



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ISOLATION AND PURIFICATION OF THE PIGMENTS FROM THE NATURAL SOURCES: A REVIEW

SHRIVASTAV A* AND SAKHIYA N

Department of Microbiology, Parul Institute of Applied Sciences, Parul University, Vadodara,
Gujarat, India

*Corresponding Author: Dr. Anupama S: E Mail Id: anupama.shrivastav82045@paruluniversity.ac.in

Received 22nd March 2021; Revised 26th April 2021; Accepted 3rd June 2021; Available online 1st Feb. 2022

<https://doi.org/10.31032/IJBPAS/2022/11.2.5879>

ABSTRACT

Colors are the most valuable attribute of any article. It is widely use in many of industries like food, textile, cosmetic, agriculture and pharmaceutical. However, toxicity caused by synthetic pigments have triggered intense search in natural colors and dye. Natural pigments are not only have the capacity to increase the market rate of the product and also display the advantageous biological activities as antioxidants and anticancer agents , additives, color intensifiers, and as well as economically friendly. Some of the Bacteria capable of producing pigment with different varieties of colors are *Agrobacterium aurantiacum*, *Staphylococcus aureus*, *Serratia marcescens*, *Chromobacterium violaceum*, *Flavobacterium sp*, *Bacillus Spp*, etc. colors are Pink-red, Purple, red, Golden Yellow, Creamy and yellow respectively. Industrial production of natural food colorants by microbial fermentation have several advantages such as production, higher yields through strain improvement, easier extraction, no lack of raw materials and no seasonal.

Keywords: Bacterial pigments, antioxidants, microorganism, non-toxic, antimicrobial activities, biocolorants

1. INTRODUCTION

Colors provide attracting appearance to marketable products such as food, textiles, and pharmaceutical products [1]. Colors are the beauty of world that means we cannot imagine world without colors. Pigments are natural sources have been obtained since long time ago, also their interest has increased due to the toxicity problems caused by the synthetic pigments. In this way the pigments from microbial sources and that are good alternative [2-5]. There are many artificial synthetic colorants, which have been widely used in foodstuff, dyestuff, cosmetic and also pharmaceutical manufacturing processes, comprise various hazardous effects. There are so many limitations of synthetic pigments [6-8]. The precursors, used in the production process of synthetic pigment has many carcinogenic hazardous effects on the workers. Also, the wastes of the production process are harmful. They are 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 [9-13]. The production of pigment 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 and independent from weather conditions. Microbial colors are available in different shades. These colors are biodegradable and environment friendly [14-18]. They also have numerous clinical characteristics like anticancer, antioxidant, 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 different industrial applications. An ideal pigment producing microorganism should be capable of using a wide range of Carbon and Nitrogen 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 Research & Development stage. So that 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 [19-24].

Furthermore, 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 with compared to the scaling up methods. Microbes having also more versatility and productivity over higher forms of life in the industrial-scale production of natural pigments and dyes.

2. 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 different 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 [13]. Microbial colors are in use in the fish industry already. Like to enhance pink color of farmed salmon. Further, some natural colorants has commercial potential for use as antioxidants. The industry is now able to produce some microbial pigments for applications in cosmetics, food or textiles. In nature, color

rich and pigment producing microorganisms (yeasts, fungi and bacteria) are common. Microorganisms produce various pigments like flavins, carotenoids, melanins, quinones, prodigiosins and more specifically monascins, violacein or indigo. It self non-environment friendly and non-biodegradable [7]. To counter these harmful effects of synthetic colorants, there is worldwide interest in process development for the production of pigments from natural sources. The utilization of natural pigments in dyestuff, foodstuff, cosmetic and pharmaceutical manufacturing processes has been increasing in recent years because of harmful effects of synthetic pigments and their industrial by-products on humans and the environment. Microorganisms and plants and are the two major sources of natural pigments. Then also 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 produced³ and the availability of cultivation technology [25].

3. List of pigment producing bacteria (Table 1)

Table 1: Pigment Producing Bacteria

Microorganism	Pigment	Color
<i>Monascus sp</i>	Ankaflavin	Yellow
<i>Blakeslea trisporia</i>	β Carotene	Yellow-orange
<i>Ashbya gossypi</i>	Riboflavin	Yellow
<i>Paracoccus zeaxanthinifaciens</i>	Zeaxanthin	Yellow
<i>Fusarium sporotrichioides</i>	β Carotene	Yellow-orange
<i>Neurospora crassa</i>	β Carotene	Yellow-orange
<i>Phycomyces blaksleeanus</i>	β Carotene	Yellow-orange
<i>Fusarium sporotrichioides</i>	Lycopene	Red
<i>Agrobacterium aurantiacum</i>	Astaxanthin	Pink-red
<i>Paracoccus carotinifaciens</i>	Astaxanthin	Pink-red
<i>Monascus sp.</i>	Rubropanctatin	Orange
<i>Rhodotorula sp.</i>	Torularhodin	Orange-red
<i>Paecilomyces sinclairii</i>	Unknown	Red
<i>Penicillium purpurogenum</i>	Unknown	Red
<i>Xanthophyllomyces dendrohous</i>	Astaxanthin	Pink-red
<i>Mucor circinelloides</i>	β Carotene	Yellow-orange
<i>Blakeslea trisporia</i>	Lycopene	Red
<i>Streptomyces echinoruber</i>	Rubrolone	Red
<i>Cardyiceps unilateralis</i>	Napthoquinone	Deep blood red
<i>Pencillium candidum</i>	Anthroquinone	Red
<i>Saccharomyces neoformis</i>	Melanin	Black
<i>Monascorubramine</i>	Monascus sp.	Red
<i>Ashbya gossypi</i>	Riboflavin	Yellow
<i>Monascus sp.</i>	Rubropanctatin	Orange

In 2011, global sales of natural colours amounted to an estimated 600 million, up by amount 29% from 2007, depicting annual growth excess of 7%. Varieties of biopigments have been produced such as prodigiosin, violancein, carotenoids, malanins, flavins, quinins, monascins. Colours provide attracting appearance to marketable products such as textiles, food products, beauty products and pharmaceutical products. Bacteria produce two types of pigments:

1. Those that predominantly remain bound to the bacterial mycelia.
2. Those that are secreted into the fermentation broth.

4. Extraction:

Purification of bacterial pigments in relatively pure and concentrated forms is the main technological challenge. Various pigments from the former class can be conventionally recovered by disrupting the filtered mycelia with acetone; secreted natural products are typically recovered by extracting the aqueous broth with large quantities of organic solvents like ethyl acetate. Pigment produced by bacteria can be separated using solvent extraction and further characterize using various instrumental based analytical techniques such as TLC, UV visible, FTIR, ESI-MS, NMR, HPLC and GPC. Most of the natural pigments are

extracted from plants and animals like tomato, grapes, beet and peprika. To mitigate environmental and health concerns associated with solvent used, alternative separation technologies such as spray drying (wide spread in the food and feed industry) and solid phase extraction (common place in the fine chemical industry) may be appropriate.

Acceptability of colour in given food in turn is influenced by cultural, geographical and sociological aspect of population. Indeed colour and other eating habits are viewed as a type of anthropology, culinary and indigenous to specific region. However, certain food groups are acceptable only if they fall within certain range of colour gamuts and their acceptability are reinforced by their economical worth judge purely on the colour value. A well textured food, rich in nutrient and flavors can be eaten unless it has the right colour. Due to the consumer awareness and concern for healthful and perfectly balanced food and increasing interest in food colorants of natural origin has been developed. The consumer wants to see the food looking natural as the synthetic colour have been proved to be carcinogenic to humans [9].

4.1 Advantage of microbial pigments:

- (1) Easy and fast growth in cheap culture medium and faster fermentation for bulk production.
- (2) Independence from weather condition and availability of cultivation technology.
- (3) Microorganisms are known as a potential for biopigments production due to their advantage over plants in terms of availability, stability, cost efficiency, labor, yield and easy downstream processes.

4.2 Disadvantage of microbial pigments:

- (1) Responsible for rotting of food.
- (2) They spread disease.
- (3) They are sometimes responsible for whooping of plants.
- (4) Responsible for tooth decay.

5. Application of the pigments:

- **Pharmaceutical industry:**
Investigation of most of the microorganisms has shown the efficiency in clinical applications of secondary metabolites (pigmented) for treating various diseases. These have many properties like antibiotic, anticancer and immune-suppressive properties. The property of bacteria to produce biopigments is used to produce medically principle products. *Adonirubin* and *astaxanthin* are the xanthophylls, which is also act as nutraceuticals. These xanthophylls by

the process of antioxidation, anti free radical or other mechanisms help to prevent carcinogenesis. The nutraceuticals functions of these xanthophylls and carotenes also help to prevent problems like heart attacks and strokes. A red pigment, astaxanthin is important carotenoids which has great commercial value and is used as pharmaceuticals feed. Furthermore, the *Monascus purpureus* produce pigments which help in the inhibition of hepatitis virus replication by interfering with viral RNA polymerase activity. A strong therapeutics molecules prodigiosins are known for their immune suppressive anticancer properties. *Hahella chejuensis* produces a pigment which is also known to have immune suppressant and antitumor properties [6].

- **Dairy industry:** *Monascus species* are known to produce nontoxic pigments, which can be used as food colorants, flavour enhancers and as a food preservative. *Monascus ruber* is used to prepare flavoured milk by utilizing rice carbohydrate for its metabolism and produces pigment as a secondary metabolite. The solid

state fermentation of rice produces red, orange and yellow pigments.

- **Textile industry:** The textile produces large amount of waste which mainly consist of synthetic dyes. These synthetic dyes are used in industries, due to their easy and cheap synthesis, temperature, stability towards light and advanced colors covering whole color spectrum. But, these synthetic dyes have many drawbacks like mutagenicity, toxicity and carcinogenicity properties leading to various health problems like skin cancer and allergies. Hence, Customers demand for dyes of natural origin like colorants.
- **Printing industry:** To conserve the forest resources and reduction of wastes it's important to reuse and recycle the papers in offices etc. The reuse of papers is important but it is also important to remove the prints from paper. Decolorize ink contains *Monascus* pigments used for inkjet printing. These pigments from *Monascus* when exposed to irradiation of visible or ultraviolet light get discolored.
- **Food colorants:** An important goal of food industry is to produce food

with an attractive appearance. Food producers are opting for natural food colors as artificial shows many negative impacts on health when used. Demand for natural food colorants are more than availability in food industry. Many natural colors are available, in which microbial colorants play important role as food coloring agent as its production and down streaming process is easy [12].

- **Nutritional supplements:** Chemical compounds in the biocolor are produced by plant cells known as vegetal active principles. These compounds are the means for obtaining biological active substances and many other natural compounds which are used in pharmaceuticals, food and cosmetics industries with having important commercial value. As β -carotene is the precursor of Vitamin A, so carotenoids can be used as Vitamins supplements. Rice is the most consumed food in under developed countries, so there is possibility of deficiency of vitamin A which causes night blindness and in serious cases to xerophthalmia. Another example of natural food grade biocolorant is Riboflavin which

is also a source of vitamins available in several leafy vegetables, milk, meat and fish. Yellow β -xanthins are used as biocolorants and can also be used as biocolorant and for introducing essential dietary amino acids into food stuffs.

5.1 Purification of pigments:

UV-Visible absorption spectrophotometer Spectral analysis can be made on a UV-Visible spectrophotometer and the extract can be scanned in the range of 400 to 800 nm to find out the maximum absorption spectra. Methanol and chloroform can be used as a blank. The method of separation are selected according to the physical and chemical properties of the biocolorants to be separated among the commonly used methods of separation in the separation of mixtures of bio colorants, may be mentioned. Purification is a process of separating the component substances from a liquid mixture by selective evaporation and condensation. Pigments were purified by various techniques like drying, evaporation, boiling etc.

5.2 Antimicrobial activity of the pigment:

The extracted pigment can be tested for antibacterial activity by disc diffusion method. Sterile Whatman's filter paper discs can be soaked in the extracted pigment and placed on sterile nutrient agar plates swabbed with 24 hour old culture of Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) and Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*). The plates can be incubated at 37°C for 24 hrs. After incubation period, the plates can be checked for zones of inhibition. The presence of inhibition zone around the discs soaked with pigment indicates antimicrobial activity.

5.3 Application of bacterial pigments:

- A. To cloth dying
- B. Making candles
- C. To make soap
- D. Used in beauty product industries
- E. Used in pharmaceutical verient products
- F. Many more used in textile industries

6. CONCLUSION

Thus there is an urgent need for the alternative colorants that are natural, cost effective and easily degradable and without production of recalcitrant intermediates when they are enter to ecosystem. And also there is an developing interest towards involving microorganisms as a possible alternate source of colorant used in food, pharma, and many other industries. In this direction, biotechnology may play a crucial role for large fermentation of Natural biocolorants. So overall the possibility of pigments production from different sources like microbial culture, vegetables, fruits, and other substrates. They are use to enhance the capability, and stability of the pigment which is beneficial to the mankind and livestock.

REFERENCES

- [1] Attalla, Mabrouk. M, Youssef and Mohamed, 2011. Production of textile reddish brown dyes by fungi. Malaysian journal of Microbiology, Vol: 7: 33-40.
- [2] Bhat SV, Khan SS, Amin T, 2013, Isolation and characterization of pigments producing bacteria from

- various foods for their possible use as biocolors, *International Journal of Recent Scientific Research*. Vol: 4(10): 1605-1609.
- [3] Bhaswati Goswami and Jayati Bhowal, 2014, Identification and characterization of extracellular red pigment producing bacteria isolated from soil, *International journal of current microbiology and applied sciences*, Vol:3(9): 169-176. ISSN: 2319-7706 Volume 3 Number 9 (2014) pp. 169-176 <http://www.ijemas.com>
- [4] Venil, Chidambaram Kulandaisamy, *et al.* "Bacterial Pigments and Their Applications." *Process Biochemistry*, no. 7, Elsevier BV, July 2013, pp. 1065–79. *Crossref*, doi:10.1016/j.procbio.2013.06.006.
- [5] Gargi Goswami, *et al.* "Effect of Ph and Temperature on Pigment Production from an Isolated Bacterium." *Chemical Engineering Transactions*, AIDIC: Italian Association of Chemical Engineering, 0 2010, pp. 127–32, doi:10.3303/CET1020022.
- [6] Goswami G, Chaudhuri S, Dutta D, 2010, Effect of pH and temperature on pigment production from an isolate bacterium, *Chemical Engineering Transactions*, Vol: 20: 127-132. <https://nopr.niscair.res.in/handle/123456789/11334>.
- [7] Joshi V.K, Devender Attri, Anju Bala and Shashi Bhushan, 2003. *Microbial Pigments*, *Indian Journal of Biotechnology*, Vol: 2, pp 362-369. -- -. *Current Research. Crossref*, doi:10.24327/ijrsr. Accessed 12 Feb. 2021.
- [8] Johnson EA, Schroeder WA. *Microbial carotenoids*, 1995, *Advances in Biochemical Engineering/ Biotechnolog*, Vol: 11: 297-326.
- [9] Kamla Malik, Jayanti Tokkas and Sneh Goyal, 2012. *Microbial Pigments*. *International Journal of Microbial Resource Technology*, pp 361-365.
- [10] "Carotenoid Actions and Their Relation to Health and Disease." *Molecular Aspects of Medicine*, no. 6, Elsevier BV, Dec. 2005, pp. 459–516. *Crossref*, doi:10.1016/j.mam.2005.10.001.
- [11] Laurent dufosse, 2006. *Microbial production of food grade pigments*. *Food Technol. Biotechnol* -

- University of Zagreb, 2019, pp. 249–59. *Crossref*, doi:10.17113/ftb.57.02.19.5980.
- [12] Madhura Nerurkar, Jyoti Vaidyanathan, Ravindra Adivarekara, Zarine Bhatena Langdana, 2013. Use of a natural dye from *serratia marcescens* subspecies *marcescens* in dyeing of textile fabrics, Vol: 1:129-135. www.sciencebeingjournal.com
- [13] Mehta, Mansi, and Gaurav Shah. “Extraction of Pigment From *Serratia Marcescens* and Its Application in Candle Industry.” *Advances in Applied Research*, no. 2, Diva Enterprises Private Limited, 2015, p. 144. *Crossref*, doi:10.5958/2349-2104.2015.00027.3.
- [14] Oren A, 2002, Diversity of halophilic microorganisms Environments, Phylogeny, Physiology, and application. *Journal of Industrial Microbiology and Biotechnology*, Vol: 28:56-63. <https://doi.org/10.1038/sj/jim/7000176>
- [15] Parekh S, Vinci V, Strobel RJ, 2005. Improvement of microbial strains and fermentation processes. Application of Microbial Biotechnology, pp 287–301.
- [16] Pritam Chattopadhyay, Sandipan Chatterjee et al; 2008, Biotechnological potential of natural food grade biocolorants, *African journal of biotechnology*, Vol 7(17):2972-2985.
- [17] Poorniammal, Parthiban, Gunasekaran, Murugesan & Thilagavathi, 2013, Natural dye production from *Thermomyces* sp. fungi for textile application. *Indian journal of Fibre & Textaile Research*, Vol: 38:276-279.
- [18] Robert PW, James AG, Donald AR, 1955, Studies on pigmentation of *Serratia marcescens*, *National academic of science*, Vol:71(1):012.
- [19] Sandmann G.1997, Carotenoid biosynthesis in microorganisms and plants, *European Journal of Biochemistry*, Vol: 223:7-24.
- [20] Suman vikas bhat, Sahara sayeed khan, Tawheed Amin, 2013. Isolation and characterization of pigment producing bacteria from various foods for their possible use of biocolors. *International Journal of Recent Scientific Research*, Vol: 3:1605-1609.

-
- [21] S.Samyuktha, Sayali Nephade, 2016, Isolation and identification of pigment producing bacteria and characterization of extracted pigments, International journal of applied research, Vol: 2(7):657-664.
- [22] Sasidharan P, Raja R, Karthik C, Ranandkumar Sharma, Indra Arulselvi P, 2014, Isolation and characterization of yellow pigment producing *Exiguobacterium* sps, Journal of Biochemical Technology. Vol:4:632-635.
- [23] Scolnik PA, Bartley GE, 1995, Nucleotide sequence of a putative geranylgeranyl pyrophosphate synthase from *Arabidopsis*, Plant Physiology, Vol: 104:1469-1470.
- [24] Sharma D, 2014, Understanding Biocolor- A Review, International Journal of Scientific and Technology, Vol: 3(1):295-299.
- [25] Sun J, Kim J, Kim G, Rhee K, Jung H, Jeun J *et al.* 2011, Inhibition of hepatitis C virus replication by *Monascus* pigment derivatives that interfere with viral RNA polymerase activity and the mevalonate biosynthesis pathway, Journal of Antimicrobial Chemotherapy, Vol: 10: 1093.
-