28°C for the production of pigment, whereas *Pseudomonas* requires 35-36°C for its growth and pigment production.
- 2) **pH** : The growth and type of pigment produced is affected by the pH of the medium in which microorganisms are grown. It differs from one microorganism to another and slight change in pH may change the shade of colour. Optimum pH for *Monascus* sp. and *Rhodotorula* is 5.5-6.5 and 4.0-4.5, respectively. Neutral to slight alkaline pH favours lycopene formation whereas acidic pH favours  $\beta$ -carotene synthesis.
- 3) **Carbon source** : The mycelial growth of pigment producing microorganism is affected by the type of carbon source like glucose, fructose, maltose, lactose, galactose, etc. Glucose and its oligosaccharides are better carbon sources for growth and pigment production. For the *Monascus* sp., the volumetric pigment formation is best on starch and dextrin, moderate on glucose and maltose but poor on fructose. In *M. purpureus*, fermentation with maltose and glucose as carbon sources gave very dark liver pigment, whereas sucrose produced a light and uneven red pigment. For pigment production from *Phaffia rhodozyma*, cello biose supported more pigmentation than others. D- mannitol also supported pigmentation, whereas glucose promoted both growth and pigmentation. The sugar-type also influences the shade of pigment.
- 4) **Nitrogen source** : The production of microbial pigments is also affected by the nitrogen source depending upon the microorganism. Ammonium chloride is the best for production of *Monascus* pigment followed by ammonium nitrate and then glutamate. Potassium nitrate is the poorest nitrogen source, while glutamate proved outstanding for the pigment production. Use of peptone plays an important role in pigment production from *Monascus* sp. In *M. purpureus*, 1.5% MSG medium produced an appealing red colour, whereas other nitrogen sources produced faint or foggy red pigment. For pigment production from *Aspergillus* sp., ammonium phosphate proved essential for the growth of the organism. The pigment production in *Bacillus subtilis* has also been stimulated by incorporation of tyrosine and histidine in the medium.
- 5) **Type of Fermentation** : Fermentation (solid or submerged fermentation) affects production of microbial pigment. The solid-state fermentation yields 3 folds more pigment than submerged fermentation. In *M. purpureus*, yields are superior in solid cultures than submerged, though media composition, pH and agitation also affect pigment production. Solid culture of *M. purpureus* gave greater production of red and yellow pigments under similar conditions *i.e.* media composition and incubation temperature in solid and submerged fermentation.
- 6) **Minerals** : Minerals play an important role in pigment production. Zn stopped the growth in liquid medium whereas in solid medium vigorous growth and pigmentation was observed. Sometimes Zn acts as a growth inhibitor and increases glucose uptake for the synthesis of pigments. Mn stimulates pigment production from *Lactobacillus plantarum* and *Streptococcus lactis*.
- 7) **Chromatic diversity** : The chromatic wonders of microbial pigments are show cased through a detailed examination of the various hues produced by different microorganisms. From the striking reds and purples of prodigiosin producing bacteria to the green chlorophylls of photosynthetic microbes, the breadth of colors in the microbial world is explored. The genetic and biochemical basis of pigment production provides insights into the mechanisms behind this captivating diversity.

### In food industry

Some fermentation-derived pigments, such as  $\beta$  - carotene from the fungus *Blakeslea trispora* in Europe or pigments from *Monascus* in Asia are now in use in the food industry (Downham *et al.*, 2015). Various pigments provide a good appearance with additional nutritive and medicinal values such as antibiotic, antioxidants. There is a craze among the peoples toward the use of natural products because of harmful effects of synthetic chemicals (pigment). For example, *Monascus* red pigments, generally produced as MFR (*Monascus*

Fermented Rice) improve the organoleptic characteristics of the food products. These pigments contain monocolins, which reduces the LDL-cholesterol and increase HDL-cholesterol. There are following microbial pigments which have a future potential and are under the research work:

### $\beta$ - Carotene production

$\beta$ - carotene is a yellowish carotenoid pigments also known as pro-vitamin A. It acts as antioxidant and has potential positive properties against certain diseases. Following microbes are mainly used for  $\beta$ -Carotene production:

1. ***Blakeslea trispora*** : Some of strains of this mould produce high level of  $\beta$ -Carotene. *B. trispora* strains are of two types: (+) mating type and (-) mating type. (-) Strains obtained by the specific ratio mating of above two mating types produce  $\beta$ -Carotene (Dufoss *et al.*, 2006). Today *B. trispora* fungal  $\beta$ -Carotene is produced by two industries, the first in Russia and Ukraine and the second in Leone, Spain.
2. ***Mucor circinelloides*** : Wild strains of *M. circinelloides*, when exposed to the light impulses of blue light, get activated due to significant change in structural genes of  $\beta$ -Carotene, hence produce it to high level.
3. ***Phycomyces blakesleeanus*** : Phycomyces is mainly used for the production of various chemicals like  $\beta$ -Carotene. They have enhanced carotenogenic potential, when grown on solid substrates or in liquid media.



**Fig. 1 :** (A) *Blakeslea trispora*, (B) *Mucor circinelloides*, (C) *Phycomyces blakesleeanus*.

### Arpink Red production

It is the red pigment produced by the strain *Penicillium oxalicum* obtained from the soil. It contains



**Fig. 2 :** *Penicillium oxalicum*.

chromophore of anthraquinone type. The amounts of red pigment Arpink Red in various food products was amount recommended by Codex Alimentarius Commission (Rotterdam meeting, March 11-15, 2002).

**Table 1 :** Arpink Red amount recommended by Codex Alimentarius Commission (Rotterdam meeting, March 11-15, 2002).

| S. no. | Food product                     | Amount of Arpink Red (in mg/kg) |
|--------|----------------------------------|---------------------------------|
| 1.     | Meat products                    | 100                             |
| 2.     | Meat and meat products analogues | 200                             |
| 3.     | Milk products                    | 150                             |
| 4.     | Ice cream                        | 150                             |
| 5.     | Confectionery                    | 300                             |

### Riboflavin (Vitamin B2) production

It is the yellow food colourant and its use is permitted in many countries. Because of its specific affinity, it is used mainly for cereal-based products. Applications of riboflavin somewhere limited due to its bitter taste and naturally slight odour. There are many microorganisms having potential to produce riboflavin by fermentation. Riboflavin fermentation can be categorized into three types: weak over producer, moderate over producer and strong overproducer. Fermentation with *Ashbya gossypi* is preferred because of higher yield and greater genetic stability; riboflavin levels of over 15g/L have been reported.

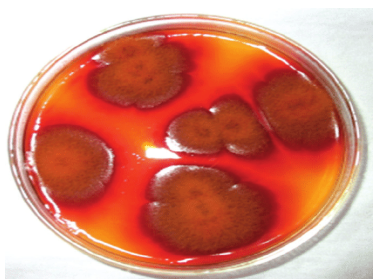


**(C)**

**Fig. 3 :** *Ashbya gossypi*.

### Monascus pigments production

*Monascus* spp. belongs to the group of Ascomycetes



**Fig. 4 :** *Monascus purpureus*.

and particularly to the family of Monascaceae. The genus *Monascus* can be divided into four species: *M. pilosus*, *M. purpureus*, *M. ruber* and *M. frigidanus*, which account for the majority of strains isolated from traditional oriental food. The common names of this fungal product are Red Yeast Rice (RYR), red rice, angkak, red leaven, benikoji (Japanese), hung-chu, hongqu, zhitai (Chinese), rotschimmelreis (Europe), red mould (USA) and MFR (*Monascus* fermented rice). *Monascus* spp. produce many pigments of industrial importance and these pigments are mainly of three types *i.e.* red colorants, orange colorants and yellowish colorants.

#### Lycopene production

It is a red open-chain unsaturated carotenoid, acyclic isomer of beta-carotene and longer than any other carotenoid. Lycopene, also known as psi-carotene is very sensitive to heat and oxidation and is insoluble in water. Because of the abundance of double bonds in its structure, there are potentially 1,056 different isomers of lycopene, but only a fraction is found in nature. In a study *cis*-isomers of lycopene were shown to be more stable, having higher antioxidant potential compared to the all-*trans* lycopene. Genetically modified fungus *Fusarium sporotrichioides* was used by Jones *et al* to manufacture the colourant and antioxidant lycopene. They used the cheap corn fiber material as the substrate. Cultures in lab flasks produced 0.5 mg (lycopene)/g of dry mass within 6 days and such a production will be increased within the next years.



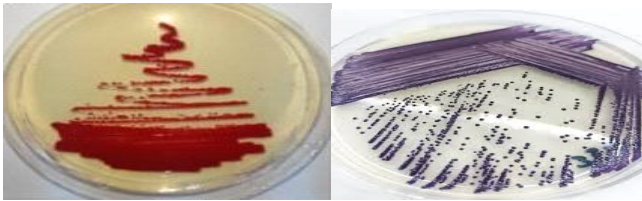
**Fig. 5 :** *Fusarium sporotrichioides*.

#### In pharmaceutical industry

Pharmaceutical industry uses many microbial pigments in their products. Many pigmented secondary

metabolites of the microorganism have significant potential clinical applications and many research works are going on for treating many diseases like cancer, leukemia, diabetes mellitus etc. These pigments may act as: antibiotics, anticancer, antiproliferative and immunosuppressive compounds. Some examples of such type of pigments are given below:

- 1) **Anthocyanin** : Anthocyanins are water soluble, flavonoid pigments. They are engaged in a wide range of biological activities *i.e.* antioxidant activity, reduce the risk of cancer, decrease and modulate immune response insult. The inhibitory effect of anthocyanins in carcinogenesis and tumor growth may be through two main mechanisms: a) Redox status modification and b) Interference with basic cellular functions (cell cycle, apoptosis, inflammation, angiogenesis, invasion and metastasis). Anthocyanin has antioxidant activity because of its phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical.
- 2) **Prodigiosin** : It is a potential pigment having many pharmacological properties. It shows a broad range of cytotoxic activity is produced by *Vibrio psychroerythrus*, *S. marcescens*, *Pseudomonas magnesorubra* and other eubacteria. Prodigiosin is a tripyrrole pigment and it was first reported from *S. marcescens* (a Gram negative bacterium). *S. marcescens* is known for the production of a non-diffusible red pigment, prodigiosin. Streptomyces or Serraria are also used for its production. It shows immunosuppressing activity and also exerts antiproliferative and cytotoxic effects on 60 cell lines of human tumor cells (derived from lung colon liver ovarian brain cancers, melanoma and leukemia). Prodigiosin was also reported as active component for prevention and treatment of diabetes mellitus.
- 3) **Violacein** : The violet pigment violacein is an indole derivative, isolated mainly from bacteria *Chromobacterium violaceum*, which exhibits important antitumoural, antiparasitary, antiprotozoan, anticancer, antiviral, antibacterial and antioxidant activities.
- 4) **Red yeast rice (RYR)** : Red Yeast Rice (RYR) is a fermented rice product produced traditionally by fermenting cooked rice kernels with yeast *Monascus* sp. (*Monascus ruber*, *Monascus purpureus*, *Monascus ruber* and *Monascus pilosus*). These *Monascus* spp. have an important characteristic to produce secondary metabolites of polyketide structure and yellow, orange and red pigments.

**Fig. 6 :** Prodigiosin.**Fig. 7 :** Violacein.

*Monascus ruber* was used for production of angkak, a fermented rice product with anticholesterol activity. RYR proved to contain many active constituents such as compounds resembling statins in its structure, unsaturated fatty acid, sterols and B-complex vitamins. Various studies also reported that RYR and statins decreases blood glucose levels in diabetes.

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