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Review Article

MICROBIAL PIGMENTS: PRODUCTION AND THEIR APPLICATIONS IN VARIOUS INDUSTRIES

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ABSTRACT

Nowadays there is a great interest of the market for the natural pigments; especially microbial pigments because of widely used synthetic pigments have harmful issues associated with the workers of industry as well as consumer. Microbial pigments have numerous beneficial properties like anticancer, antiproliferative, immunosuppressive, antibiotic, biodegradability etc. Many microorganisms, including bacteria, fungi, yeast and mould etc. are employed for the industrial production of various pigments by using fermentation technology. These microbial pigments have broad area of application, mainly in food industries, pharmaceutical industries and textile industries. Food grade pigments such as β-carotene, Arpink Red, Riboflavin lycopene and Monascus pigments are used in food industry. In pharmaceutical industry pigments like Anthocyanin, Prodigiosin and Violacein are widely used to treat diseases. Several microbial pigments are also used in textile industry.

Keywords: Microbial pigments, β-carotene, Monascus pigments, Anthocyanin, Prodigiosin.

INTRODUCTION

Colours provide attracting appearance to marketable products such as food products, textiles, and pharmaceutical products. Colours are the beauty of world that means we cannot imagine world without colours. Pigments from natural sources have been obtained since long time ago, and their interest has increased due to the toxicity problems caused by the synthetic pigments. In this way the pigments from microbial sources are a good alternative. There are many artificial synthetic colorants, which have widely been used in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes, comprise various hazardous effects. There are many limitations of synthetic pigments. The precursors, used in the production process of synthetic pigment, have many carcinogenic hazardous effects on the workers. The wastes of the production process are also harmful. They are itself non-environment friendly and non-biodegradable.

To counter these hazardous effects of synthetic colorants, there is worldwide interest in process development for the production of pigments from natural sources[1]. The utilization of natural pigments in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes has been increasing in recent years because of harmful effects of synthetic pigments and their industrial byproducts on humans and the environment[2].

Plants and microorganisms are the two major sources of natural pigments. Yet the natural pigments from plants also have drawbacks such as: instability against light, heat or adverse pH, low water solubility and are often non-availability throughout the year. Hence the microbial pigments are of great interest owing to the stability of the pigments produced[3] and the availability of cultivation technology[4-5].

In course of the limitation of synthetic as well as natural pigments, the microbial pigments came into preference. Microbial pigments have many advantages over artificial and inorganic colors. The pigment production from microorganisms is efficient and beneficial process as compare to chemical synthesis of pigments. Microbes can grow easily and fast in the cheap culture medium[6] and independent from weather conditions. Microbial colours are available in different shades. These colours are biodegradable and environment friendly. They also have numerous clinical characteristics like antioxidant, anticancer, antiproliferative, immunosuppressive, treatment of diabetes mellitus etc.

Hence, microbial pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial applications[7].An ideal pigment producing microorganism should be capable of using a wide range of C and N sources, must be tolerant to pH, temperature, and minerals, and must give reasonable color yield. Most of the bacterial pigment production is still at the R&D stage. Hence, work on the bacterial pigments should be concerned especially to finding cheap and suitable growth medium in order to reduce the cost and increase its applicability for industrial production. Fermentation is an inherently faster and more productive process as compared to other chemical processes so it is more beneficial to use it for industrial production. Moreover, pigment production from microbial sources has gained attention owing to public sensitivity regarding "synthetic food additives."

Microorganisms can be genetically engineered because their relatively large strands of genes can be easily manipulated. As a result, microbial pigment production can be increased in geometric proportions through genetic engineering, compared to the scaling up methods of chemists. Microbes have also more versatility and productivity over higher forms of life in the industrial-scale production of natural pigments and dyes. Fermentation process has been increased by genetic engineering and further research for nontoxic microbial pigment can make quantum leaps in the economics of microbial pigments.

Possible aspects of microbial pigments

Microorganisms have been used for a long time for production of numerous molecules as diverse as antibiotics, anticancer, antioxidants, enzymes, vitamins, texturizing agents and so on. There is also a growing interest in the industries for the production of various types of pigments. Ingredients like colors when derived from biological sources like plants or microorganisms are considered as natural and also they are safe for health as well as environment friendly. Microbial colors are in use in the fish industry already, for example to enhance the pink color of farmed salmon. Further, some natural colorants have commercial potential for use as antioxidants. The industry is now able to produce some microbial pigments for applications in food, cosmetics or textiles. In nature, color rich and pigment producing microorganisms (fungi, yeasts, and bacteria) are common. Microorganisms produce various pigments like carotenoids, melanins, flavins, quinones, prodigiosins and more specifically monascins, violacein or indigo[8].

Factors affecting Microbial Pigment Production

1. Temperature

Temperature of incubation is the main factor which depends on the type of microorganism. The growth of *Monascus spp.* requires 25-28°C for the production of pigment, whereas *Pseudomonas* requires 35-36°C for its growth and pigment production.

2. pH

pH of the medium affects the growth of the microbe and type of pigment production. Optimum pH for *Monascus sp*. and *Rhodotorula* is 5.5-6.5 and 4.0- 4.5 respectively[9]. Neutral to slight alkaline pH favours lycopene formation whereas acidic pH favours β-Carotene synthesis.

3. Carbon source

The mycelial growth of pigment producing microorganism is affected by the type of carbon sources like glucose, fructose, maltose, lactose, galactose, etc.

4. Type of Fermentation

The solid state fermentation yields 3 fold more pigment than submerged fermentation[9]. In *M. purpureus*, yields are superior in solid cultures than submerged, though media composition, pH and agitation also affect pigment production.

5. Minerals

Minerals play an important role in pigment production. Zn (2×10^{-3} M and 3×10^{-3} M) stopped the growth in liquid medium whereas in solid medium vigorous growth and pigmentation was observed[9].

6. Nitrogen source
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.

7.Moisture content
In solid state fermentation, the higher level of pigment production by *Monascusruber* occurs at the 70% initial moisture level in substrate i.e. rice.

8. Aeration rate
When the Monascus pigments are produced from the solid state fermentation,the bed of rice is continuously aerated by sparging with humidified air (95–97% relative humidity).A forced aeration rate of higher than 0.5 L min-1 reduces the production of the pigments and biomass as a consequence of water loss from the bed. Highest levels of pigments are obtained at forced aeration rates of between 0.05 and 0.2 L min^{-1}.

In food industry
Some fermentation-derived pigments, such as b-carotene from the fungus *Blakesleatrispora*in Europe or pigments from *Monascus* in Asia are now in use in the food industry[10-12]. 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) [12].
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[13].
There are following microbial pigments which have a future potential and are under the research work:

β –Carotene production
β –Carotene is a yellowish carotenoid pigments also known as pro-vitamin A (Fig. 1). It acts as antioxidant and has potential positive properties against certain diseases. Following microbes are mainly using for β –Carotene production:

1. ***Blakesleatrispora***: Some of strains of this mould produce high level of β –Carotene. *B. trispora* strains are of two types: (+) mating type and (-) mating type.(Fig.2) (-) Strains obtained by the specific ratio mating of above two mating types produce β –Carotene[14].Today *B. trispora* fungal β –Carotene is produced by two industries, the first in Russia and Ukraine, and the second in Leone, Spain.
2. ***Mucorcircinelloides***: Wild strains of *M. circinelloides*, when exposed to the light impulses of blue light, get activated due to significant change in structural genes of β –Carotene, hence produce it to high level[15].
3. ***Phycomyces blakesleeanus***: *Phycomyces* is mainly used for the production of various chemicals like β –Carotene. They have enhanced carotenogenic potential when grown on solid substrates or in liquid media.

ArpinkRed[tm]Production
It is the red pigment produced by the strain *Penicilliumoxalicum* obtained from the soil. It contains chromophore of anthraquinone type[14] The amounts of red pigment Arpink Red in various food products was amount recommended by Codex AlimentariusCommmision (Rotterdam meeting, March 11-15, 2002).(Table.1)

Riboflavin (Vitamin B_2) 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. Its structure is given in following Figure (Fig. 3).There are many microorganisms having potential to produce riboflavin by fermentation. Riboflavin fermentation can be categorized into three types: weak overproducer, moderate overproducer and strong overproducer. (Table 2)[16-18]
Fermentation with *Ashbyagossypi* is preferred because of higher yield and greater genetic stability; riboflavin levels of over 15g/L have been reported.

Monascus pigments production
*Monascus*spp. belongs to the group of Ascomycetes and particularly to the family of Monascaceae. The genus *Monascus* can be divided into four species: *M. pilosus, M.purpureus, M.*

*ruber*and *M. froridanus*, 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 (Table 3). *M. purpureus*can be easily distinguished by its ascospores which appeared to be spherical in shape of 5 microns in diameter or slightly ovoid (6×5 microns). *Monascus* fungi, organisms produce angkak can convert starchy substrates into several metabolites such as alcohols, antibiotic agents, antihypertensives, enzymes, fatty acids, flavor compounds, flocculants, ketones, organic acids, pigments and vitamins. Thus, the implementation of *Monascus* pigment as a coloring agent in food provided an additional advantage of specific flavor in the products.

Generally, pigment production in industrial scale has been carried out using submerged fermentation (SmF). However, solid-state fermentation (SSF) systems appear to be promising due to the natural potential and many advantages.

Carbon and nitrogen sources are nutritional sources required for mycelial growth. Glucose is considered as the best carbon source for production of pigment[19]. The nitrogen source such as ammonium and peptone provide good growth and higher pigment concentration. *Monascus* growth and ankaflavin synthesis were favored at low pH (pH 4.0) whereas production of other pigments was relatively independent of pH[20].

For production of Monascus pigments from *Monascusruber*solid state fermentation is used. The initial moisture level and aeration rate affects the level of colour production. Highest levels of pigments of about ≥ 98 AU per g dry matter in the bed, were obtained at forced aeration rates of between 0.05 and 0.2 L min-1. An initial moisture level of 70% gave the highest pigment production in the fermented dry solids and also the highest pigment productivity, which were 1,415 AU per g dry matter and 101 AU per g dry matter per day, respectively[21].

Lycopene Production

It is a red open-chain unsaturated carotenoid, acyclic isomer of beta-carotene, and longer than any other carotenoid (Fig 4). 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 *Fusariumsporotrichioides*was used by Jones *et al* to manufacture the colourant andantioxidant lycopene. They used the cheap corn fiber material as the substrate.Cultures in lab flasks produced 0.5 mg (lycopene)/g ofdry mass within 6 days and such a production will be increased within the next years.

In parmaceutical 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, antiploriferative 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[22-24], decrease and modulate immune response insult[25]. 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[26] is produced by *Vibrio psychroerythrus*[27], *S. marcescens*, *Pseudomonas magnesiorubra*, and other eubacteria[28]. 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[29, 31]. *Streptomyces* or*Serrarita* are also used for its production. It shows immunosuppressing activity[32] and also exerts antiploriferative and cytotoxic effects on 60 cell lines of human tumor cells (derived from lung colon liver ovarian brain cancers, melanoma and leukemia)[33]. Prodigiosin was also reported as active component for prevention and treatment of diabetes mellitus[34].

3) **Violacein:**The violet pigment violacein is an indole derivative, isolated mainly from bacteria *Chromobacteriumviolaceum*, which exhibits important antitumoural, antiparasitary, antiprotozoan[35], anticancer[36, 37], antiviral[38], antibacterial[39, 40] and antioxidant activities[41].
4) **Red yeast rice(RYR):** Red Yeast Rice (RYR) is a fermented rice product produced traditionally by fermenting cooked rice kernels with yeast *Monascusspp(Monascusrubur, Monascuspurpureus*, *Monascusruber*and *Monascuspilosus*). These *Monascus spp*. have an important characteristic to produce secondary metabolites of polyketide structure and yellow, orange and red pigments.*Monascusruber*was used for production of angkak, a fermented rice product with anti cholesterol activity. RYR proved to contain many active constituents such as compounds resembling statins in its structure, unsaturated fatty acid, sterols and B-complex vitamins[42]. Various studies also reported that RYR and statins decreases blood glucoselevels in diabetes[43].

In Textile industry

The use of dyes for coloring the garments of cloths was known during Indus civilization. Textile industries use large amount of pigments mostly the synthetic ones. Synthetic dyes are widely available at an economical price and produce a wide variety of colours but they cause skin allergy and other harmfulness to human body, produces toxicity/chemical hazards during its synthesis, releases undesirable/hazardous/toxic chemicals etc. Due to these hazardous effects of synthetic pigments, there is more market demand and customer interest toward natural or microbial pigments(known as natural dying). Use of natural pigments in the textile industry is eco-friendly and non-carcinogenic. For successful commercial use of natural dyes for any particular fiber, the appropriate and standardized techniques for dyeing for that particular fiber natural dye system need to be adopted. Therefore to obtain newer shade with acceptable colour fastness behaviour and reproducible colour yield, appropriate scientific dyeing techniques/procedures are to be derived. Some of details of application of pigments in textile industry is given in Table 4.

DISCUSSION AND CONCLUSION

The success of any pigment produced by fermentation depends upon its acceptability in the market, regulatory approval, and the size of the capital investment required to bring the product to market. Microbial pigments are not only used as food colorant, flavoring agent and dying agents they are widely applied in medicinal aspects. Apart from food and textile coloring they have been used in clinical therapy to lower the blood cholesterol concentration, Anti-Diabetic Activity, Anti-Inflammation etc. A few years ago, some expressed doubts about the successful commercialization of fermentation-derived food grade pigments because of the high capital investment requirements for fermentation facilities and the extensive and lengthy toxicity studies required by regulatory agencies. Public perception of biotechnology-derived products should also be taken into account. Nowadays some fermentative food grade pigments are in the market and also the algae-derived or vegetable-extracted pigments are successful marketed.

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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

Table 2: Different level of Riboflavin production from microbes

S.No.	Producer microorganism	Amount produced	Examples
1.	Weak overproducer	100 mg/L or less	*Clostridium acetobutylicum*
2.	Moderate overproducer	Upto 600mg/L	*Candidaguilliermundii*or *Debaryomycessubglobosus*
3.	Strong overproducer	Over 1g/L	*Eremotheciumashbyii*and *Ashbyagossypi*

Table 3: Pigments obtained from *Monascus spp.*

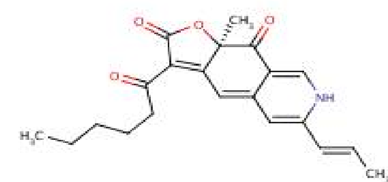
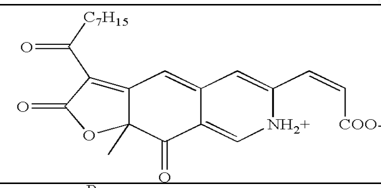
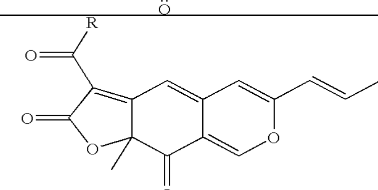
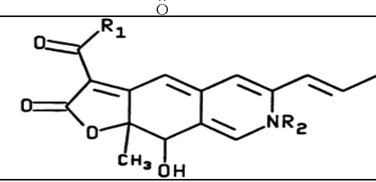
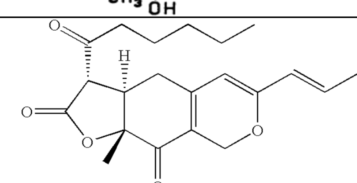
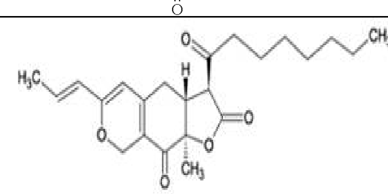
S. No.	Colorants	Name of pigments	Chemical Formula	Chemical structure
1.	Red colorants	Rubropunctamine	$C_{21}H_{23}NO_4$	
		monascorubramine	$C_{23}H_{27}NO_4$	
2.	Orange Colorants	rubropunctatin	$C_{21}H_{22}O_5$	
		monascorubrin	$C_{23}H_{26}O_5$	
3.	Yellowish colorant	Monascin	$C_{21}H_{26}O_5$	
		Ankaflavin	$C_{23}H_{30}O_5$	

Table 4: Sources and uses of dyes in textile industry

S. No.	Name of dye	Source	Application
1.	Prodigiosin[44]	*Vibrio spp.*	Wool, nylon, acrylics and silk
2.	Red Prodigiosin[45]	*Serratiamarcescens*	Acrylics, polyester, polyester microfiber, silk and cotton
3.	Pigments from	*Janthinobacteriumlividum*	Silk, cotton and wool (bluish-purple, all natural fibers) and nylon and vinylon (both synthetic fibers)
4.	Violacein (violet pigment)[46]	*Chromobacteriumviolaceum*	Pure cotton, pure silk, pure rayon, jacquard rayon, acrylic, cotton, silk stain and polyster
5.	Pink pigment	*Roseomonasfauriae*	Cotton fabrics
6	Pigments from	*Fusariumoxosporum, Trichoderma viride*and *Alternaria spp.*	Cellulosic fibers
7.	Anthraquinone	*Dermocybe sanguine.*	Wool fibers.
8.	Pigment from	*Trichoderma spp.*	Wool and silk fibers.

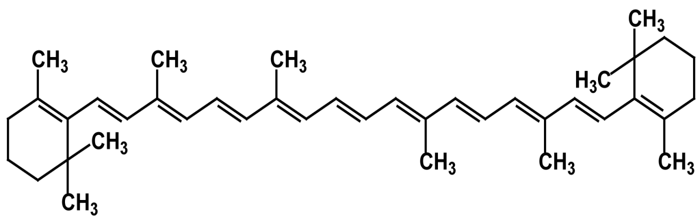


Fig. 1: Structure of β –Carotene

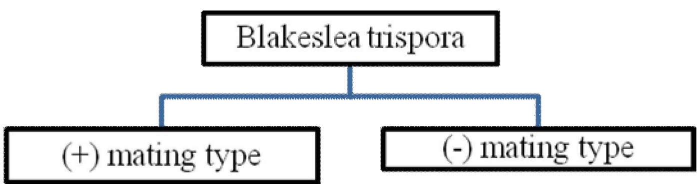


Fig. 2: Strains of *Blakesleatrispora* used for production of β-carotene

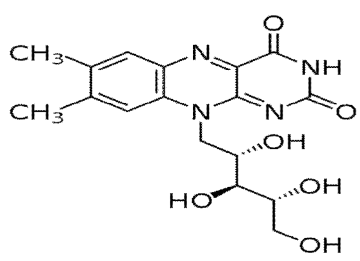


Fig. 3: Structure of Riboflavin

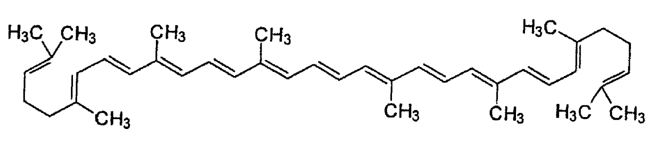


Fig. 4: Structure of Lycopene

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