Valorization of Agri-Food Industry Waste for the Production of Microbial Pigments: An Eco-Friendly Approach

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Abstract

Globally, over one-third of total food production is wasted along the food value chain, which amounts to 1.7 billion tons per year that also includes agro-industrial residues such as fruits and vegetable waste in the form of peels, seeds, liquid, and molasses. A major portion of this waste is either anaerobically digested or utilized for animal feed and is dumped into landfills which contribute to greenhouse gas emissions that adversely affect the environment. The valorization of food waste can be achieved through the biorefinery processing of biomass into high-value components and energy. Microbial biocolors are the coloring agents that are derived from biological sources such as biomass and agricultural residues by microorganisms. Bicolor production from the microbial origin is beneficial in terms of nontoxic and superior quality, biodegradability, compatibility with the environment, and independence from seasonal variation. Thus, biotechnological production of natural colors with low-cost substrates such as agro-industrial residues is the cheapest source of natural color production. In addition to food

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color, natural colors also act as antimicrobial, antioxidant, antimutagenic, and precursor of vitamins, which help to reduce cancer, chronic diseases, macular degeneration, cataract and are used for the production of biopharmaceutics and cosmetic products. Natural colors have a very high market value; thus, the extraction of these pigments from waste can lead to high market revenue. This chapter covers a comprehensive review of the biotechnological production of microbial colorants from agro-industrial waste, discusses their physicochemical properties and applications in different industries.

Keywords

Agro-industrial waste · Food colorant · Microbial pigments · Fermentation · Novel technologies

8.1 Introduction

Biocolor is derived from a combination of two words "Bio" which means natural and "color" which provides a hue to the substance. Biocolorants or biopigments are the natural coloring agents obtained from the biological origin such as plants, animals, microorganisms such as bacteria, algae, fungi, yeasts, and insects (Parmar and Phutela 2015). Natural pigments obtained from biological origin are safe, renewable, environment friendly, and are biodegradable. Bio colorants are the coloring agents known for their safe usage in the food, nutraceutical, pharmaceutical, and cosmetic industries (Aruldass et al. 2018). These natural pigments are considered as potent green pigments to replace synthetic dyes. Nevertheless, artificial or chemically synthesized colorants are cheaper and more stable than the biocolorants, but excess usage of synthetic colorants in manufacturing of food products and drugs causes carcinogenic, toxicological, teratogenic, and allergenic problems (Heer and Sharma 2017).

On the other hand, synthetic color extraction depends on petroleum-based organic solvents and non-renewable sources. In addition to this, WHO and U.S. FDA had imposed the guidelines on usage or recommended daily intake of synthetic colorants in food products, nutraceuticals, cosmetics, and drug products (Tuli et al. 2015). Hence, to avoid the adverse effects of synthetic colorants, many research efforts have been made to produce natural colorants or biopigments.

Biocolorants can be synthesized from different renewable sources, i.e., plants, animals, and microbes. Plant pigments are produced by the photosynthesis process, which uses chlorophyll and carotenoids for their functioning. Examples of plant pigments are curcumin, lutein, lycopene, carotenoids, anthocyanin, betanin, chlorophyll, and bixin (Rodriguez-Amaya 2016). Most animals produce biological pigments or biochromes such as melanin in mammals, pterin, porphyrin, and flavonoids (Heer and Sharma 2017). Microbial production of pigments is the potential source of biocolorant producers due to their benefits over plants and animals such as independence of geographical and environmental conditions, throughout the year availability, more stability, cost-effective, good yield, easy and solvent-free downstream processing (da Costa Cardoso et al. 2017). Microbial fermentation produces a variety of biopigments such as carotenoids, anthocyanin, quinines, flavins, monascins, violacein, prodigiosin, and indigoidine (Ganguly et al. 2019).

Microbial production of biopigments is considered the cheapest source of pigment production due to their effective growth on low-cost medium substrates. Hence a low-cost medium provides a thumb rule to produce low-cost pigments (Panesar et al. 2015). Among low-cost substrates, a wide range of agro-industrial residue or waste can be considered the ideal source providing carbon, nitrogen, and minerals in potent amounts (Lopes et al. 2013).

Agro-industrial waste or residue is the waste generated during the post-harvest or industrial processing of agricultural produce. The waste obtained during the agricultural practices in the field gives straw, stem, leaves, husk, stubbles, shell, and hulls produced throughout the year (Hernández-Alcántara et al. 2016). These agrowastes are rich source of nutrients which allows its use as raw material for solid-state fermentation. Hence, this waste provides a low-cost alternative substrate to produce high-value bioactive components and control environmental pollution (Zuin and Ramin 2018). On the other hand, waste or by-products from the agro-food processing industries produce bagasse, peels, brewer's spent grains, spent coffee grounds, etc. Liquid waste such as corn steep liquor and whey can be used as efficient medium component for carbon, nitrogen, and minerals in microbial pigment production processes (Lopes and Ligabue-Braun 2021).

The biocolors produced from microorganisms have Pro-Vitamin A and other medicinally important properties apart from being natural and safe to use. Microbial pigments provide other biological functions such as antimicrobials, antioxidants, antiproliferative, antiparasitic, and anticancer. Hence, the application of microbial pigment is not restricted to food industries but can be applied to the pharmaceutical, nutraceutical, cosmetic, and textile industries (Sen et al. 2019).

However, the microbial production of natural colorants is a cumbersome task with the fermentation conditions. Hence, with modern biotechnological methods, the extraction efficiency can be improved to meet the growing demand for natural colorants (Rymbai et al. 2011). This chapter will provide detailed information on sustainable microbial production of pigments from low-cost substrates such as agro-industrial waste, downstream processing to recover the pigments, and their application in different industries.

8.2 History of Microbial Pigments

Production of biocolorants from microbial sources is considered as a novel method for cheaper pigment production. The oldest use of natural colorants as the dye was recorded dated 2600 BC in China. In the Indus valley period (2500 BC), clothes of red color and madder dye traces were found in the destroyed sites of Mohenjodaro and Harappa civilization (3500 BC), which gave the proof of dye or color invention at that period (Lopes and Ligabue-Braun 2021). Natural colorants were the primary or exclusive origin of colors prior to synthetic dyes. In 1856, Perkin prepared synthetic pigments, which was cheaper, easy to produce, and independent of the weather conditions (Joshi et al. 2003). Hence, with the advancement of technology and chemical methods, the production of synthetic pigments has become easy, cheaper, and higher production rate. Despite all these, excessive use of synthetic pigments in food, pharmaceuticals, and cosmetics can cause toxic effects to humans due to their carcinogenic nature. All these side effects from the synthetic colorants have shifted the interest of people towards natural and safe edible colorants from the last five decades (Venil et al. 2013).

8.3 Classification of Microbial Pigments

Microbial pigments can be majorly categorized based on color, microorganism type, and solubility of the pigments in different solvents (Table 8.1).

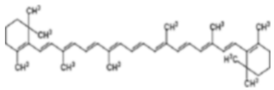
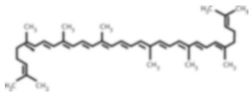
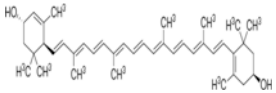
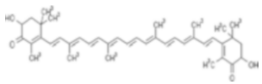
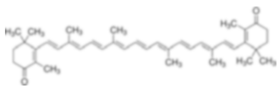
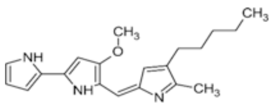
1. Based on the color of the pigment.
   (a) Yellow pigments (Carotenoids, Riboflavin).
   (b) Red pigments (Carotenoids, prodigiosin, porphyrin, arpink red).
   (c) Blue pigments (Anthocyanin, indigoidine, violacein, melanin).
2. Based on microbial sources.
   (a) Bacteria, algae, yeasts, fungus, protozoa, and molds.
3. Based on solubility.
   (a) Water and fat-soluble.
   (b) Polar and non-polar solvents solubility.

8.3.1 Carotenoids

Carotenoids are the major class of natural pigments containing isoprenoid structure and exhibit yellow to orange color. These red, orange, and yellow color pigments are mainly synthesized by bacteria, fungi, yeast, microalgae, and plants. Major carotenoids pigments of huge market interest, safe usage in the food industry, and synthesized by microorganisms are lycopene, β-carotene, lutein, astaxanthin, and canthaxanthin (Mussagy et al. 2021).

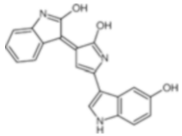
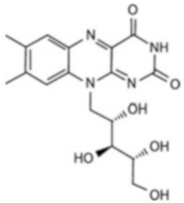
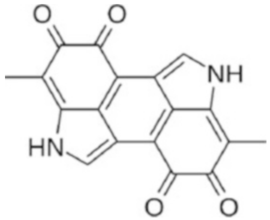
Agro-industrial waste such as grape must, carrot and other orange and yellow colored vegetable peels, corn steep liquor, beet molasses, glycerol, sugarcane molasses, glucose syrup, gram waste such as soybean flour, moong bean flour, and cereals waste can be considered as a low-cost carbon, nitrogen, and mineral sources for carotenoid production by microorganisms (Roukas et al. 2003).

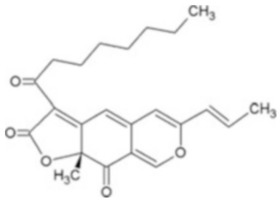
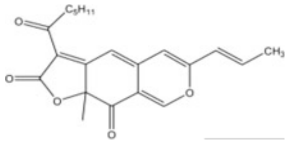
Table 8.1 Chemical structure and microbial sources of pigments

Microbial pigment	Color	Chemical Structure	Microbial sources	References
β-Carotene	Yellowish orange		• *Blakeslea trispora* • *Rhodotorula glutinis* • *Dunaliella salina* • *Rhodotorula rubra* • *Mucor circinelloides* • *Phycomycesblakes leeanus* • *Rhodotorula rubra*	Kaur et al. (2019)
Lycopene	Red		• *Blakeslea trispora* • *Fusarium sporotrichioides*	Zuorro et al. (2011)
Lutein	Yellowish-red		• *Chlorella* sp.	Lopes and Ligabue-Braun (2021)
Astaxanthin	Pink, Pink-red		• *Xanthophyllomyces dendrorhous* • *Agrobacterium aurantiacum* • *Paracoccus carotinifaciens*	Lopes and Ligabue-Braun (2021)
Canthaxanthin	Dark-red		• *Bradirhizobium* sp. • *Haloferax alexandrines* • *Gordonia jacobaea*	Dufossé (2006)
Prodigiosin	Red		• *Serratia marcescens* • *Pseudoalteromonas rubra* • *Streptomyces* sp. • *Vibrio gaogenes*	Kumar et al. (2015)
Phycocyanin	Light blue		• *Aphanizomenonflos-aquae* • *Spirulina* sp.	Dufossé (2016)

(continued)

Table 8.1 (continued)

Microbial pigment	Color	Chemical Structure	Microbial sources	References
			• *Pseudomonas* sp. • *Arthrospira* sp.	
Violacein	Purple		• *Chromobacterium violaceum* • *Janthinobacterium lividum* • *Pseudoalteromonas* sp.	Sigurdson et al. (2017)
Riboflavin	Bright yellow		• *Bacillus subtilis* • *Ashyba gossupi* • *Xanthophyllomyces dendrorhous* • *Candida guilliermondii* • *Debaryomyces subglobosus* • *Clostridium acetobutylicum*	Dufossé (2006)
Melanin	Black		• *Bacillus thuringiensis* • *Saccharomyces* sp. • *Neoformans* sp. • *Streptomyces virginae* • *Saccharomyces nigricans*	Venil et al. (2013)
Arpink red	Red		• *Penicillium oxalicum*	Kumar et al. (2015)

				
Monascus pigments	Red		• *Monascus pilosus* • *Monascus purpureus* • *Monascus ruber* • *Monascus froridanus*	Dufossé et al. (2005)

8.3.1.1 Carotenoid Synthesis from Bacteria, Yeast, and Fungi

Carotenoids are naturally occurring in bacteria, fungi, yeast, and microalgae. The colorants obtained from these microbial sources range from yellow to red. The synthesis of carotenoids from fungi and yeast can be improved by manipulating the media composition, substrates and light stimulation. As yeast and fungi are heterotrophic organisms, the culture medium needs to be optimized along with the fermentation conditions and medium composition. Agro-industrial waste serves as the cheapest nutrient source for microbial pigment production with higher yield and minimizes production costs. Different agro-industrial residues and by-products serve as the low-cost carbon source for microbes production (Papaioannou and Liakopoulou-Kyriakides 2012).

β-Carotene production by *Blakeslea trispora* (fungus) and the use of two mating species play a major role in the large-scale production of carotenoids (Ribeiro et al. 2011). Yeast species *Rhodotorula glutinis* plays a major role in the large-scale production of carotenoids such as β-carotene, astaxanthin, and zeaxanthin from agro-industrial waste such as fruit peels, cereals, raw stalks, bran and pulses husk, etc. (Malisorn and Suntornsuk 2008).

Lycopene is a dark red colored pigment and an acyclic isomer of conjugated carotenoid structure of β-carotene. It is more stable and has high antioxidant potential in comparison to other carotenoids such as trans-lycopene and β-carotene. Lycopene can be synthesized by fungus sp. *Fusarium sporotrichioides* on corn fibers and *Rhodotorula glutinis* yeast and *Blakeslea trispora* on agro-industrial residues such as tomato peels, etc. (Chandi et al. 2010). Zeaxanthin, a carotenoid alcohol, is yellowish-orange in color, majorly a bacterial pigment synthesized by *Staphylococcus aureus*, *Bacillus*, *Flavobacterium* sp., *Corynebacterium* sp. (Ganguly et al. 2019). On the other hand, astaxanthin, classified as a xanthophyll, is a reddish-orange lipid-soluble pigment mainly found in yeasts such as *Xanthophyllomyces dendrorhous*, and microalgae *Haematococcus pluvialis* (Bi et al. 2010). Bacterial sources for astaxanthin production may include *Paracoccus marcusii*, *Paracoccus carotinifaciens*, *Agrobacterium aurantiacum,* etc. (Dufossé 2006).

8.3.1.2 Carotenoids Synthesis from Microalgae

Astaxanthin, a yellowish-orange keto-carotenoid pigment, represents strong antioxidant activity compared to other carotenoids such as lycopene, lutein, β-carotene, and zeaxanthin. It has enormous demand in the pharmaceutical and nutraceutical industries (Honda et al. 2019). Astaxanthin can be produced in freshwater microalgae *Haematococcus pluvialis* by two-stage culture. Such intracellular production of pigment involves the morphological transformation, which turns green vegetative cells into dark-red astaxanthin-rich components (Chattopadhyay et al. 2008).

Agro-industrial wastewater can be treated with the production of well-nourished *Haematococcus* and *Dunaliella* sp. which further reduces the cost of algal biomass by valorizing it for the production of high-value bioactives and other bio-energy products (Spolaore et al. 2006). Agro-industrial by-products or waste materials such as cassava processing wastewater, corn steep liquor, and ethanol effluent can be used as a growth media for the production of microalgae (Babitha et al. 2006).

During the treatment of wastewater with conventional biological processes, external carbon sources are incorporated to convert the excess nitrates into nitrogen gas and biomass. While the growth of microalgae in the wastewater assimilate nitrates present in it and convert this into nitrogen which helps in the production of astaxanthin pigment. Research reported by Kang et al. (2006) revealed that *Haematococcus* algae cultivation in the wastewater completely removed the inorganic wastes and helps in the conversion of green vegetative cells into red astaxanthin pigments (Brar et al. 2013).

Canthaxanthin, orange to dark pink colored microbial synthesized, is a keto-carotenoid pigment soluble in lipids. Microalgae species *Nannochloropsis gaditana* and *Chlorella zofingiensis* have been reported to produce canthaxanthin from agro-industrial residues such as corn steep liquor, glucose, and these carotenoids are considered as a natural antioxidant to prevent lipid oxidation (Rana et al. 2021).

8.3.2 Anthocyanin

Anthocyanin is the blue-purple color of natural pigments and belongs to the flavonoid group of polyphenols. Anthocyanin is a water-soluble rich intensity coloring pigments and has high antioxidant and antimicrobial activities (Rodriguez-Amaya 2019). Anthocyanin pigment is not stable at normal conditions, and its production is also not sustainable due to the variation in plant species. So metabolic engineered or engineered microorganisms such as *E. coli*, *Candida utilis*, and *Pichia pastoris* have been used to produce anthocyanin at an industrial scale using agro-industrial residue as carbon and nitrogen source (Ganguly et al. 2019).

8.3.3 Prodigiosin

Prodigiosin is a natural red colored pigment and secondary metabolite alkaloid mainly produced by bacteria. Prodigiosin is tetrapyrrole structured antibiotic pigments synthesized by *Serratia marcescens*, gram-negative bacteria such as *Pseudomonas magneslorubra*, *Rugamonas rubra*, *Hahella chejuensis*, *Vibrio psychroerythrus,* and *V. gazogenes* (Sánchez-Muñoz et al. 2020). This pigment represents biological functions such as antimicrobial, antiviral, anticancer, and antimalarial (Rana et al. 2021). This pigment is unstable at normal atmospheric conditions such as sensitivity to high temperature, poor solubility, and pH instability. Hence, to mitigate these limitations, prodigiosin is spray-dried and encapsulated in microcapsules to enhance stability (Darjily et al. 2016).

8.3.4 Violacein

Violacein is a naturally occurring di-indole-pyrrole violet-blue colored pigment that possesses numerous biological functions such as antimicrobial, antiviral, anticancer, antiulcerogenic, anti-leishmanial, and enzyme modulation properties (Narsing et al. 2017). Violacein is biosynthesized by bacterial species such as *Chromobacterium violaceum*, *Collimonas* sp., *Pseudoalteromonas* sp., *Pseudomonas aeruginosa*, and *Janthinobacterium* sp. This natural pigment is used extensively in the cosmetic, food, pharmaceutical, and textile industries (Baiano 2014).

8.3.5 Indigoidine

Indigoidine is a blue-violet organic pigment related to the Azaquinones group and synthesized by bacterial strains. It is biosynthesized by bacterial species such as *Streptomyces chromofuscus*, *E. coli*, and *Corynebacterium insidiosum* (Ganguly et al. 2019). It is used as a food colorant in cereal, baking, and ice-cream industries.

8.3.6 Phycocyanin

Phycocyanin is a distinct blue color photosynthetic and water-soluble pigment. This pigment is produced by photosynthetic microorganisms such as blue-green algae, *Spirulina platensis*, *Synechocystis* sp., and *Aphanizomenon flos-aquae* (Narsing et al. 2017). Phycocyanin provides various biological functions such as antibacterial, antiviral, antifungal, and anti-alzheimeric agents (Jayaseelan et al. 2014).

8.3.7 Melanin

Melanin is a nitrogenous indolic polymer known as eumelanins, allomelanins, and pheomelanins (Banerjee et al. 2011). Melanin provides photoprotection from UV radiations by absorbing radiations from the electromagnetic spectrum, also effective against chemical stress and high temperature. Due to these properties, it is used in cosmetics, eyeglasses, and pharmaceutical products. Melanin pigment is biosynthesized by several microorganisms such *as Magnoporthe grisea*, *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, *Vibrio cholera*, *Aspergillus fumigates*, *Alteromonas nigrifaciens*, and *Streptomyces* sp. (Sánchez-Muñoz et al. 2020).

8.3.8 Arpink Red

Arpink red is an anthraquinoid pigment majorly obtained from *Penicillium oxalicum*. Its structure is similar to cochineal caramine and a major alternative for insect-derived pigment. It is used in food and pharmaceutical industries due to its nontoxic nature. It provides various biological functions such as antibacterial, antiviral, anticancer properties (Kumar et al. 2015).

8.3.9 Monascus

Monascus pigments are secondary metabolites mainly synthesized by filamentous fungi such as *Monascus* genus. *Monascus* sp. produce red, yellow, and orange colored pigments from various fungal species such as *Monascus pilosus*, *M. purpureus*, *M. ruber*, and *M. rubropunctatus*. This pigment is used as a natural food colorant in wines, yogurt, meat products (hams, sausages, and red meat). Apart from their usage in food industries, they also possess biological functions such as antimicrobial, anticancer, antioxidant, and anti-ulcerous agent (Malik et al. 2012).

8.3.10 Riboflavin

Riboflavin, also known as vitamin B_2, is a water-soluble vitamin and exhibits greenish-yellow in color. This natural pigment has been reported to be synthesized by *Candida guilliermondii*, *Eremothecium ashbyii*, and *Debaryomyces subglobosus*. This pigment is used as an additive in dairy products, fruit juices, baby foods, and canned fruits (Dufossé 2006).

8.4 Valorization of Agri-Food Industrial Waste for Production of Microbial Pigments

8.4.1 Fruits and Vegetable Processing Industry

Fruits and vegetable processing industries produce a huge amount of waste or by-products in pulp, peels, bagasse, seeds, stem, pulp, wastewater effluents, etc. These by-products contain number of nutrients that can be used for microbial growth and fermentation. Fruits and vegetable processing waste contain a high amount of carbohydrates, cellulose, dietary fibers, soluble sugars, minerals, and organic acids, which may be considered the best substrate for solid-state or submerged fermentation for microbial pigment production (Kaur et al. 2019).

In previous studies, carotenoids production from fruit processing by-products such as sugarcane molasses, sugarcane juice, corn syrup, and fruits and vegetable residues, i.e., papaya, carrots, tomato, watermelon, peaches, orange, and kinnow, was obtained with different microbes, i.e., *Blakeslea trispora*, *Rhodotorula glutinis*, and *Rhodotorula rubra* (Papaioannou and Liakopoulou-kyriakides 2012; Bhosale and Bernstein 2004; Buzzini 2001; Malisorn and Suntornsuk 2008).

The utilization of citrus fruit peels such as kinnow peel powder has been considered as an excellent low-cost substrate for the production of monascus pigments (Dufossé 2006). Apple pomace, a rich source of sugars, minerals, and organic acids, has been used to produce carotenoids and violacein pigments. Wine industry waste also provides a cheap and affordable substrate for red, yellow, and blue pigments. Grape pomace waste was used to produce anthocyanin pigment by *Monascus purpureus* (Panesar et al. 2015). Tomato waste, a rich source of carbohydrates, proteins, and crude fat, serves as an excellent medium for the growth of yeast species such as *Rhodotorula* sp. for carotenoid production (Chandi et al. 2010).

8.4.2 Cereal Industry

The cereal processing industry produces by-products from three different processes, dry milling (for flour production), wet milling (for production of starch and glucose), and brewing industry. Thus, the by-products obtained from the cereal processing industry include germ meal, bran, gluten meal, husk, corn steep liquor (CSP), etc. (Charalampopoulos et al. 2002). The corn wet-milling industry produces corn steep liquor as a by-product that can be used to produce penicillin, β-galactosidase enzyme, and ethanol. They provide nitrogen, sugars, amino acids, and vitamins to the fermentation medium, which can further be used to produce microbial pigments. CSP obtained from corn industry contains a rich amount of nitrogen and salts, which helps in the production of red pigments by *Monascus ruber* (Papaioannou and Liakopoulou-Kyriakides 2012).

8.4.3 Dairy Industry

The dairy industry produces several by-products such as whey, skim milk, buttermilk, and residues during cream, butter, cheese, and ghee processing. The major waste of the dairy industry is whey, obtained during the processing of cheese after the removal of casein from milk. Whey is a rich source of milk protein and sugar (lactose) that serves as an excellent medium for the growth of microbes. Cheese whey is used to produce different fungal strains of filamentous fungus to produce yellow or carotenoid pigments (Lopes et al. 2013). Whey protein and coconut water have been used for submerged fermentation (SmF) of *Rhototorula rubra* to produce yellowish-pink pigments (Kaur et al. 2012).

8.4.4 Agricultural Residues and Agro-Industrial By-Products

The post-harvesting operations of crops give several by-products such as husk, bran, hulls, bagasse, cobs, molasses, germ meal, starch, corn steep liquor, soybean meal, and oil processing waste, etc. CSP and cassava liquid waste obtained from the corn and starch processing industry is considered as a low-cost substrate for the growth of *Serratia marcescens* for red pigments and prodigiosin production (De Araújo et al.

2010) (Table 8.2). Different nitrogen sources such as pea pod powder, green gram, okra waste, soy, and taro leaves have been reported to produce microbial pigments. Among all these, pea pod powder gave higher production of pigments with *Monascus purpureus* (Sehrawat et al. 2017). Corn cob powder has been reported as an excellent source of carbon in fermentation medium for the production of microbial pigment by *Monascus* sp. On the other hand, sugarcane bagasse and cornmeal have been reported as an excellent source of starch and carbon required for pigment synthesized by *Monascus* sp. (Moussa et al. 2018).

8.4.5 Poultry and Other Miscellaneous Waste

The fermentation medium constituents such as sugar, nitrogen, and minerals are expensive in their pure form, and hence enhance the cost of natural pigments produced using synthetic media culture. The most expensive component of the media composition is nitrogen provided by peptone and beef extract. To mitigate this high cost, experiments have been done to extract peptone from chicken feathers by acid hydrolysis. This was used as a substrate for carotenoids production by *Rhodotorula glutinis* (Taskin et al. 2011).

Waste obtained from the oil processing industries such as peanut, sesame, and coconut oil along with peanut seed powder and sesame seed powder has been tested for the production of prodigiosin by *Serratia marcescens*, which revealed that peanut seed powder gave a maximum yield of prodigiosin pigment than synthetic media (Shahitha and Poornima 2012).

8.5 Improvement of Quality of Microbial Pigments by Biotechnological Method

For biotechnological production of microbial colors, two approaches have been examined: to find out the source of natural pigment and further increase the disposition of color production. Hence, to increase the yield of microbial pigments, the primary step is to improve or develop the strain and optimize the fermentation or growth parameters. To enhance the yield of microbial pigments, a well-optimized process is needed for fermentation with a metabolic engineering approach (Negi 2019).

8.5.1 Strain Development

Conventionally, strain development was the major task achieved by mutagenesis and selection of the suitable strain. In the previous year's studies, the techniques related to gene deletion helped in the efficient inactivation of genome DNA that helps in metabolisms of bacteria. The industrial development of strain is economical as the wild strain of microorganisms produces a very low yield for economic processes.

Table 8.2 Bibliographic review of agro-industrial waste from different industries used for microbial pigment production

Agro-industrial by-products/ Waste	Pigment type	Microorganism sp.	Fermentation type	Fermentation/Optimization conditions	References
Cereal Industry					
Rice bran	β-carotene	*Rhodotorula glutinis*	SSF	Carbon: Nitrogen 4:1, pH = 5.0, moisture content: 70%	Roadjanakamolson and Suntornsuk (2010)
Bakery waste (Bread)	Orange and yellow pigments	*Monascus purpureus*	SSF, and submerged fermentation	Temp: 30 °C, time: 84–90 h, rpm: 250, moisture 60%	Haque et al. (2016)
Corn steep liquor	Carotenoids	*Sporodiobolus pararoseus*	Submerged fermentation	Temp: 25 °C, time: 84–90 h, rpm: 180, Corn steep liquor: 5.5%	Leite et al. (2013)
Bengal gram husk	Red colored pigment	*Talaromyces* sp.	Submerged fermentation	Temp: 30 °C, time: 10 days, rpm: 110, pH: 5.5	Pandit et al. (2019)
Corn meal	Red and yellow pigments	*Monascus purpureus*	SSF	Temp: 30 °C, time: 14 days, pH: 5.5, glucose conc: 8%	Nimnoi and Lumyong (2011)
Fruits and vegetable processing industry					
Onion peels/ mung bean husk	β-carotene, and phytoene	*Rhodotorula mucilaginosa*	Submerged fermentation in bioreactor	Temp: 26 °C, time: 84–90 h, rpm: 120	Sharma and Ghoshal (2020)
Papaya, orange, and carrot peels	β-carotene	*Blakeslea trispora*	SSF	Temp: 28 °C, time: 96 h, hours, rpm: 200	Kaur et al. (2019)
Orange peels	Carotenoids	*Monascus purpureus, Penicillium purpurogenum*	SSF and Submerged fermentation	Moisture 65%, Temp: 25 °C, time: 96 h, hours.	Kantifedaki et al. (2018)
Pineapple waste	Carotenoids	*Chryseobacterium artocarpi*	Submerged fermentation	Temp: 28 °C, pH: 7.0 rpm 200, time: 36 h	Aruldass et al. (2016)
Olive pomace	Astaxanthin	*Xanthophyllomyces dendrorhous*	SSF	Temp: 15 °C, pH: 4.5, time: 12 days, moisture: 90%	Eryilmaz et al. (2016)

Grape waste	Carotenoids	*Monascus purpureus*, *Penicillium chrysogenum*	Submerged fermentation	Temp: 30 °C, pH: 6.5, rpm: 125, time: 7 days, moisture: 90%	Lopes et al. (2013)
Sugarcane juice	Carotenoids	*Rhodotorula rubra*	Submerged fermentation	Temp: 30 °C, pH: 6.5, rpm: 200, time: 7 days	Bonadio et al. (2018)
Sugarcane bagasse	Red pigment	*Monascus ruber*	Submerged fermentation	Temp: 30 °C, pH: 6.5, rpm: 150, time: 12 days	Terán Hilares et al. (2018)
Cassava waste	Carotenoids	*Rhodotorula glutinis*	Submerged fermentation	Temp: 30 °C, pH: 6.5, rpm: 200, time: 120 h	Santos Ribeiro et al. (2019)
Dairy industry					
Cheese whey	Carotenoids	*Blakeslea trispora*	Submerged fermentation	Temp: 26 °C, pH: 6.5, rpm: 150, time: 4 days, β-ionene: 0.2%	Roukas et al. (2014)
Whey sugar	Yellowish pink	*Rhodotorula rubra*	Submerged fermentation	Temp: 25 °C, rpm 250, time: 3 days	Kaur et al. (2012)
Miscellaneous waste					
Peanut seed oil	Prodigiosin	*S. marcescens*	Submerged fermentation	Temp: 28 °C, rpm 160, time: 36 h	Hernández-Velasco et al. (2020)
Coffee husk	Carotenoids	*R. mucilaginosa*	Submerged fermentation	Temp: 28 °C, rpm 160, time: 120 h	Moreira et al. (2018)
Corn cob	Orange and red pigments, carotenoids	*Monascus purpureus*	Submerged fermentation	Temp: 30 °C, rpm 150, time: 10 days, pH: 4.5	Embaby et al. (2018)
Brewery wastewater	Carotenoids	*Rhodotorula glutinis*	Submerged fermentation	Temp: 25 °C, rpm 115, time: 7 days	Schneider et al. (2013)
Potato chips waste	Red, yellow, and orange pigments	*Monascus purpureus*	SSF	Temp: 30 °C, time: 15 days, pH: 6.5, moisture 60–70%	Ramadan and Mahmoud (2016)
Chicken feathers	Carotenoids	*Rhodotorula glutinis*	Submerged fermentation	Temp: 30 °C, rpm 200, time: 5 days, 0.8% peptone obtained from chicken feathers	Taskin et al. (2011)

Hence, pure strain isolation for pure pigment recovery is the major requirement for a cost-effective process. Thus, strains can be improved and purified by mutagens, i.e., EMS (ethyl methanesulfonate), NTG (1-methyl-3-nitro-1-nitrosoguanidine), and ultraviolet (UV), and further can be used for an increase in the production of pigments (Venil et al. 2013).

8.5.2 Fermentation

Fermentation is a metabolic process for producing a metabolite by the mass cultivation of microbial cells that convert complex substances into simpler ones. Production of microbial pigments by fermentation is a great interest that helps in the biotechnological production of natural pigments in the safest and pure form. The fermentation method depends on the type of organism and pigment produced, i.e., in solid-state fermentation (SSF) and submerged fermentation (SmF) (Joshi et al. 2003).

The solid-state fermentation (SSF) is defined as the phenomenon in which microbes grow on moist solid medium in the absence of free-flowing water. In SSF, the solid matrix or the dry material serves as both support and nutrient source to the fermentation medium. The solid matrix in the fermentation medium provides an inert substrate as a base material for the fermentation. Agricultural waste or food processing industries by-products such as rice bran, wheat bran, germ meal, gram husk, pea pods, etc., provide a complete nutritious medium for microbial growth. SSF technique is affected by different parameters like physical properties of the substrate (particle size, shape, porosity, consistency, etc.) and fermentation conditions (moisture content, relative humidity, temperature, pH, dissolved oxygen, and nutrient composition) (Vidyalakshmi and Mohan 2011). Hence, SSF is cost-effective, uses cheaper substrates from residues, saves wastewater, and gives a higher yield of the natural pigments. Several pigments have been produced using SSF, such as Monascus pigment by *Monascus purpureus* utilizing rice bran, red rice, and wheat by-products (Dufossé et al. 2005). Filamentous fungus such as *Blakeslea trispora*, *Monascus* sp., and *Penicillium* sp. have been reported to produce yellow to red color pigments using agro-industrial residues by SSF (Papaioannou and Liakopoulou-kyriakides 2012; Lopes et al. 2013).

While in SmF, microbes are cultivated and isolated aerobically in the presence of free-flowing water with pre-set agitation system for homogenous growth of cell mass and mixing of media components (Heer and Sharma 2017). López-Nieto et al. 2004 reported production of lycopene pigment by mated fermentation of *Blakeslea trispora* plus (+) and minus (−) strains in submerged media. Malisorn and Suntornsuk (2009) also reported the production of carotenoids in SmF medium by *Rhodotorula glutinis* using the waste generated from vegetable processing industry such as radish brine, carrots, and tomato.

Numerous bacterial strains have shown potential in the production of pigments through the utility of agro-industrial wastes; these include *Serratia marcescens*, *Serratia rubidaea*, *Vibrio psychroerythrous*, *Vibrio gazogenes*, *Rugamonas rubra*, *Pseudomonas magneslorubra,* and *Streptomyces longisporus* (Venil et al. 2020).

SmF is currently followed for microbial pigment extraction; however, solid-state fermentation (SSF) has been found to have more potential in pigment extraction (Kumar et al. 2015; Venil and Lakshmanaperumalsamy 2009). A comparative analysis was done by Sehrawat et al. (2017). In *Monascus purpureus* through solid-state fermentation, pigment accumulation up to 9.0 CVU/g was achieved on day 9 compared to SmF, where 5.1 CVU/g accumulation was achieved on day 15.

8.5.3 Downstream Processing of Pigments

The quality characteristics of the microbial pigments need to be improved for their usage in biological or food industries. The separation and purification processes for the production of pure microbial pigments still have many bottlenecks that need to be considered and constrain their large-scale implementation. The conventional method of separation and purification of microbial pigments was the extraction of pigments from the fermentation medium using organic solvents. Current strategies for pigment extraction include HHP (high hydrostatic pressure) and PEF (pulse electric field), membrane technology, sonication assisted extraction, and gamma irradiation enzymatic extraction; however, extraction is not limited to these techniques only (Parmar and Phutela 2015). Hence, during the extraction using organic solvents from the fermentation broth, many organic solvents were exhausted, which gives a very low yield of pigments due to the binding of pigments with the bacterial or fungal envelopes (Venil et al. 2013).

To mitigate the limitations of extraction using organic solvents, non-ionic resins have been used to extract and purify organic macromolecules such as proteins, peptides, nucleic acids, and other organic compounds. In this process, the target components can be adsorbed on the surface of non-ionic resin from the fermentation broth. This process will remove the cell disruption, separation, and extraction steps which further lowers the cost of operation by reducing the usage of organic solvents. In previous research, 86% of the recovery of prodigiosin pigment directly from the broth culture was observed using non-resin adsorbents (Wang et al. 2004). Hence, this process gave higher recovery compared to the conventional extraction methods and silica gel chromatography. In addition to this, extraction with vegetable oils can also be used to extract non-polar pigments, which could help prevent toxic reactions with the use of organic solvents. Sunflower oil is reported to be a green solvent for carotenoid pigment extraction from the fermentation broth (Dufossé 2006).

An economical method was developed to meet the demand for violacein pigment from the other species. A marine bacterium *Pseudoalteromonas* sp. gave thirteen times higher yield from the cell mass when the pigment was extracted from slurry with hot solvent such as methanol (Venil et al. 2013). Hence, several new technological advancements and developments are still required to efficiently recover microbial pigments from the cell culture by cost-effective and energy-efficient methods.

8.6 Metabolic or Genetic Engineering Approach for Industrial Production of Pigments

Natural pigment production by microbes is limited for industrial production as the wild strain of microbes gives the lower concentration of the pigment. Hence, genetic engineering or mutation approach is required to produce hyper-produced strains at an industrial scale. An easy method to produce mutant strains is mutagenesis, which gives a higher pigment yield with a shorter fermentation period. This technique is used to create genetic mutations by manipulating and altering the sequences of genes (Siddique et al. 2011). Production of metabolites by mutagenesis can be improved by creating genetic modulations with physical methods such as UV radiations, gamma radiations, and treatment to chemicals such as NTG, EMS, and antimycin A (Venil et al. 2013). Hence, the selection of suitable microorganisms is the foremost step for the biotechnological production of strain which can be improved by mutagenesis and increase the production of metabolites (Lopes and Ligabue-Braun 2021).

Several studies have been done on the application of metabolic engineering to increase the yield of microbial pigments. The modified strain of *Saccharomyces cerevisiae* yeast has been used to produce carotenoids—such as astaxanthin, canthaxanthin, β-carotene, and lycopene due to inoculation of carotenogenic genes from the various microorganisms, i.e., *Xanthophyllomyces* sp., *Agrobacterium aurantiacum,* and *Erwinia uredovora,* into yeast (Ungureanu et al. 2012). Similarly, increased production of lycopene from the *Xanthophyllomyces dendrorhous* has been reported to incorporate carotenogenic genes into the organism (Verwaal et al. 2007). The use of non-carotenogenic yeast *Pichia pastoris* was also reported to increase the production of carotenoids by mutation. Hence, gene encoding of two different plasmids has also been reported to increase the yield of carotenoids (Araya-Garay et al. 2012).

Genetic engineering is being encouraged in the industrial production of pigments wherever strain development is required by the adoption of result-oriented strategies (Saini et al. 2020). CRISPR CAS9 has brought new trends in genetic engineering and is widely used nowadays. It can be used for metabolic engineering in bacteria, fungi, and yeast by injecting a colorant gene, leading to cost-effective production of natural colorants (Donohoue et al. 2018; Sen et al. 2019).

8.7 Application of Microbial Pigments in Pharmaceutical Industries

Microbial pigments possess important properties that include immune-suppressive, antimicrobial, and anticancer. These have shown potential in diagnosing several diseases such as leukemia, diabetes mellitus, cancer, etc. (Kumar et al. 2015). The red pigment from microbes has shown the highest antibacterial property, followed by orange and then green colored pigments (Soliev et al. 2011). Bacterial pigments are a potential source of anticancer and deserve further investigation (Srilekha et al.

Table 8.3 Biological activity and health-promoting benefits of microbial pigments

Microbial pigments	Biological activity/health benefits	References
Prodigiosin	Cytotoxic activity, Immunosuppressing activity, apoptosis in cell cancer lines in humans, used in the treatment of Diabetes mellitus	Furstner (2003), Kim et al. (2003)
Carotenoids	Treatment of disorders like erythropoietic protoporphyria, Cancer prevention- Breast, prostate, ovary, and liver cancer Prevent the risk of Cardiovascular diseases (CVD), blood pressure issues, and stroke Prevent the risk of neurodegenerative diseases such as Parkinson's, Alzheimer, and Dementia Helps in healthy fetal growth during pregnancy	Leong et al. (2018), Kirti et al. (2014)
Violacein	Antibacterial, Anticancer, Antiviral properties	Sanchez et al. (2006), Ferreira et al. (2004)
Marennine	Antiviral, Anticancer, Antimicrobial and Antioxidant	Gastineau et al. (2014)
Monascins	Effective against obesity-related inflammation	Fujimoto et al. (2012)
Canthaxanthin	Antioxidant, anticancer	Dufossé (2006), Ram et al. (2020)
Ankaflavin	Anti-allergic activity in mice lung cell line (A549) as well as lungs ovalbumin (OVA)	Lee et al. (2013)
Flexirubin	Used to treat chronic skin disease, gastric ulcers, eczema	Venil et al. (2015)
Fucoxanthin	Anticancer, Anti-obesity, Anti-inflammatory properties	Borowitzka et al. (2016)
Rubrolone	Antimicrobial	Venil et al. (2020)
Azaphenanthrne	Antibacterial, anticancer	Banerjee et al. (2011)

2018). Human skin is protected from harmful UV radiations by bacterial pigment melanin and hence is being used in sunscreens (Narsing et al. 2017). Similarly, adonirubin and astaxanthin (xanthophylls) play a role in heart attack, cancer, and stroke prevention (Kim et al. 2012). There are several other pigments with potential application in the pharmaceutical industries. Table 8.3 below enlists some of them.

8.8 Application of Microbial Pigments in the Food Industry

The word "organic" is being interchangeably used for "safe" in the current times, be it for food or any of the daily essentials of our lives. Due to increasing awareness about the environmental hazards and the side effects that have been observed over the years because of synthetic materials, efforts are being made to replace the synthetic materials with something organic that is friendly to our bodies as well as the whole environment (McCann et al. 2007; Potera 2010; Oplatowska-Stachowiak and Elliott 2017; Gebhardt et al. 2020). However, this shift is not easy since we have become habitual of the practices both at the commercial level and the domestic level.

Cancer, which is the second fatal disease in the world, and about ten million people die from it every year, has a genetic reason as well as epigenetic among which exposure to synthetic products is a major cause (Kim et al. 2019; Hofseth et al. 2020; Ahmed et al. 2021). When we talk of cancer through food, color is considered one of the researched reasons that cause it. Food coloration is a practice that goes decades back. By 1900, the food coloring industry has completely transformed as earlier used natural dyes were unstable and not as efficient as the synthetic ones but with time, the side effects that it posed became disastrous, and researches carried on made the government impose laws against their use and even now the synthetic color use is restricted to some countries while others use it freely due to economy depending on it and the inefficiency of natural colors production (according to the regulations by organizations like the United States FDA, World Health Organization (WHO), and the European Food Standards Authority (EFSA) (Wrolstad and Culver 2012; Galaffu et al. 2015; Oplatowska-Stachowiak and Elliott 2017; Coultate and Blackburn 2018; Shanmugasundaram and Rujaswini 2019). However, microbial products and plant pigments gain popularity due to increasing explorations and high-tech techniques for purification and stability. The demand for natural colors has increased so much that it is estimated to increase by 7% annually, and almost all the natural pigments are being used at this time for at least one department of the food industry (Clark 2011; Scotter 2011, 2015; Faustino et al. 2019).

Microbial pigments are the natural pigments that have quite extra advantages over the remaining classes of natural pigments like microbial handling is very easy and adequate without needing large spaces for their growth and care. Their environment can be easily regulated and are not hypersensitive to seasonal changes. Its exponential power of division would provide a sufficient amount in a limited time, a cheap practice relatively as their maintenance costs are less. The product yield is high, and thus, their commercial applications are promising (Panesar et al. 2015; Sen et al. 2019). To add to these advantages, these colors could also be beneficial to us by providing nutraceutical benefits in acting as antimicrobial, anticancer, or antioxidants and thus be added to food items as functional food ingredients additives manifesting the function of color along with various other benefits. A few examples include flavins, carotenoids [Lutein and Zeaxanthin (Lin et al. 2015); Sarcinaxanthin, Decaprenoxanthin—not synthesized by plants] (Dufossé 2018), Melanins, Azaphilones like *Monascus* Red, Anthraquinones like Fungal Natural Red, etc. (Downham and Collins 2000; Rajguru et al. 2016; Narsing et al. 2017; Heer and Sharma 2017). The present era of genomics and proteomics could take microbial pigment biosynthesis to an altogether new level where genes could be overexpressed or made stable through appropriate editing tools and in integration with nanotechnology is proving to be a success (Venil et al. 2013; Barnawal et al. 2017; Jixian et al. 2017; Lin et al. 2017; Pailliè-Jiménez et al. 2020).

At present, the challenge for microbial pigments to flourish in the commercial market is essential, and their competition is the synthetic colors. Their commercial success is dependent on their efficient generation, purification, stability, and approval by the food regulating authorities (Mapari et al. 2010; Tuli et al. 2015; Jurić et al. 2020). These factors that determine the success of microbial pigments are few but have been worked upon by scientists for years. To build such qualities in organic products is practically very difficult as every organic product is prone to be affected by both biotic and abiotic factors very quickly, unlike synthetic colors. Use of synthetic color has been going on for years, and their stability, coloring effect, and shelf-life remain unchanged for months altogether, but microbial pigments need relatively large amounts of raw material, which further leads to their high-cost disparity (about 20 times more than synthetic pigments) (Sigurdson et al. 2017). The trials of their use are also not global, and hence their effects on different groups of populations remain unknown, along with what effects they create when used with different types of food items across the globe. The interactions between microbial pigments and the other organic biomolecules within the food items can vary, and uniformity is not possible as we observe that vitamin C is very compatible with microbial carotenoids but causes degradation of anthocyanins (Wrolstad and Culver 2012; Chaitanya Lakshmi 2014; Kirti et al. 2014; Rodriguez-Amaya 2016) and at the same time, both the pigments are destroyed under conditions of exposure to oxygen or light (Mayne 1996; Laos et al. 2007; Qiu et al. 2018). Similarly, authorities rarely permitted fungal pigments into commercial business because of the toxic effects due to mycotoxins (Frisvad et al. 2004). The *Monascus* species produce efficient red and yellow polyketide pigments, which have efficient commercial power in the coloration of sea foods like fish paste and surimi and also meats like hams and sausages. Due to the presence of mycotoxin, citrinin, they are not approved by European Union and the US food authorities (Dufossé 2006). Thus, a shift of synthetic to microbial pigments is essential and needed but making it possible requires great efforts. Such efforts have been going on from the past few years where nanotechnology (making nano-emulsions), biochemistry (making micro encapsulations), and metabolic engineering. Regulation has paved the way for giving microbial pigments a chance and make our lives better and our environments sustainable.

8.9 Application of Microbial Pigments in Nutraceutical Industries

Application of microbial pigments in food coloration imparts coloration of varied cuisines like processed meats, fish and their products, varied vegetable-based foods, improving wine quality, desserts, and even flavored milk (Dufossé 2006). Besides these diverse applications, microbial pigments, unlike synthetic pigments, prove beneficial not only to the environment due to their organic nature but also nutraceutical in nature. That is, in addition to making our foods colorful and attractive, they possess health benefits as well.

8.9.1 Antioxidant Activity

Various microbial pigments have been found to exhibit antioxidant activity (Chandra et al. 2020). For instance, violacein known to protect the lipid bio membranes from the free radical activity is produced by *Pseudoalteromonas* sp. and *Chromobacter violaceum* (Konzen et al. 2006), and it also activates the mucosal defense mechanisms (De Azevedo et al. 2000; Antonisamy and Ignacimuthu 2010). Monascus pigments (Vandamme and Revuelta 2016), (rare C_{50} carotenoids like sarcinaxanthin and its derivatives from bacterium *Micrococcus yunnanensis*) (Osawa et al. 2010), phenolic carotenoids (3,30-dihydroxyisorenieratene from *Streptomyces mediolani*) (Martin et al. 2009), cyanobacterial pigments (lycopene, lutein, phycocyanins), and phycobiliproteins have all been reported to act against the oxidative damage and hence can be used as potential antioxidants (Sonani et al. 2016).

8.9.2 Antimicrobial Activity

Many microbial pigments show the property of antibiotics and even in some cases proved better than synthetic antibiotics like an endophytic fungus pigment proved more effective than Streptomycin (Visalakchi and Muthumary 2009). Similarly, violacein displays antifungal, antiprotozoal, and antiviral activities (Nakamura et al. 2003; Lopes et al. 2009; Sen et al. 2019). Gram-negative and gram-positive bacteria are effectively being attacked by prodiginine compounds produced by various strains of *Serratia marcescens* and have also been effective against several classes of fungi (Stankovic et al. 2014; Suryawanshi et al. 2017; Ji and Kim 2019). Marine bacteria like *Pseudoalteromonas tunicate* produce antibacterial and antifungal compounds called Tambjamines (Franks et al. 2005; Kim 2013). Phenazine compounds obtained from *Pseudomonas* and *Streptomyces* species have been observed to show antibacterial, antiviral, and antifungal properties (Schneemann et al. 2011; Saeed et al. 2019). Several quinones and anthraquinones are reported to show antibacterial and antiviral properties (Margalith 1999; Koyama 2006; Gessler et al. 2013).

8.9.3 Anticancer Activity

Various pigments have been shown to exhibit anticancer properties by causing apoptosis of the uncontrollably growing cell lines. A few examples are Prodigiosin pigments (Yip et al. 2019), violacein (Liu and Nizet 2009; Choi et al. 2021), bacterial phenazines (Chincholkar and Thomashow 2013; Hussain et al. 2019), *Monascus* pigments (Vandamme and Revuelta 2016), and phycobiliproteins (Sonani et al. 2016).

8.10 Conclusion

Consumer demand and perception have now increased towards the use of a healthy, safe, green, and eco-friendly nutritious diet that provides metabolic, physiological, and functional benefits. Natural pigments obtained from microbial sources are safe, eco-friendly, cheaper, and provide various biological benefits such as antioxidants, antimicrobial, anticancer, and anti-inflammatory agents. For the production of microbial pigments, agri-food industrial residue is considered as the safe and excellent medium for fermentation. These residues provide carbon, nitrogen, and minerals in potent amounts for fermentation and help in the sustainable management of food waste as well. Although in the past few decades, extensive research has been done on the production of microbial pigments using low-cost substrates, sustainable processing methods for strain improvement, or genetic modification of the strains for the synthesis of pure pigments. But the large-scale production and downstream processing of the pigments at the industrial level is still a challenge. Hence, there is a need to develop technologies to produce safe and clean microbial strains which can be used to synthesize colorants at a large scale to meet the increased market demand for natural colors.

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