The Realm of Microbial Pigments in the Food Color Market

Babita Rana¹, Malini Bhattacharyya², Babita Patni², Mamta Arya¹ and Gopal K. Joshi¹*

¹ Department of Biotechnology, Hemvati Nandan Bahuguna Garhwal University, Srinagar, India, ² High Altitude Plant Physiology Research Centre, Hemvati Nandan Bahuguna Garhwal University, Srinagar, India

Colors are added to food items to make them more attractive and appealing. Food colorants therefore, have an impressive market due to the requirements of food industries. A variety of synthetic coloring agents approved as food additives are available and being used in different types of food prepared or manufactured worldwide. However, there is a growing concern that the use of synthetic colors may exert a negative impact on human health and environment in the long run. The natural pigments obtained from animals, plants, and microorganisms are a promising alternative to synthetic food colorants. Compared to animal and plant sources, microorganisms offer many advantages such as no seasonal impact on the quality and quantity of the pigment, ease of handling and genetic manipulation, amenability to large scale production with little or no impact on biodiversity etc. Among the microorganisms algae, fungi and bacteria are being used to produce pigments as food colorants. This review describes the types of microbial food pigments in use, their benefits, production strategies, and associated challenges.

Keywords: microbial pigment, food, fermentation, bio-colorant, market

INTRODUCTION

The world cannot be imagined without colors and this is equally true for the food that we eat. Some food materials such as fruits, vegetables etc. have striking natural shades and hues and therefore, do not require any further coloration. However, for many food items addition of food colorants is an integral part of their recipe before final packaging or serving. Food colorants enhance the visual appeal and grant unique identity to the food so that it could look more attractive and seem enjoyable to eat. Many times, food color is also associated with the flavor, safety and nutritional value (Sigurdson et al., 2017). The market of food colorants was estimated to be USD 3.88 billion in 2018 and it is estimated to reach USD 5.12 billion by 2023 with a compound annual growth rate (CAGR) of 5.7% [Food Colors Market by Type (Natural, Synthetic, Nature-Identical), Application (Beverages, Processed Food, Bakery & Confectionery Products, Oils & Fats, Dairy Products, Meat, Poultry, Seafood), Form, Solubility, and Region—Global Forecast to 2023]. Food coloring has been known to be in practice as early as 1,500 BC (Burrows, 2009). Earlier, all the colorants used were of natural origin such as saffron, paprika, turmeric, various flowers etc. (Burrows, 2009). In the midst of nineteenth century, synthetic colors were started to be produced and owing to their low production cost, high tinctorial strength, and chemical stability they became popular as food colorants (Sigurdson et al., 2017). However, in later years several health issues were realized with the
use of many potentially hazardous synthetic chemicals as food colorants which led to the banning of various such food color additives e.g., Quinoline Yellow, Yellow 2G, Ponceau SX, Brilliant Black B etc.². At present, although strict regulations are in practice in different countries toward approval of a synthetic colorant for intended use as food additive, people with the growing awareness about personal health and environment are now more inclined toward their substitute obtained from natural sources. However, there is still a substantial share of synthetic colors in the market of food colorants. Mono- and di-azo dyes are the most commonly used synthetic food colorants approved by FDA and EU. The other approved food grade colorants include Triarylmethane derivatives, xanthenes dyes, quinophthalones, and indigoid compounds (Corradini, 2018).

Natural pigments, the colored compounds synthesized by plants, animal, microorganisms or derived from mineral ores are a promising alternative to the synthetic food colorants (Corradini, 2018). Titanium dioxide (E171), calcium carbonate (E170), iron oxides (E172) are some examples of mineral pigments approved as food colorants by FDA³. Although for similar shades the cost of synthetic colors is on lower side for most cases in comparison to natural colors but the mass production of natural colors may fill this gap. Unlike synthetic colorants, they have nutritional values and associated with cytotoxic, antioxidant, antimicrobial, antimalarial, anticancer, antitumor, and antifouling activities (Ramesh et al., 2019) (Figure 1). Not only the natural colorants/pigments but their identical compounds made by chemical processes are also exempted from the certification process before use as food additive (Sen et al., 2019). Although plants are a major source of natural pigments, pigments obtained from microbial sources offer special advantages. Compared to plants, microbial pigments are more stable, cost-effective, unaffected by seasonal variations, amenable to yield improvement, and smoothly extractable (Nigam and Luke, 2016). Also, the excessive use of microbial culture for pigment production is not likely to harm the biodiversity and environment. Currently, a variety of different food color additives produced through fermentative processes are available in the market. Monascus pigments, Astaxanthin from Xanthophyllomyces dendrorhous, Arpink Red from Penicillium oxalicum, Riboflavin from Ashbya gossypii, and carotene from Blakeslea trisporatrispora (Venil et al., 2013) are the examples of some microbial food grade pigments.

Pigment production is one of the strategies of bacteria to escape from adverse effect of UV radiations. The photoprotective pigments help bacteria to cope up with prolonged exposure to UV radiation (Wynn-Williams et al., 2002). Some of these pigments are also well-known for their ability to provide protection against oxidative damage which helps in stimulation of immune response and cancer inhibition (Krisnky and Johnson, 2005). Symbiotic pigmented bacteria are known to protect their host from other pathogens (Egan et al., 2002). Fungi also produce pigments as a protection strategy against abiotic stresses like UV radiation and desiccation (Issac, 1994). Endophytic fungi have been reported to protect the host plant from insects or other microorganisms by producing the pigment Anthraquinones (Gessler et al., 2013). In microalgae, pigments are known to play light harvesting, photo protective and structural roles and they are also involved in carbon and energy storage (Mulders et al., 2014). Microbial pigments are thus more than simple coloring compounds because of the associated biological activities that can be of potential human benefit in case of their use as food additives. This article comprises the up to date details about the types, advantages and challenges related to the production and use of microbial food pigments.

### TYPES OF MICROBIAL PIGMENTS AS FOOD COLORANTS

Among microorganisms, fungi, bacteria, and microalgae are well-known to produce a range of natural pigmented substances having marked variation in chemical compositions, function, stability and solubility. These naturally occurring pigments are reflection of the secondary metabolites with great commercial value in food & dairy, cosmetics, pharmaceutical, textile, and dyeing industry (Narsing Rao et al., 2017). They belong to distinct categories based on their chemical composition, functional activities and natural occurrence such as derivatives of flavonoids, pyrroles, carotenoids, etc. Riboflavin, Beta-carotene, Canthaxanthin, Prodigiosin, Phycocyanin, Melanin, Violacein, Astaxanthin, and Lycopene are the major pigments reported from microbial sources having application as food colorants (Sen et al., 2019). The important natural food pigments reported to be produced by microorganisms and their advantages are discussed below and their details are also summarized in Tables 1, 2.

1. Riboflavin, also known as B2 vitamin, is a water soluble pigment of yellow color having applications as a dietary supplement and food additive in dairy products, sauces, baby foods, fruit, and energy juices. It helps body break the dietary polymeric compounds such as carbohydrates, proteins, and fats to generate energy and use oxygen. Microorganisms such as Candida guilliermondii, Debaryomyces subglobosus, Eremothecium ashbyii, Ashbya gossypii, Clostridium acetobutylicum have been reported to produce it (Unagul et al., 2005; Hong et al., 2008; Nigam and Luke, 2016; Dufossé, 2018).

2. Beta-carotene, a red-orange water insoluble organic pigment, is a very good source of vitamin A for human body that boosts immunity, prevents aging and helps in night vision issues (Eroglu et al., 2012). Several microorganisms such as Dunaliella salina, Blakeslea trispora, Mucor circinelloides, Phycymyes blakesleeanus, Rhodotorula glutinis, Rhodotorula gracilis, Rhodotorula rubra are known to produce it (Ruegg, 1984; Nigam and Luke, 2016; Sigurdsone et al., 2017; Dufossé, 2018). Some natural colorants belonging to carotenoid family obtained from Haematococcus pluvialis and Phaffia rhodozyma are extensively used as food additives for animals and fish as well as in pharmaceuticals and aquaculture fields (Stafsnes et al., 2010).

---

² [http://importedfoods.afdo.org/food-color-additives-banned-in-the-usa.html](http://importedfoods.afdo.org/food-color-additives-banned-in-the-usa.html)
³ [http://fic-solutions.com/food-coloring/mineral-pigments](http://fic-solutions.com/food-coloring/mineral-pigments)
### TABLE 1 | Structural details of important microbial pigments used as food colorants.

| Pigment      | Molecular formula | Chemical structure | References                                      |
|--------------|-------------------|--------------------|-------------------------------------------------|
| Canthaxanthin| C_{40}H_{52}O_{2}  | ![Canthaxanthin](#) | National Center for Biotechnology Information. PubChem Compound Summary for CID 5281227, Canthaxanthin¹. |
| Astaxanthin  | C_{40}H_{52}O_{4}  | ![Astaxanthin](#)  | National Center for Biotechnology Information. PubChem Compound Summary for CID 5281224, Astaxanthin². |
| Prodigiosin  | C_{20}H_{25}N_{3}O | ![Prodigiosin](#)  | National Center for Biotechnology Information. PubChem Compound Summary for CID 135455579³. |
| Phycocyanin  | C_{33}H_{38}N_{4}O_{6} | ![Phycocyanin](#) | Ramesh et al., 2019                             |

(Continued)
| Pigment   | Molecular formula | Chemical structure | References                                                                 |
|-----------|-------------------|--------------------|---------------------------------------------------------------------------|
| Violacein | C_{20}H_{13}N_{3}O_{3} | ![Violacein molecule](image) | National Center for Biotechnology Information. PubChem Compound Summary for CID 11053, Violacein⁴. |
| Riboflavin| C_{17}H_{20}N_{4}O_{6} | ![Riboflavin molecule](image) | National Center for Biotechnology Information. PubChem Compound Summary for CID 493570, Riboflavin⁵. |
| Beta-carotene | C_{40}H_{56} | ![Beta-carotene molecule](image) | National Center for Biotechnology Information. PubChem Compound Summary for CID 5280489, beta-Carotene⁶. |
| Melanin   | C_{18}H_{10}N_{2}O_{4} | ![Melanin molecule](image) | National Center for Biotechnology Information. PubChem Compound Summary for CID 6325610, Melanin⁷. |
TABLE 1 | Continued

| Pigment   | Molecular formula | Chemical structure | References |
|-----------|-------------------|--------------------|------------|
| Lycopene  | C_{40}H_{56}       | ![Lycopene Structure](image1) | National Center for Biotechnology Information. PubChem Compound Summary for CID 446925, Lycopene. |
| Ankafflin | C_{23}H_{30}O_{5}  | ![Ankafflin Structure](image2) | National Center for Biotechnology Information. PubChem Compound Summary for CID 15294091, Ankafflin. |
| Arprink Red | C_{22}H_{18}O_{6} | ![Arprink Red Structure](image3) | Ramesh et al., 2019 |

1. Canthaxanthin is orange to dark pink colored, keto-carotenoid and lipid soluble pigment. There are reports of its production by Bacteriochlorophyll containing microbes such as *Bradyrhizobium* sp. and *Halobacterium* sp. (Jaswir et al., 2011; Surai, 2012; Chuyen and Eun, 2017). The production of canthaxanthin from microalgae like *Nannochloropsis gaditana* (Millao and Uquiche, 2016) and *Chlorella zofingiensis* (Li et al., 2006) has also been reported. Canthaxanthins are effective antioxidants and inhibit the oxidation of lipids in liposomes (Woodall et al., 1997).
### TABLE 2 | Microbial sources, bioactivity and applications of natural pigments.

| Pigment       | Source organism                          | Bioactivities known          | Applications                                      | References                                      |
|---------------|------------------------------------------|------------------------------|--------------------------------------------------|------------------------------------------------|
| Canthaxanthin | • Bradyrhizobium Sepp, • Halobacterium sp. | • Antioxidant                | Food colorant, salmon food and poultry feed      | Jaswir et al., 2011; Surai, 2012; Chuyen and Eun, 2017; Hamidi et al., 2017 |
|               |                                          | • Anticancer                 |                                                   |                                                 |
| Astaxanthin   | • Halobacterium salinarium, • Agrobacterium aurantiacum • Paracoccus carotinifaciens • Yeast • Microalgae | • Antioxidant                | Animal and fish food and food colorants          | Dufossé, 2009, 2016; Guedes et al., 2011; Asker, 2017; Pogorzelska et al., 2018 |
|               |                                          | • Photoprotector             |                                                   |                                                 |
|               |                                          | • Anti-inflammatory          |                                                   |                                                 |
|               |                                          | • Antimicrobial              |                                                   |                                                 |
| Prodigiosin   | • Serratia marcescens • Pseudoalteromonas rubra | • Anticancer                | Coloring agents in yogurt, milk, and carbonated drinks | Nagpal et al., 2011; Namazkar and Ahmad, 2013; Kamble and Hiwarale, 2012 |
|               |                                          | • Antimetastatic activity    |                                                   |                                                 |
|               |                                          | • Immunosuppressant          |                                                   |                                                 |
|               |                                          | • Antimalarial               |                                                   |                                                 |
| Phycocyanin   | • Aphanizomenonflos-aquae • Spirulina sp. • Pseudomonas spp. | • Cytotoxicity               | Sweets and ice cream                             | Barsanti et al., 2008; Eriksen, 2008; Cuellar-Bermudez et al., 2015; Matz et al., 2004; Konzen et al., 2006; Durán et al., 2012; Dufossé, 2018 |
|               |                                          | • Apoptosis                  |                                                   |                                                 |
|               |                                          | • Anti-alzheimer Activity    |                                                   |                                                 |
|               |                                          | • Antioxidant                |                                                   |                                                 |
| Violacein     | • Chromobacterium violaceum • Janthinobacterium lividum • Pseudoalteromonas tunicata • Pseudoalteromonas spp. | • Anticancer                | Used in food, cosmetic, and textile industries  |                                                   |
|               |                                          | • Antioxidant                |                                                   |                                                 |
|               |                                          | • Antifungal                 |                                                   |                                                 |
|               |                                          | • Antiviral                  |                                                   |                                                 |
|               |                                          | • Anti-tuberculosis          |                                                   |                                                 |
|               |                                          | • Ant parasitic              |                                                   |                                                 |
|               |                                          | • Antiprotozoal              |                                                   |                                                 |
|               |                                          | • Anti-HIV                   |                                                   |                                                 |
|               |                                          | • Anti-malarial              |                                                   |                                                 |
| Riboflavin    | • Candida guilliermundii, • Debaryomyces subglobosus, • Eremothecium ashybi, • Ashbya gossypii, • Clostridium acetobutylicum | • Anticancer                | In food industry                                 | Powers, 2003; Unagui et al., 2005; Hong et al., 2008; Dufossé, 2018 |
|               |                                          | • Antioxidant                |                                                   |                                                 |
|               |                                          | • Protection against         |                                                   |                                                 |
|               |                                          | cardiovascular diseases      |                                                   |                                                 |
|               |                                          | • In vision                  |                                                   |                                                 |
| Beta-carotene | • Dunaliella salina, • Blakeslea trispora, • Muscor cincheloides, • Phycomycesblakes levanus, • Rhodotorula glutinis, • Rhodotorula gracilis, • Rhodotorula rubra | • Anticancer                | Food colorant, Vitamin A source                 | Ruegg, 1984; Terao, 1989; Kot et al., 2016; Sigurdson et al., 2017 |
|               |                                          | • Antioxidant                |                                                   |                                                 |
|               |                                          | • Suppression of cholesterol synthesis |                                                   |                                                 |
| Melanin       | • Saccharomices, • Neoforman           | • Antioxidant                | Eye glasses, Cosmetic creams and food items       | Vinarov et al., 2003; Valla et al., 2004; Surwase et al., 2013 |
| Lycopene      | • Lycolpersicon esculentum, • Fusarium sp. • Sporotrichioides • Blakesleatrispora | • Antioxidant                | Meat colorant                                     | Di Mascio et al., 1989; Giovannucci et al., 2002 |
| Arpink red    | • Penicillum oxalicum | ........ | Food colorant | Kumar et al., 2015 |
| Monascus pigments (ankafavine, monascine) | • M. pilosus, • M. purpureus • M. ruber • M. tironidas | • Antimicrobial | Food colorant | Joshi et al., 2003; Feng et al., 2016 |
|               |                                          | • Anticancer                 |                                                   |                                                 |
|               |                                          | • Anti-obesity activities    |                                                   |                                                 |

4. Prodigiosin, a red colored multipurpose pigment, is reported to be produced by *Serratia marcescens*, *Vibrio psychoerythrus*, *Rugamonas rubra*, *Streptoverticillium rubrireticuli*, and other eubacteria (Nagpal et al., 2011). It is used in yogurt, milk and carbonated drinks (Namazkar and Ahmad, 2013). Prodigiosin has been shown to have insecticidal, antifungal, antibacterial, anticancer, and anti-malarial activities (Kavitha et al., 2010; Kamble and Hiwarale, 2012).

5. Phycocyanin is a blue colored photosynthetic pigment produced by blue-green algae that contain chlorophyll A (Sen et al., 2019). It is water soluble and an accessory pigment to chlorophyll. It is found in *Aphanizomenon flos-aquae* and *Spirulina* sp. (Barsanti et al., 2008; Cuellar-Bermudez et al., 2015). It is used in sweets, ice creams and also as a dietary supplement rich in proteins. Pyocyanin also acts as bio-control agent that have anti-bacterial,
anti-fungal and anti-alzheimeric activity (Jayaseelan et al., 2014).

6. Melanin is a natural pigment which is known to be produced by a wide variety of microorganisms such as Colletotrichum lagenarium, Aspergillus fumigates Vibrio cholerae, Shewanella colwelliana, Alteromonas nigrificans (Soliev et al., 2011). This pigment is also present in animals and plants. Besides several other uses such as in eye glasses, cosmetic creams, pharmaceuticals, they are also added in food items (Sen et al., 2019). The pigment is also reported to be associated with anti-HIV activity (Surwase et al., 2013).

7. Violacein is a versatile purple colored pigment that possess numerous biological activities. Various bacteria like Chromobacterium violaceum, Pseudalteromonas, Calliononas, Janthinobacterium, Microbulbifer are known to produce this pigment (Choi et al., 2015). It is highly demanded at large scale in cosmetics, food, medicine and textiles (Dufossé, 2018). The pigment is associated with several useful bioactivity including antibacterial, anticancer, antiviral, enzyme modulation, anti-ulcerogenic, and anti-Leishmanial (Soliev et al., 2011).

8. Astaxanthin is a lipid soluble orange-red pigment present in yeast, microalgae, marine organisms, and in feather of some birds (Sen et al., 2019). It is also reported to be produced by various bacteria such as Halobacterium salinarum, Agrobacterium aurantium, Paracoccus carotinifaciens (Guedes et al., 2011; Asker, 2017; Zuluaa et al., 2017; Pogorzelska et al., 2018). It is associated with anti-aging and memory improving activities and used as coloring agent in animal and fish foods (Capelli and Cysewski, 2013).

9. Lycopene is an approved meat coloring agent in several countries. It is a water insoluble biopigment belonging to carotene. It is present in tomato and other red fruits and vegetables (Di Mascio et al., 1989; Giovannucci et al., 2002) and can also be chemically synthesized. However, microbial production of lycopene is comparatively more economical and sustainable and has been produced in microbial hosts like Blakeslea trispora, E. coli, and yeasts by genetic engineering methods (Chen et al., 2016).

10. Arprink red is a red colored extracellular metabolite of the anthraquinone class produced by Penicillium oxalicum. It is also suggested to have anticancer effects when used as food supplements (Sardaryan, 2002). It is used as food colorant in various food products in different amounts as recommended by Codex Alimentarius Commission (Kumar et al., 2015).

11. Monascus pigments are a group of fungal secondary metabolites called azaphilones produced by filamentous fungi belonging to the genus Monascus of Ascomycetes group (Chung et al., 2008). They are red (monascorubramine and rubropunctamine), yellow (ankaflavin and monascin) and orange (rubropunctatin and monascorubrine) colored pigments (Vendruscolo et al., 2013). These pigments are extracted from various species of this fungi i.e., M. pilosus, M. purpureus, M. ruberand, M. froridanus, etc. and being used as food colorants for many years in red wines, yogurt, sausages, hams, and meats (Dufossé et al., 2005). They are also known to exhibit antimicrobial, anticancer, anti-obesity, and antioxidant activities (Vendruscolo et al., 2013).

**FERMENTATION CONDITIONS FOR MICROBIAL PIGMENT PRODUCTION**

Microbial pigments intended to be used as food additive or colorants are being commercially obtained from bacteria, fungi, and algae (Nigam and Luke, 2016). For industrial production of pigments the desired microorganism should possess properties like acceptability of wide range of carbon and nitrogen sources and tolerance of process pH and temperature (Kirti et al., 2014; Kumar et al., 2015). In addition, the yield should be sufficiently high enough to make it a cost-effective affair. The microorganisms capable to produce promising pigments can be isolated and screened through bioprospecting programs in different environment. Alternatively, the known pigment producing microorganisms can be subjected to strain improvement techniques for the desired yield and properties. A combination of the aforesaid two approaches can also be applied. Various factors mainly the type of fermentation, media components (carbon, nitrogen sources, and minerals), pH, temperature, time of incubation, moisture content and aeration rate (Figure 2) affect the growth and yield of pigments through microbial fermentation. Solid state and submerged fermentation approaches are used for the production of microbial pigments (Tuli et al., 2015). Solid state fermentation offers the advantages of higher yield and productivity as well as the direct applicability of the fermented product as a colorant without isolating the product (Babitha, 2009). SSF is particularly suitable for the growth of fungi and the use of this technique also leads to savings in wastewater and yield of the metabolites (Tuli et al., 2015). The optimum value of various factors affecting the fermentation conditions vary with the microorganism used for pigment production. For instance, optimum temperature range for pigment production by Monascus spp. is 25–28°C whereas Pseudomonas sp. prefers the temperature of 35–36°C (Kumar et al., 2015). The maximum growth and production of carotenoid from Sarcina sp after an incubation period of 72 h was reported by Joshi et al. (2011). The same incubation period has been found as the optimum for pigment production by Rhodotorula and Micrococcus (Attri and Joshi, 2005; Joshi and Attri, 2006). On the other hand, an incubation period of 48 hr has been reported as optimum for Chromobacter for pigment production by Attri and Joshi (2006).

The pH can affect the pigment production and its shade. Monascus sp. produces pigments optimally at pH between 5.5 and 6.5 whereas Rhodotorula does it as pH 4.0–4.5. Lycopene formation occurs at neutral to slightly alkaline pH whereas β-carotene formation occurs at acidic pH (Joshi et al., 2003). Carbon sources have impact on the microbial growth and shade of the microbial pigment. Depending on the species used monosaccharide or their polymers can be the optimum carbon source choice for pigment production (Joshi et al., 2003). A range of inorganic and organic nitrogenous compounds may be preferred by different microbes for maximum pigment
production. In addition, various minerals have also been documented to affect microbial pigment production (Joshi et al., 2003).

Although synthetic media can be used for the microbial production of pigments but use of agrochemical waste is suggested to be much better in terms of reducing the overall production cost (Panesar et al., 2015). Hamano and Kilikian (2006) demonstrated the use of corn steep liquor favorable for the production of pigment by Monascus ruber. A waste stream cellulose culture medium was utilized and optimized for pigment production by Penicillum sp. (Sopandi et al., 2013). Tinoi et al. (2005) produced carotenoid pigment by culturing Rhodotorula glutinis on hydrolyzed mung bean waste flour. Whey and soya protein have also been successfully used as raw material for the production of microbial pigments (Kaur et al., 2008; Panesar et al., 2015). Various food and vegetable waste products have also been utilized for microbial pigment production (Nigam and Luke, 2016).

**TECHNICAL ADVANCEMENT TOWARD MICROBIAL PIGMENT PRODUCTION**

Numerous technical advancements have been reported in recent years toward successful production of microbial pigments intended for various industrial applications. The progress in
various aspects related to fermentative production of pigments is discussed below.

**Strain Improvement**
Microbial pigments of desired characteristics with increased yield can be obtained through the use of established strain improvement techniques. Often a wild microbial strain is associated with limited production of pigment which negatively affects the economy of the process. The routinely used method of random mutagenesis and selection has resulted in the increase of pigment yield. Exposure to UV light and other mutagens such as 1-methyl-3-nitro-1-nitrosoguanidine, Ethyl methyl sulfonate is known to cause several fold increase in microbial pigment production (Nigam and Luke, 2016). Fakorede et al. (2019) have reported 5 fold increase in pigment production by *Serratia marcescens* (GBB151) after mutagenic treatment with Ethidium bromide. Techniques of genetic engineering have also been successfully employed for enhancing the microbial pigment yield and alter its molecular structure and color (Sen et al., 2019). Although information on the complete blueprint of
the biochemical synthetic pathways and the intermediates is generally a prerequisite for all such genetic manipulations targeting the rate limiting step for enhancing production. Bartel et al. (1990) and McDaniel et al. (1993) have reported such genetic alteration for blue pigment Actinorhodin, produced by *Streptomyces coelicolor*. Increased production of Zeaxanthin and other pigments by genetic engineering of *Synechocystis* sp. Strain PCC 6803 has been reported by Lagarde et al. (2000). There are reports of the development of cell factories using heterologous expression for the production of microbial pigments (Nielsen and Nielsen, 2017; Sankari et al., 2018). Technique of transposon mutagenesis applied on *Pseudomonas fluorescens* revealed 8 genes involved in blue pigment production and antioxidant protection (Andreani et al., 2019).

### Optimization of Fermentation Conditions and Downstream Processing

The optimization of fermentation conditions and development of economic downstream processing can lead to the cost-effective production of microbial pigments. Media optimization includes variation in fermentation conditions like temperature, pH, incubation time, nutritional sources, aeration, and agitation rate etc. for selection of conditions that provide best yield. The technique of Response surface methodology (RSM) has many advantages over classical methods used for media optimization. Fewer experiments are required to derive an optimum combination of all the variable factors under investigation. Optimization of fermentation conditions thus require less time and efforts leading to reduction in the overall cost. Hamidi et al. (2017) determined the optimum values of temperature, pH and saline concentration and the effect of light on total carotenoid production by *Halorubrum* sp. TBZ126 using response surface methodology. Optimization of culture medium for yellow pigments production with *Monascus anka* mutant using response surface methodology has been reported by Zhou et al. (2009). Artificial neural network (ANN) is another technique that can be used to study the impact of fermentation conditions as well as their optimization for microbial pigment production. Singh et al. (2015) have investigated the application of Artificial Neural Network (ANN) in modeling a Liquid State Fermentation (LSF) for red pigment production by *Monascus purpureus* MTCC 369 and reported that ANN model can be used to predict the effects of fermentation parameters on red pigment production with a high correlation.

The conventional method of organic solvent extraction of pigments from fermentation broth is a complicated and time-consuming process with disadvantages of high cost, low yield and possible solvent leftover in the purified product as contaminants (Sen et al., 2019). Sen et al. (2019) have also mentioned that use of non-ionic resin due to their high loading ability is particularly suitable for large scale recovery of pigments and the technique also offer the advantage of direct absorption of compounds form the culture broth, thereby reducing the overall cost. Wang et al. (2004) have described the use of a non-ionic adsorbent (X-5) resin in the presence of Tween 80 for direct recovery of prodigiosin from the culture broth of *S. marcescens.*

A novel approach of perstraction for recovery of intracellular pigments through submerged fermentation of *Talaromyces* spp. in a surfactant rich media has been described by Morales-Oyervides et al. (2017).

### Pigment Stabilization

Stability against light, pH, temperature, UV radiation, and food matrices is an important issue with regard to the suitability of a microbial pigment for industrial applications and strategies like microencapsulation, nanoemulsion, and nanoformulations have been suggested for this purpose (Sen et al., 2019). In encapsulation solids, liquids, or gaseous materials are packaged in matrices (encapsulants) which sustain and release their contents under specific conditions (de Boer et al., 2019). The technique offer multiple advantages such as protection against light, moisture, or heat and also increase the brightness of the natural colorant and enhance their stability for many industrial applications (de Boer et al., 2019). The commonly used techniques for encapsulating colorants are spray-drying, electrospaying and anti-solvent precipitation (de Boer et al., 2019). A number of reports are available on the microencapsulation of microbial pigments and their enhanced applicabilities. Lycopin and carotenoids have been reported to be encapsulated by the methods of spray drying and lyophilization, respectively (Rocha et al., 2012; Nogueira et al., 2017). The strategy of nano-encapsulation or nano-emulsions which employs the droplet sizes of 100 nm or less with water, soil and emulsifier can be used for encapsulating microbial pigments which offers further advantages of stronger kinetic stability and resistance to chemical and physical changes because of larger surface area per unit (Gupta et al., 2016; Sen et al., 2019). There are various studies about the positive impact of nanoemulsion on stability of β carotene (Yi et al., 2014; Sen et al., 2019). Although various nanostructures are known to confer stability to carotenoid pigments, encapsulated polymeric nanocapsules are most utilized due to its stability during storage, high efficiency to encapsulate and to control the release of the carotenoid (Dos Santos et al., 2018). Bazan et al. (2015) have explored some nanoencapsulation techniques such as emulsification, coacervation, inclusion complexation and nanoprecipitation for lycopine. Other strategies for enhancing pigment stability that has been worked upon are like additions of copigment compounds, such as polymers, phenolic compounds, and metals as well as the exclusion of *O₂* during processing (Cortez et al., 2017). Various patents have been filed for novel methods of pigment stabilization such as the hard candy coating for various colors (Cortez et al., 2017). The technique of genetic engineering has also been applied for enhancing the natural pigment stability. A novel method of gene-encoded acyltransferase of aromatic acyl groups has been filed for patent by Tanaka et al. (2011).

### Analysis and Detection of Microbial Pigments

Various analytical techniques developed over the years are in use to detect and analyze microbial pigments. TLC, UV–VIS spectrophotometry, FTIR, NMR, HPLC are the techniques in
routine use for identification and characterization of microbial pigments. A handheld Raman spectrometer, working on the principle of excitation laser, has been employed for the detection of microbial pigments in various environments (Jehlicka and Oren, 2013; Kumar et al., 2015). Mass spectrometry coupled with electrospray ionization can be used for classifying the pigments producing fungi (Smedsgaard and Frisvad, 1996).

MAJOR CHALLENGES ASSOCIATED WITH THE USE OF MICROBIAL PIGMENTS AS FOOD COLORS

Both extracellular and intracellular production of pigments is known in microorganisms. Although commercial production of food pigments from microorganism offers special advantages, the species under use must be amenable to culture with a fast growth rate and productivity in limited space and time (Ramesh et al., 2019). In addition, it must be non-toxigenic, non-pathogenic, and able to grow on a wider range of cheaper raw materials with stability under harsh physical and chemical process conditions (Ramesh et al., 2019). Due to various factors natural colors are more expensive as compared to their synthetic counterparts. In confectionary items biopigments can be 20 times more expensive as synthetic pigments (Sigurdsdson et al., 2017). Microbial pigments may also be associated with the tendency to react with the other food components and may generate unwanted odors and flavors (Sen et al., 2019). Extraction and purification of microbial pigments from fermentation broth is a time consuming, low yielding and costly affair (Nigam and Luke, 2016) and use of organic solvent may itself overcome the idea of obtaining natural pigments (Hicketier and Buchholz, 2002).

The use of synthetic media for microbial production can overprice the production cost although cheap agro-industrial residues such as coconut residue, soybean meal, corn syrup, starch, cheese whey, rice water, jackfruit seed extract, mustard waste, sugar beet molasses, etc. are promising alternative media substitutes for pigment production (Venil et al., 2014). However, the availability of such byproducts throughout the year at many places may be difficult. The another issue with natural pigments is their stability and sensitivity toward light, pH, UV, temperature, oxygen, heat, and other environmental conditions that may lead to color loss and a reduced shelf life (Sen et al., 2019). Various encapsulation strategies as well as genetic engineering methods have been developed to address this issue. In future the development of novel techniques like the combination and evaluation of new pigment stabilizing material will further enhance their prospects to be used as value-added natural food pigments (Cortez et al., 2017). Since all food additives are under very strict legislation and approval mechanism, it is of paramount importance that microbial pigment production and its purification process must not allow any unwanted toxic or allergic metabolite in the final product (Gao et al., 2003).

CONCLUSION

Given the growing public perception and concern over the use of safe food ingredients, the industrial demand of natural pigments is expected to increase in future by many folds. Microbial pigments are attractive alternative to synthetic food colorants not only because of their natural origin but also due to their several proven health benefits. Although a plethora of microorganisms have been reported to produce food grade pigments at laboratory level, large scale production and purification of the products from many of them is still a challenge. More studies are required with respect to media and fermentation condition optimization for sufficient production and easy recovery of microbial pigments. In addition, classical strain improvement methods as well as the advance techniques of genetic or metabolic engineering can be used for sustainable production of microbial pigments of high use. The strain improvement methods can also be preceded by bioprospecting programs to screen and identify novel pigment producing microbial strains from different environments in adherence to the Nagoya protocol and other applicable state rules. Exploration of traditional fermentative food in isolated or tribal region can also lead to the identification of promising pigment producing isolates. Although only non-pathogenic microbes are acceptable for food grade pigments, co-production of toxic or undesirable compound by so called “safe” organism is also possible and therefore, appropriate cost effective purification strategies are to be devised.

AUTHOR CONTRIBUTIONS

BR, MB, and BP prepared the first draft of the manuscript. GJ critically evaluated the same and prepared the final version with the help of all of them and MA. All authors contributed to the article and approved the submitted version.

REFERENCES

Andreani, N. A., Carraro, L., Zhang, L., Vos, M., and Cardazzo, B. (2019). Transposon mutagenesis in Pseudomonas fluorescens reveals genes involved in blue pigment production and antioxidant protection. Food Microbiol. 82, 497–503. doi: 10.1016/j.fm.2019.03.028

Asker, D. (2017). Isolation and characterization of a novel, highly selective astaxanthin-producing marine bacterium. J. Agri. Food Chem. 65, 9101–9109. doi: 10.1021/acs.jafc.7b03556

Attri, D., and Joshi, V. K. (2005). Optimization of apple pomace based medium and fermentation conditions for pigment production by Micrococcus species. J. Sci. Ind. Res. 64, 598–601.

Attri, D., and Joshi, V. K. (2006). Optimization of apple pomace based medium and fermentation condition for pigment production by Chromobacter species. J. Food Sci. Technol. 43, 515–520.

Babitha, S. (2009). "Microbial pigments," in Biotechnology for Agro Industrial Residues Utilization, eds. N. Singh, P.S. Nigam, and A. Pandey (Dordrecht: Springer), 147–162. doi: 10.1007/978-1-4020-8480-5_17

Bartsani, L., Coltell, P., Evangelista, V., Frassanito, A. M., Passarelli, V., Vesentini, N., et al. (2008). "Oddities and curiosities in the algal world," in Algal toxins: nature, occurrence, effect and detection (Dordrecht: Springer), 353–391. doi: 10.1007/978-1-4020-9942-7_8

Bartel, P. L., Zhu, C. B., Lampel, J. S., Dosch, D. C., Connors, N. C., Stohl, W. R., et al. (1990). Biosynthesis of anthraquinones by interspecies cloning of actinorhodin biosynthesis genes in streptomyces:
Tuli, H. S., Chaudhary, P., Beniwal, V., and Sharma, A. K. (2015). Microbial pigments as natural color sources: current trends and future perspectives. J. Food Sci. Technol. 52, 4669–4678. doi: 10.1007/s13197-014-1601-6

Unagul, P., Wongsa, P., Kittakoop, P., Intamas, S., Srikitikulchai, P., and Tantricharoen, M. (2005). Production of red pigments by the insect pathogenic fungus Cordyceps unilateralis BCC 18669. J. Induct. Microbiol. Biotechnol. 32, 135–140. doi: 10.1007/s10295-005-0213-6

Valla, A., Cartier, D., Valla, B., Le Guillou, R., Andriamialisoa, Z., Breithaupt, D., et al. (2004). “New synthesis of natural carotenoid isoreinieratene,” in Peer-Reviewed Proceedings of the 3rd International Conference on Pigments in Food (Quimper: Pigments Publishing, Université de Bretagne Occidentale), 67–69.

Vendruscolo, F., Müller, B. L., Moritz, D. E., de Oliveira, D., Schmidell, W., and Ninow, J. L. (2013). Thermal stability of natural pigments produced by Monascus ruber in submerged fermentation. Biocatalysis Agri. Biotechnol. 2, 278–284. doi: 10.1016/j.bcab.2013.03.008

Venil, C. K., Aruldass, C. A., Dufossé, L., Zakaria, Z. A., and Ahmad, W. A. (2014). Current perspective on bacterial pigments: emerging sustainable compounds with coloring and biological properties for the industry—an incisive evaluation. RSC Adv. 4, 39523–39529. doi: 10.1039/C4RA06162D

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Rana, Bhattacharyya, Patni, Arya and Joshi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.